Contribution of urea to urinary concentrating ability in the dog

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SCHMIDT-NIELSEN, BODIL, AND ROSCOE R. ROBINSON. Contribution of urea to urinary concentrating ability in the dog. Am. J. Physiol. 218(5): 1363-1369. 1970.—Dogs were maintained on balanced high- and low-protein diets for 3 weeks prior to the experiments. Urea clearance, insulin clearance, and solute concentrations in the various zones of the kidney were determined. The glomerular filtration rates and osmolar clearances were approximately equal in the two dietary groups. Maximum osmolar urine-to-plasma ratios were far higher in the high- (8.3) than in the low-protein dogs (3.0). This difference was primarily due to urea accumulation in the inner zone of the renal medulla. Blood urea concentration was 2.5 times higher in high- than in low-protein dogs, whereas the amount of urea per gram urea-free dry tissue at the medullary crest was 6 times higher in the high- than in the low-protein dogs. Uphill transport out of the collecting duct was indicated by a higher urea concentration in the tissue of the inner medulla than in the urine in the low-protein dogs. Compared to other mammals that conserve urea during low-protein feeding, the mechanism in the dog appears to be less pronounced.

MAXIMAL URINARY CONCENTRATING ABILITY is, in some mammals, influenced greatly by the relative contribution of urea and nonurea solutes to the total solute composition of the final urine. Gamble et al. (6) first observed in the rat that maximal urinary concentration was achieved when urea excretion accounted for approximately 60% of the total excreted solute. This observation has since been confirmed in the rat (4, 13), and observations that urea enhances concentrating ability have been made in other mammalian species as well, i.e., man (6), dog (17), opossum (21), and sheep (26) and unpublished observations). Since maximal concentrating ability is thought to be a direct function of the effective osmolarity of medullary interstitial fluid at the end of the collecting ducts, it is logical to expect that a relative increase of urea excretion and an associated enhancement of concentrating ability are accompanied by an appropriate rise of medullary tonicity.

The present studies were initiated in the dog to further evaluate the relationships between changes of dietary protein intake and those of urea excretion, medullary solute composition, and maximal concentrating ability. Particular attention was directed toward characterizing the changing medullary solute pattern during high- and low-protein feeding in an effort to gain further insight into possible mechanisms by which increased medullary tonicity might be achieved during high-protein feeding when urea excretion and urinary concentrating ability are highest. Further emphasis was also placed on reassessment of the presence or absence of an uphill urea-concentration gradient from collecting-duct fluid to the medullary interstitium during low-protein feeding—a circumstance that has now been observed in several other animal species (2, 25, 29) during similar dietary conditions. The presence of this phenomenon seemed likely in the dog because previous clearance studies (23) and unpublished observations) have indicated that this species exhibits renal tubular regulation of urea excretion (decreased fractional urea excretion at low rates of urine flow) during low-protein feeding—a common parallel finding in low-protein rats where net urea reabsorption against an uphill concentration gradient has now been documented clearly (3, 16, 32).

METHODS

Renal clearance studies and chemical analyses of kidney tissue were performed on 13 healthy dogs weighing between 8 and 18 kg. Four dogs were maintained on a high-protein diet (70% protein, 3% salt), two dogs on a medium-protein diet (36% protein, 7.8% salt), and seven dogs on a low-protein diet (7.3% protein, 11.8% salt). The diets were specially prepared by the General Biochemicals Co., Chagrin Falls, Ohio, according to formulas that have been described previously (29). The protein and sodium chloride composition of these three diets is varied so that all three diets contain the same amount of total solute to be excreted per calorie. Four of the thirteen dogs were pair fed since total food consumption is often reduced during prolonged low-protein feeding; two of these four dogs were permitted ad libitum access to the low-protein diet while the two additional animals received a high-protein diet that provided a total solute and caloric load that was identical to that consumed by the low-protein dogs.

All animals were allowed free access to water throughout the period of dietary adaptation until 24 hr prior to the experimental day. Dietary water was withdrawn at that time and each animal subsequently received an intramuscular injection of Pitressin tannate in oil (0.1 U/kg) about 12 hr prior to study. In the nine dogs that were not pair fed, each experiment was initiated by the intravenous administration of a single dose of inulin (0.25 g/kg) on the
morning of the experimental day; in two of these nine dogs, inulin administration was accompanied by the simultaneous intravenous injection of urea-14C (25 μCi/kg). Thereafter, bladder urine was collected for about 60 min via free flow into a small test tube that was attached to the end of the indwelling bladder catheter; each dog was permitted to move about freely throughout this initial collection and equilibration period. Immediately thereafter, the bladder was emptied utilizing an air wash, and a timed urine collection of 10–15 min duration was obtained; a sample of peripheral venous blood was secured at the midpoint of this collection period. Upon termination of the collection period, each dog was killed by the intravenous injection of phenobarbital (150 mg/kg), the abdomen was then incised rapidly, and both kidneys were removed quickly for immediate processing and subsequent measurement of total osmolality and the concentrations of sodium, potassium, and urea in renal tissue.

The four pair-fed dogs were handled somewhat differently on the experimental day. After water deprivation and Pitresin administration as described above, each of these animals was first anesthetized with intravenous pentobarbital (25 mg/kg). Inulin was administered intravenously (0.25 g/kg) as a single dose, the abdomen was then incised, and the left ureter was cannulated with polyethylene tubing. The tip of the ureteral catheter was placed within 5 cm of the left renal pelvis so that high ureteral urine might be collected directly and thus minimize any possible loss of urinary urea via diffusion across the ureteral or bladder epithelium. After an equilibration period of 30–45 min duration, three urine-collection periods of 10 min duration were obtained sequentially and a sample of peripheral venous blood was obtained at the midpoint of each clearance period. Upon termination of the third clearance period, the hilus of the left kidney was clamped and the organ was removed rapidly for immediate processing.

**Analytical methods.** Two transverse slices were taken from the midportion of each kidney immediately after its removal. In the four pair-fed animals, each of the two transverse slices was frozen immediately in a mixture of Dry Ice and acetone (O-25 g/w as a single dose, the abdomen was then incised, and the left ureter was cannulated with polyethylene tubing. The tip of the ureteral catheter was placed within 5 cm of the left renal pelvis so that high ureteral urine might be collected directly and thus minimize any possible loss of urinary urea via diffusion across the ureteral or bladder epithelium. After an equilibration period of 30–45 min duration, three urine-collection periods of 10 min duration were obtained sequentially and a sample of peripheral venous blood was obtained at the midpoint of each clearance period. Upon termination of the third clearance period, the hilus of the left kidney was clamped and the organ was removed rapidly for immediate processing.

**Analytical methods.** Two transverse slices were taken from the midportion of each kidney immediately after its removal. In the four pair-fed animals, each of the two transverse slices was frozen immediately in a mixture of Dry Ice and acetone and then, while still frozen, was divided into seven adjacent sections or zones progressing inward from the outer cortex to the crest of the medulla (Fig. 1). Each section (50–400 mg) from one of the slices was placed in a preweighed 10-ml Erlenmeyer flask containing 2.0 ml of distilled water and then reweighed. Each piece of tissue was dried for 48 hr at 105 C and then reweighed preparatory to calculating the water content of each section. The dried tissue was dissolved in 1.0 ml of concentrated nitric acid and the concentrations of sodium and potassium were measured in the supernatant.

Similar observations were made on two transverse slices of renal tissue from each of the nine dogs which were not pair fed. One slice was frozen prior to sectioning (Fig. 1), and each of the divided frozen sections was then placed in a weighed Erlenmeyer flask and handled in a manner identical to that outlined above. The other slice was sectioned without prior freezing since it has been suggested that freezing and thawing may effect an increase in tissue osmolality (1). Nonfrozen tissue sections were placed directly in weighed Erlenmeyer flasks and handled thereafter as outlined above. The results of the chemical analyses on frozen and nonfrozen tissue did not differ significantly, so the results of the two analytical approaches were combined and averaged in each of the experiments.

Sodium and potassium concentrations in plasma, urine, and tissue diffusate or supernatant were measured by standard flame photometry; urea concentrations were determined by the microdiffusion method of Conway, and osmolality was measured cryoscopically using a Fiske or Advanced osmometer. Inulin concentrations in plasma and urine were measured by a modified resorcinol method (28), or by the microanthrone method of Fuhr, Kaczmarczyk, and Krüttgen (7). Urea-14C activity was determined in a liquid scintillation counter; 35 μl of tissue diffusate, plasma, or urine were placed in a counting vial to which 0.2 ml of Hyamine was then added and mixed. An additional 15.0 ml of scintillation mixture (29) was added preparatory to final counting.

**RESULTS**

The blood urea concentration averaged 3.5 mm in the low-protein dogs and 9.0 mm in the high-protein animals. These changes were accompanied by similar qualitative differences in urea excretion between the two groups as shown in Table 1. The low-protein animals exhibited renal conservation of urea as indicated by the lower urea-to-inulin clearance ratio, 25% in the low-protein animals and 49% in the high-protein animals (P < 0.02). The difference is more significant than it appears since the urine flow was twice as high in the low- (0.020 ml/kg per min) as in the high-protein animals (0.011 ml/kg per min). This would normally give a higher rather than a lower urea-to-inulin clearance ratio. Average values for inulin clearance and osmolal clearance were similar in the two dietary groups: inulin clearance equaled 5.9 and 5.0 ml/kg per min, and osmolal clearance was 0.63 and 0.59 ml/kg per min during high- and low-protein feeding, respectively. Overall, urinary urea excretion accounted for 58% of the total excreted sodium.
solute in high-protein dogs, and 23% of the total excreted solute in low-protein dogs.

Maximal concentrating ability and tissue osmolality. High- and low-protein feeding was attended by marked differences in maximal concentrating ability. The urea osmolality averaged 2,643 mOsm/kg in the four high-protein dogs and only 947 mOsm/kg in the seven low-protein dogs (Table 1 and Figs. 2 and 3). The observed differences in maximal concentrating ability were accompanied by similar differences in medullary osmolality. However, in both dietary groups, a progressive rise of medullary osmolality toward the crest of the medulla was always observed and maximum values were achieved in inner zone 3 (IZ3). In low-protein dogs, the maximum urea osmolality was less than that observed in IZ3, whereas during high-protein feeding the total tissue urea osmolality was consistently higher than that observed in IZ3 (Figs. 2 and 3). Presumably, in both dietary groups, the interstitial fluid at the medullary crest achieves osmotic equilibrium with terminal collecting-duct fluid during antidiuresis; however, since IZ3 is not infinitely thin, the average osmolality of the entire zone may not accurately reflect the actual osmolality at the crest. For this reason, it is logical to assume that an average IZ3 osmolality which is higher than that of urine may well reflect a progressive decrease of osmolality throughout IZ3 as the actual medullary crest is approached. Conversely, in high-protein dogs, an average IZ3 osmolality less than that of the final urine may reflect a progressive rise of IZ3 osmolality toward the medullary crest.

Water content of renal tissue. The water content of renal tissue from each of the several zones was determined in the four pair-fed dogs (Fig. 4). The observed water content of all zones was similar in both high- and low-protein animals. All of the dogs exhibited an abrupt increase of tissue water content during the transition from the outer to the inner medullary zone; however, in each dog, a reduction of tissue water content always appeared apparent in IZ3 as the medullary crest was approached.

<table>
<thead>
<tr>
<th>TABLE 1. Data on urea excretion</th>
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<tbody>
<tr>
<td>Blood urea, mM</td>
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<tr>
<td>3.5 ± 0.4</td>
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<tr>
<td>Urine urea, mM</td>
</tr>
<tr>
<td>Urine osmolality, mOsm/kg H2O</td>
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<tr>
<td>Urea in % of total solute in urine</td>
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<tr>
<td>Inulin U/P</td>
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<tr>
<td>Urea U/P / Inulin U/P × 100%</td>
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</table>

Seven dogs on low-protein diet, four dogs on high-protein diet. Values are means ± SE. Values in parentheses indicate the number of clearance periods. * P < 0.01. † P < 0.02.

Urea. The urea concentration in urine and in each of the zones of renal tissue is always higher during high-protein than during low-protein feeding (Figs. 2 and 3). The difference between the total osmolality in medullary tissue in high- versus low-protein dogs could be explained almost entirely by the observed differences in tissue urea concentration—although a minor contribution of undetermined solute could not be excluded.

Overall, in all animals, the tissue urea concentration appeared to rise progressively toward IZ3, although the rise was less pronounced in the low- than in the high-protein animals.

The maximum papilla urea concentration was roughly proportional to the urine urea concentration in all of the experimental animals, however, in five of the seven low-protein dogs, the urinary urea concentration was distinctly less than that observed in IZ3 (Fig. 5), whereas it always exceeded that observed in IZ3 during medium- or high-protein feeding. The tissue-to-plasma urea ratios in each of the medullary zones were not significantly different in high- and low-protein animals (Fig. 3), whereas the urine-to-plasma urea ratio was significantly lower in the low- than in the high-protein dogs (P < 0.02).

From the data on pair-fed dogs, it can be seen (Fig. 4) that the dietary related changes of tissue urea concentration were also associated with marked differences in the absolute amount of urea per gram urea-free dry tissue solids within the various zones. In the high-protein dogs, the urea content rose progressively toward the medullary crest and in IZ3 it was approximately 6 times higher than in the comparable zone from the low-protein dogs. Conversely, in low-protein dogs, the amount of urea per gram dry weight in IZ3 was slightly lower than in IZ2.

The experiments with urea-14C indicated no significant...
intrarenal production of urea. The specific activity in the renal tissue, divided by the specific activity of the plasma measured 1 hr after the injection of urea-\(^{14}\)C, was close to unity in all of the five medullary zones in both dietary groups (average 1.13 and 1.02 in high- and low-protein animals, respectively). Similar observations have been made on rats and sheep on comparable dietary regimens (29).

Sodium and potassium. The tissue concentrations of sodium and potassium were altered very little by high- and low-protein feeding despite large differences in tissue osmolality between the two dietary groups. On the average, the medullary sodium concentration was somewhat higher in high-protein dogs; nevertheless, this apparent difference was not observed consistently as can be seen in Fig. 6, where the \(IZ3\) sodium concentration is plotted against the \(IZ3\) urea concentration. The \(IZ3\) sodium concentration was low only in those dogs that exhibited an unusually low \(IZ3\) concentration of urea; in contrast, the \(IZ3\) sodium concentration achieved maximal levels that were independent of urea concentration when the \(IZ3\) urea concentration exceeded 350 mM. Examination of the pair-fed dogs (Fig. 4) revealed that the absolute amount of sodium per gram urea-free dry solids was slightly higher in the high protein than in the low-protein animals. In both groups, the amount of sodium was approximately constant throughout the entire inner zone, as found previously by Ruiz-Guinazu et al. (22).

The tissue potassium concentrations were similarly unaffected by dietary manipulation; zonal differences between high- and low-protein dogs were not observed (Figs. 3 and 4). The concentration of potassium decreased markedly within the inner medullary zones of both dietary groups. The absolute amount per gram urea-free dry solids was remarkably constant in all zones, but decreased slightly toward the crest.

DISCUSSION

Previous observations in antidiuretic dogs have demonstrated that the fraction of filtered urea excreted is reduced substantially during low-protein feeding. For example, at normal antidiuresis fractional urea excretion was found to equal 30% of the filtered urea load in dogs receiving a normal diet plus 3% urea as drinking water, 30% of the filtered load in animals ingesting a normal diet without supplemental urea, and only 15% of the filtered load in dogs maintained on a low-protein diet (23) and unpublished observations). The present observations confirm these earlier findings and demonstrate that reduced fractional urea excretion during low-protein feeding is also accompanied by the appearance and maintenance of an inner medullary urea concentration which exceeds that of high-ureteral urine. Although this combination of findings in the dog does not localize the renal tubular regulation of urea ex-

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**FIG. 3.** Average relationship between tissue and plasma osmolality and concentrations of urea and electrolytes during high- and low-protein feedings. Left panel depicts the ratios between tissue and plasma concentrations; right panel depicts actual tissue concentrations within various zones. Mean values ± SEM.
cretion to the collecting duct or establish the occurrence of net urea reabsorption from collecting-duct fluid against an uphill concentration gradient, the data are certainly suggestive of such a possibility. It seems particularly likely since renal micropuncture studies in the rat (3, 16, 32) have shown that net urea reabsorption in the collecting duct does occur during similar experimental circumstances (low-protein feeding and reduced fractional urea excretion in the presence of an uphill medullary concentration gradient). Furthermore, it has been shown that it accounts quantitatively for the difference in the fraction of filtered urea excreted in high- and low-protein rats (5).

Earlier observations in the dog (17) have also suggested that low-protein feeding is accompanied by medullary urea concentrations which equal or somewhat exceed those of bladder urine. Since low-protein feeding in those experiments was attended by a relative reduction of filtration rate and urine flow as compared to comparable observations during high-protein feeding, the finding of urine urea concentrations slightly lower than those of the medulla was ascribed to the diffusional loss of urea from ureteral urine, as a smaller volume of urine passed through the ureter. Although such a mechanism may have been operative in those earlier experiments, it would not appear to play an important role in the present studies for at least two principal reasons: 1) urine flow was similarly reduced in both low- and high-protein animals (in fact, on the average, urine flow was higher in low-protein dogs), and 2) an uphill urea-concentration gradient between urine and medullary tissue was still observed when urine was collected from a site near the ureteropelvic junction—thus obviating the possible diffusional loss of urea along the length of the ureter.

In our results on medium- or high-protein dogs, the tissue urea concentration was distinctly lower than the urine urea concentration. Goldberg et al. (10), however, have demonstrated that the tissue urea concentration in dogs on normal protein intake may exceed that of the urine when the renal sodium gradient is abolished by ethacrynic acid. They suggest that the mechanism for urea transport out of the collecting duct may be operating at low levels even in dogs on normal protein diet.

The ratios between tissue and plasma urea concentrations were similar in both high- and low-protein dogs throughout all of the medullary zones except for the innermost zone, I23. In the high-protein dogs, the T/P urea ratio was about 50% higher in I23 than in I22. In the low-protein dogs, it was approximately the same as in I22 (Fig. 3). In the pair-fed animals (Fig. 4) low-protein feeding was accompanied by a slight reduction of urea content in the innermost medullary zone as compared to the adjacent medullary zone, I22; such was not the case during high-protein feeding where the absolute urea content of I23 was markedly higher than in I22. High- and low-protein feeding in rats and sheep is associated with qualitative differ-
ences in the pattern of intrarenal urea distribution which resemble, but are more pronounced than, those that were observed in the dogs, i.e., initial medullary accumulation of urea followed subsequently at some point by a leveling off or relative reduction of the urea concentration. Thus, the maximum tissue urea concentration appears within the outer medulla of low-protein sheep (23), in the outermost zone of the inner medulla of low-protein rats (27), and in the innermost zone of the inner medulla of low-protein dogs. These qualitative similarities and quantitative differences are correlated with the efficiency with which the kidney of each of the species conserve urea during low-protein feeding (shown in Table 2). The sheep has by far the most effective urea conservation mechanism (98% of filtered urea reabsorbed), the rat less, and the dog still less.

In the following a tentative explanation for this correlation is attempted, attributing the differences between the species in conserving urea to differences in transport of urea out of the collecting duct.

Net efflux of urea from the collecting duct apparently can take place in two ways: by diffusion down a concentration gradient and by transport against a concentration gradient. There is evidence that the net movement out of the collecting duct is mostly down the concentration gradient in the normal or high-protein rat, but is against the concentration gradient in the low-protein rat. Observations in other species are similar, but not as well substantiated. In water diuresis the collecting duct has a low permeability to urea. With increasing antidiuresis the lower part of the collecting duct becomes permeable to urea by the action of antidiuretic hormone (10, 12, 19, 20). (The permeability to urea of the cortical part of the collecting duct does not increase with antidiuretic hormone, although the water permeability increases (11).)

Urea leaving the collecting duct is known to be recycled by entering the loops of Henle and returning to the collecting ducts (3, 18, 30, 31). According to the careful anatomical investigations by Kriz of the kidneys of several mammalian species (14, 15), the ascending thin limbs of the loops of Henle, in all species examined, are found in close juxtaposition to the collecting duct in the inner medulla. In the outer medulla, on the other hand, ascending limbs are separated from the collecting duct by capillary nets. Consequently, the closer to the crest the tip urea leaves the collecting duct, the greater the probability that it is recycled and returned to the inner medulla. Conversely, urea leaving the outer medulla will enter the capillaries and return to the blood, and only a smaller fraction will be recycled within the kidney.

During normal or high-protein feeding, dog, rat, and sheep behave quite similarly (2, 4, 26, 29) and Table 2. The fraction of filtered urea excreted is about 50% in all three species and is very little influenced by urine flow in the range from maximum water diuresis (inulin U/P 10) to antidiuresis (inulin U/P up to 500). In water diuresis there is little recycling of urea in the kidney. With increasing antidiuresis the recycling increases (30) as the lower part of the collecting duct becomes permeable to urea (9, 12, 19, 20). The findings that the fraction of filtered urea excreted does not decrease appreciably with increasing antidiuresis over a wide range of urine flows, in spite of the fact that the lower part of the collecting duct becomes quite permeable to urea, indicate that urea leaving the lower part of the collecting duct is extremely effectively recycled and returned to the renal medulla. In this respect there is no difference between the three species discussed. Also, in all the three species, the urea concentration of the renal tissue increases progressively toward the innermost zone of the medulla.

In low-protein animals, the fraction of filtered urea is lower and is highly dependent on urine flow (23, 96, 29); in addition, the medullary urea concentration does not increase progressively toward the crest or papilla tip. The degree to which the three species exhibit these features differs (Table 2). It is during low-protein feeding that the active movement out of the collecting duct is most pronounced and that the urea concentration in the medullary tissue becomes higher than that of the urine. If the active transport of urea out of the collecting duct takes place over the entire length of the collecting duct then the urea concentration inside the collecting duct will become lower than that of the tissue at some point along its longitudinal axis. Since the collecting duct is also permeable to urea (19) a point might be reached at which active efflux of urea and passive influx of urea will be equal and no net movement of urea is taking place.

The more pronounced the uphill transport of urea is, the more toward the cortex the point will be at which the net delivery of urea to the countercurrent system ceases. This point along the longitudinal axis of the collecting duct may determine the point of maximum urea concentration in the renal medulla, since a simple countercurrent system would tend to maintain the highest concentration at the point of solute delivery. (This is, of course, an oversimplification since the medullary anatomy is quite complex and may differ in the three species.)

If the uphill transport out of the collecting duct is most pronounced in the sheep and least pronounced in the dog, we might expect maximum tissue urea concentration to be more toward the cortex in the sheep and more toward the crest in the dog, and due to the decrease in recycling we should expect a smaller fraction of filtered urea to be excreted in the sheep than in the dog. Our findings are consistent with this prediction.

### TABLE 2. Correlation between site of maximum urea concentration in renal medulla and renal urea conservation

<table>
<thead>
<tr>
<th>Species</th>
<th>Percent of Filtered Urea Reabsorbed</th>
<th>Site of Maximum Urea Concentration</th>
<th>Urea at Crest, Tissue/Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep*</td>
<td>98</td>
<td>Z72</td>
<td>150</td>
</tr>
<tr>
<td>Rat†</td>
<td>95</td>
<td>Z22</td>
<td>35</td>
</tr>
<tr>
<td>Dog</td>
<td>75</td>
<td>Z23</td>
<td>85</td>
</tr>
<tr>
<td>Normal or high-protein diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep*</td>
<td>50</td>
<td>Z23</td>
<td>80</td>
</tr>
<tr>
<td>Rat†</td>
<td>50</td>
<td>Z23</td>
<td>150</td>
</tr>
<tr>
<td>Dog</td>
<td>30</td>
<td>Z23</td>
<td>85</td>
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