Structure and concentrating mechanism in the mammalian kidney

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According to the countercurrent hypothesis (1, 2), the loops of Henle act as a countercurrent multiplier system, which creates the increase in osmotic concentration in the kidney tissue from cortex toward the papilla. The final concentration of the urine is supposed to be brought about by passive diffusion of water from the collecting ducts to the interstitium as the urine passes through regions of increasing osmotic pressure. All experimental evidence obtained so far is consistent with the countercurrent hypothesis (2-6), and, in principle, there can no longer be much doubt about its validity. However, most mammalian kidneys consist of both long- and short-looped nephrons. Whether these two types of nephrons operate in essentially the same manner or if their functions differ is not known. Similarly, the functions of the various parts of the loops of Henle are not understood. There is good experimental evidence for sodium transport out of the thick ascending limb of the loop of Henle (4, 5, 7, 8) in the outer zone of the medulla, but it is not known with certainty if sodium is also actively pumped out of the ascending part of the thin limb of the loop in the inner zone of the medulla. It has, so far, not been possible to demonstrate any histological difference between the ascending and descending part of the thin limbs, and this makes it difficult to conceive that sodium should be reabsorbed in the ascending and not in the descending limb.

The maximal concentration that a uniform countercurrent multiplier system can achieve is directly related to the length of the multiplier system. We should therefore expect the ability to concentrate the urine to be closely related to the length of the loops that act as multiplier systems. If only the outer zone of the medulla were active, a thick inner zone should not appreciably increase the concentrating ability. If, on the other hand, the entire inner zone also acts as a multiplier system, one should expect the concentrating ability of the animal to be related to the combined thickness of the outer and inner zone of the medulla.

The present study was undertaken primarily to determine if such a relationship exists, and to determine if the relative numbers of short- and long-looped nephrons play any decisive role in the concentrating ability. Data on maximal concentrating ability from a number of mammals were obtained from the literature and previous studies: man (9), dog (F. H. Epstein, personal communication), cat (10, 11), rat (12, 13), kangaroo rat (14-16), and jerboa (manuscript in preparation).

The kidneys of these animals represent a considerable range in relative medullary thickness, and maximal urinary concentrations have been determined under a variety of physiological conditions giving values which, within limits, appear to be reasonably reliable.

In order to include kidneys of extreme morphological types, we examined the beaver, the pig, and the African rodent Psammomys. The beaver kidney has no inner zone of the medulla and 100% short-looped nephrons. The pig kidney also has a thin renal medulla. It has a short inner zone, and only 3% of the nephrons are long-looped. The Psammomys kidney represents the other extreme, it has a well developed thick inner zone and 100% long-looped nephrons. Further evidence for this assumption was furnished through studies of the distribution of urea and electrolytes in different kidney types during antidiuresis. The beaver with 100% short-looped nephrons, the rabbit with 44% long-looped nephrons, and the desert rodent Psammomys with 100% long-looped nephrons were studied. In all three kidney types sodium and urea concentrations increased to approximately the same level in the outer zone of the medulla. In the rabbit and Psammomys kidneys a considerable increase in sodium concentration was found through the inner zone of the medulla. This can best be explained if we assume that the sodium pump, which has been demonstrated in the thick ascending limb of the loop of Henle, functions in a similar manner in the thin limb.
looped nephrons. Thus, by including these mammals with the others from the literature, it is possible to extend the range in both directions.

The results of the comparison indicated a close correlation between the urinary concentrating ability and the relative medullary thickness. On the other hand, the relative numbers of long- and short-looped nephrons did not seem to have any significant role in this relationship.

Another approach to the study of the function of short- and long-looped nephrons was the determination of the distribution of electrolytes and urea in the various kidney types. The beaver, rabbit, and *Psammomys* were chosen for this part of the study. The beaver and *Psammomys* kidneys represent the two extremes, 100% short-, and 100% long-looped nephrons, respectively. The rabbit kidney has a mixed population of nephrons, with 44% long-looped nephrons. The kidneys were removed from the animals during the state of antidiuresis. They were cut into slices perpendicular to the axis of the papilla, and each slice was analyzed for urea and electrolytes. The data obtained in this manner can be used to calculate the average concentration of solutes in the tissue water in a cross section of the kidney. They do not give any information concerning the concentration differences between the fluid inside the nephrons, and the capillaries, the interstitial fluid, and the intracellular fluid. They do, however, give us information concerning the gradient from cortex through the outer and inner zone of the medulla, and concerning the difference between the average renal tissue water on the one hand and the urine and the blood on the other hand.

The particular advantage of this method is that the animal can be maintained in a completely normal physiological state until the moment it is killed and the kidneys removed for analysis. Thus the interferences with normal function from anesthesia and surgery are avoided. Furthermore, parts of the kidney, e.g., the important outer medullary region, that are not accessible to micropunctures can be investigated.

All three kidney types established, during antidiuresis, about the same sodium and urea concentration in the outer zone of the medulla. In the kidneys with inner zone the sodium and urea concentration continued to increase throughout the inner zone, thus reaching the highest values in the kidneys with the thickest medulla. The findings indicate that there is not an important qualitative difference in the function of short- and long-looped nephrons in the parts that are structurally alike. Furthermore, these findings, as well as the above-mentioned correlation between concentration ability and medullary thickness, indicate that the inner zone of the medulla also functions as a countercurrent multiplier system.

### METHODS

**Animals.** The work with the beavers, *Castor canadensis*, was carried out at Mount Desert Island Biological Laboratory, Maine, on five adults and three young. The beavers were trapped on the island and maintained in outdoor cages. The pigs, *Sus scrofa domestica*, were studied at Duke University, where two immature female

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**Table 1**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Kidney Size, mm</th>
<th>% Long-Looped Nephrons</th>
<th>Relative Medullary Thickness</th>
<th>Max. Freez. Pt. Depression in Urine ΔC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaver</td>
<td>36</td>
<td>0</td>
<td>1.3</td>
<td>0.96</td>
</tr>
<tr>
<td>Pig</td>
<td>66</td>
<td>3</td>
<td>1.6</td>
<td>2.00</td>
</tr>
<tr>
<td>Man</td>
<td>64</td>
<td>14</td>
<td>3.0</td>
<td>2.60</td>
</tr>
<tr>
<td>Dog</td>
<td>40</td>
<td>100</td>
<td>4.3</td>
<td>4.85</td>
</tr>
<tr>
<td>Cat</td>
<td>24</td>
<td>100</td>
<td>4.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Rat</td>
<td>14</td>
<td>48</td>
<td>5.8</td>
<td>4.85</td>
</tr>
<tr>
<td>Kangaroo rat</td>
<td>5.9</td>
<td>27</td>
<td>8.5</td>
<td>10.4</td>
</tr>
<tr>
<td>Jerboa</td>
<td>4.5</td>
<td>33</td>
<td>9.3</td>
<td>12.0</td>
</tr>
<tr>
<td><em>Psammomys</em></td>
<td>13</td>
<td>100</td>
<td>10.7</td>
<td>9.2</td>
</tr>
</tbody>
</table>
animals was not determined in the rabbits. The
Psammomys obesus work was done partly at Duke
University on animals imported from the Nile valley,4
and partly in the Sahara.6 About 30 animals were used.
Rabbits, Oryctolagus cuniculus, were obtained from a local
dealer. They were maintained in the laboratory for a
few days only, prior to the experiments. Maximal
concentrating ability was not determined in the rabbits.

**Concentrating ability.** In order to obtain maximal
urinary concentrations, the animals were, at different
times, exposed to water deprivation (with or without
administration of Pitressin), urea loading, and salt
loading. However, because of the profound differences
in feeding habits of this diverse group of mammals it
was not possible to treat them in a uniform manner.
The beavers were fed aspen branches, of which they
consumed the leaves, smaller stems, and bark. Even
the bark contained a considerable amount of water
(about 30 %), and, consequently, water deprivation
without simultaneous starvation was not possible.
However, when given water ad libitum the beavers
consumed large amounts (the beaver’s habit of im-
mersing itself in its drinking water made it difficult to
obtain accurate determinations of the daily consump-
tion). When given food, but deprived of drinking water,
the beavers lost weight and the plasma concentrations
increased, showing that water balance could not be
maintained without drinking water. Furthermore,
Pitressin administration during water deprivation did
not increase the urinary concentration any further.

Because of the limited concentrating ability and
selective eating habits of the beavers, urea loading could
best be accomplished by giving a limited amount of 1 %
urea solution as drinking water. This form of urea loading
resulted in a twofold increase in plasma-urea concen-
tration.

The pigs were maintained on commercial pig feed
(10 % protein). For salt loading, the pig feed was mixed
with 100 g of NaCl/kg of feed, and for urea loading,
200 g urea/kg of feed.

The natural diet of the rodent, *Psammomys*, consists
of the succulent, salty plants, Traganum and Salicornia.
The total electrolyte concentration of the sap of these
plants is about 900 mEq/liter (in preparation). The
highest salt concentrations in the urine were obtained
in the Sahara experiments when the animals were given
their normal food plants. In the laboratory at Duke
they were maintained on fox checkers, and a 5 % NaCl
solution for drinking water. For urea loading in the
laboratory, ground checkers were mixed with 15 g
of urea/100 g of checkers and given for 1–2 weeks, with
plain water for drinking. Then water was removed for
a period of 24–36 hr prior to urine collections. Salt
loading was accomplished by giving a mixture of 10 g
of NaCl/100 g of ground checkers.

1 These animals were kindly furnished by Dr. Harry Hoogstraal,
N.A.M.R.U. No. 3, Cairo, Egypt.
2 The work in the Sahara was done in collaboration with Dr.
Knut Schmidt-Nielsen at Centre de Recherches Sahariennes,
Beni Abbes, Algerie.

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**FIG. 2.** Schematic drawing of three different kidney types. Dotted lines separate the three zones, cortex, outer zone of the medulla, and inner zone of medulla. Type of nephrons occurring in the kidneys are shown greatly enlarged, but in their correct position relative to the zones of the kidney.

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**FIG. 3.** Sodium concentrations determined in the tissue water of the kidneys of two beavers, two rabbits, and two *Psammomys* plotted against the zones of the kidney. All six animals were producing a urine that was nearly maximally concentrated (Table 2). Abscissa has been divided in following way: cortex and the outer zone of the medulla have been given equal length. This is correct for the rabbit and for the *Psammomys*, but not for the beaver, where the cortex is twice as thick as the medulla. However, the thickness of the cortex is unimportant for this comparison. Inner zone of the medulla has been given a length on the abscissa that corresponds to its thickness relative to the cortex and the medulla. In the rabbit, 60% in the *Psammomys*, 71 % of the combined thickness of cortex and medulla.

Urea, ammonia (17), true endogenous creatinine (18), and total electrolytes (19) were determined in all urine and blood samples. In addition, sodium, potassium (Baird flamephotometer) and osmolality (Fiske osmometer) were determined in all samples from the pig experiments, and sodium in the samples from the *Psammomys*. In urine samples from beaver and *Psammomys* the osmolality was estimated as the sum of the molar concentration of urea and 2 times the sum of the total electrolyte concentration.
Determination of solute distribution in the kidneys. The animals were killed about 25–30 min after a voluntary urination. Thus, a urine sample formed within the last 25 min prior to death could be collected from the bladder. Blood was taken from the heart. The kidneys were removed immediately and the middle section of the kidney cut out and frozen instantly in a mixture of dry ice and acetone. In the beaver, which has several papillae in each kidney, two or three sections, each containing a papilla, were cut out. The time that elapsed from death to the time all kidney slices were frozen was less than 5 min. While still frozen, the kidney slices were cut with a sharp scalpel into slices perpendicular to the axis of the papilla. The number of slices cut from cortex, outer and inner zone, varied with the species of animals, as indicated in the graphs. The slices were weighed on a Roller-Smith balance. Their weights ranged from 4–100 mg. The slices from one kidney were ground in mortars with sand and 1 ml of water and analyzed for urea and ammonia by the Conway method (17). The slices from the other kidney were cut in the same manner, weighed and placed in 10-ml Erlenmeyer flasks with 1 ml of water added. The flasks with sample and water were weighed, then heated to boiling in a water bath. After cooling, water was added to the flasks to the same weight as before boiling. The flasks were closed tightly and left for 24 hr for diffusion to take place. The supernatant was then analyzed for sodium, potassium, and total electrolytes. In a later stage of the investigation we also analyzed these samples for urea and found good agreement between this procedure and the method of grinding the samples with sand.

Calculations of concentration in renal tissue water are based on determinations of wet and dry weights of the kidneys analyzed. All values are presented as concentrations in mM or mN per liter of tissue water. The dry weight determinations were made on two or three kidneys from each species.

The osmotic concentration (given in mOs) in the tissue water has been estimated as the sum of the urea concentration in mM + 2 × electrolyte concentration in mM. This, of course, can only give an approximate figure.

RESULTS

Concentrating ability and medullary thickness. In Table 1 the maximal freezing-point depression calculated in the urine of beaver, pig, and Psammomys is listed together with similar values from other mammals. These values are compared with Sperber’s data for relative medullary thickness, kidney size, and nephron population (20). In Fig. 1, maximal freezing point depression of the urine, and the maximal values for urine-urea and electrolyte concentrations, are plotted against relative thickness of the medulla. The reason for choosing the relative

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**TABLE 2**

<table>
<thead>
<tr>
<th>Beaver 6</th>
<th>537</th>
<th>108</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaver 7</td>
<td>438</td>
<td>118</td>
</tr>
<tr>
<td>Rabbit 2</td>
<td>1390</td>
<td>214</td>
</tr>
<tr>
<td>Rabbit 3</td>
<td>914</td>
<td>159</td>
</tr>
<tr>
<td>Psammomys 13</td>
<td>3023</td>
<td>150</td>
</tr>
<tr>
<td>Psammomys 18</td>
<td>2199</td>
<td>290</td>
</tr>
</tbody>
</table>

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Rather than the absolute thickness of the medulla for the comparison is that the diameter of the nephrons increases somewhat with the size of the kidney.

It should be emphasized here that the maximal values for urine concentrations obtained in our studies as well as from the literature are, of course, approximate only. The variability between individuals of the same species and the long-term effects of diet and environment make it impossible to give any sharply defined upper limit for the concentrating ability of a certain species. Furthermore, in the case of the beavers and the pigs the number of individuals used was very small.

It can be seen from the graph and from the table that in spite of the crudeness of such a comparison a definite increase in maximal freezing-point depression of the urine is found with increasing medullary thickness. However, one animal, the Psammomys, does not show quite as high concentrating ability as one could expect from the medullary thickness.

There is a rather good correlation between the ability to concentrate urea and the medullary thickness, but again, the Psammomys has a distinctly lower concentrating ability for urea than the other rodents with comparable medullary thickness.

When the maximal electrolyte concentration is plotted against medullary thickness, the correlation is much better and the value for the Psammomys does not fall out of line. The relative number of long- and short-looped nephrons in the kidney has no effect upon this relationship. In the beaver, pig, and Psammomys, the ability to concentrate electrolytes is distinctly better than the ability to concentrate urea. It should be noted, in contrast, that in all the other animals the ability to concentrate urea is considerably better than the ability to concentrate electrolytes.

Neprhon structure and distribution of solutes in the kidney. The animals used for this part of the study were beaver, rabbit, and Psammomys. The three kidney types are shown in schematic drawings in Fig. 2.

The kidney of the European beaver (Castor fiber) has been described by Sperber (20): "There are two short papillae, only slightly projecting into the pelvis. The cortex is about 7 mm thick, the medulla is 14 mm. The former contains thick medullary rays. The medulla is not divided into zones."

The kidneys of Castor canadensis examined by us differed in two respects from those described by Sperber. Instead of two papillae, there were often three to four papillae in each kidney. The cortex was thicker and was twice the thickness of the medulla. As described by Sperber, there were no long-looped nephrons and thus

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6 Under certain physiological conditions (e.g., high protein diet) some other solute is present in the tissue in sufficiently large amounts to make the figure for the estimated osmolarity much too low.
the medulla consisted only of what corresponds to the outer medullary zone in other mammals.

Sperber describes the nephrons as follows: “There are cortical nephrons (loops that turn in the cortex) and short looped nephrons; the kidney seems to be devoid of long loops. . . . The cortical nephrons mostly have no thin segment. The loops always turn in the thick segment which is differentiated into a thick and a thinner part in those nephrons whose loops enter the medulla.”

According to Sperber, the kidney of the rabbit, *Oryctolagus cuniculus*, has, on the average, the following thickness of the zones: cortex 3-4 mm, outer zone 3-4 mm, and inner zone 9-10 mm.

There are both short- and long-looped nephrons, approximately 44% of the loops being long. The short loops normally turn in the inner part of the outer zone only. They often turn where the thin segment turns into the thick segment. The various segments of the long-looped nephrons had, on the average, the following lengths: proximal tubules, 38% of total length; thin segment, 47%; thick segment, 9%; distal tubule, 6%.

The kidney of *Psammomys obesus* is described by Sperber (20) as follows: “The cortex is, after maceration 2.2 mm thick, the outer zone 2.5 mm, the inner zone 1.2 mm. Before maceration the layers could not be clearly distinguished but their joint depth was 16 mm. The papilla is very strongly developed, long and broad and projects into the ureter. There are long loops only.” Sperber describes the thin segment as “relatively enormous as compared with normal forms.” He has estimated the length of a nephron reaching the apex of the papilla as follows: “The proximal tubule occupied 23% only of the total lengths, the thin segment 62%, the thick segment 10%, and the distal tubule 3%.”

In Fig. 3, the sodium concentration determined in the tissue water of the kidneys of two beavers, two rabbits, and two *Psammomys* are plotted against the zones of the kidney. All six animals were producing a urine that was nearly maximally concentrated, this can be seen from the figures for urine osmolarity and creatinine U/P ratio given in Table 2. In all six animals, sodium rose to approximately the same value in the outer zone of the medulla. In the rabbit and *Psammomys*, sodium concentration continued to rise through the inner zone of the medulla, and in the *Psammomys*, with a thicker inner zone of the medulla, the final value was higher than in the rabbit. Thus, it appears that maximal sodium concentration that can be reached in the renal papilla is directly dependent upon the relative thickness of the medulla.

In Fig. 4, the concentration ratios in the renal tissue of the three different types of kidneys are given. It is seen that the urea concentration also rises through the inner zone of the medulla, and that the maximal urea concentration that can be attained in the tissue appears to be in some way related to the thickness of the medulla.

In the beaver kidney, where there are only short looped nephrons, the urea concentration in tissue water rose to its highest value at the tip of the papilla (Fig. 5). In all beavers the most marked increase occurred in the innermost zone of the medulla, i.e., the inner stripe of the outer zone of the medulla, where the descending limb of the loops of Henle and the ascending part of the thick segment are located. In the rabbit kidney, with a mixed population of long- and short-looped nephrons, a similar increase in urea concentration occurred in the same anatomical zone of the kidney as in the beaver, but urea concentration continued to increase in the outer zone of the medulla, although it did not increase to the very tip of the papilla. In the *Psammomys* kidney, where there are long-looped nephrons only, the distribution was essentially the same as in the two other kidney types, the only important difference being that the osmotic concentration at the tip of the papilla was rather enormous compared to that in the beaver kidney.
The mechanism that sets up the osmotic gradient in the kidney presumably is the pumping of sodium out of the ascending limb of the loop of Henle. This is indicated by the low osmotic concentration (4, 5) and low sodium concentration found in the early distal convoluted tubule (7), and by the sodium gradient in the renal medulla. The sodium that is pumped out may diffuse into the descending limb or it may remain in the interstitium. If the latter is the case, it must then cause water to move out of the descending limb passively (7). The sodium reabsorption in the ascending limb will in either case cause the osmotic concentration in the descending limb to rise, and will, because of the multiplier effect, cause a rise in the sodium concentration toward the papilla. What causes the urea concentration to increase toward the tip of the papilla is not yet clear (21). That the mechanism is distinctly different from that which causes sodium concentration to rise is evident from microtubule studies (7, 22) and from the different distribution patterns found in this study.

Do both short- and long-looped nephrons pump sodium? In kidneys with exclusively short-looped nephrons as well as in those with both types of nephrons, and those with long-looped nephrons only, the urea and sodium concentration showed an increase to about the same value in the zone corresponding to the outer zone of the medulla. Thus, it appears that both types of loops function in essentially the same manner in the parts that are structurally alike, viz., the parts located in the inner and outer stripe of the outer zone of the medulla.

Is the sodium pump restricted to the thick ascending limb of the loop of Henle or does sodium reabsorption also take place in the thin limbs? It is quite evident that there is a close correlation between the relative thickness of the medulla and the ability to concentrate the urine. It also seems that the ability to concentrate electrolytes is more closely related to the thickness of the medulla than the ability to concentrate urea. The Psammomys, which has, relatively, the thinnest inner zone of the medulla, shows a poorer ability to concentrate urea than the jerboa and the kangaroo rat, but with respect to concentrating electrolytes, its ability exceeds the ability of the other two animals.

In the rabbits and Psammomys with long-looped nephrons sodium concentration was found to increase all the way to the tip of the renal papilla. It has been suggested by Ullrich (23) that sodium concentration in the tissue of the renal papilla may be uniformly the same throughout the inner zone and that the increasing concentration found in the kidney slices is due to the fact that the slices represent the collecting-duct fluid as well as the tissue water. It is unlikely that this is correct because a) in the animals with a high potassium but low sodium concentration in the urine, the same steep increase in sodium concentration throughout the inner zone of the medulla is found (21; Psammomys 16 and 18, and rabbit 8) and b) quantitatively, it would be difficult to explain that the sodium concentration at the tip of the papilla had almost three times the sodium concentration in the outer zone, if the sodium concentration in the loop of Henle and in the interstitium did not continue to increase throughout the inner zone.

Thus, it seems more likely that the thin limbs of the loops of Henle act as countercurrent multiplier systems all the way to the tip of the papilla. This assumption is also consistent with the finding that maximal concentrating ability is directly related to the total thickness of the medulla. Further evidence for this assumption has recently been furnished by micropuncture studies (24) on Psammomys, where a progressive increase in osmolality toward the tip of the papilla was found in the thin limb of the loop of Henle.

REFERENCES