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# Functional heterogeneity of the descending limbs of Henle's loop

## II. Interspecies differences among rabbits, rats, and hamsters

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**Abstract.** Permeability properties of the descending limbs of Henle's loop were compared among rabbits, hamsters, and rats by measuring transepithelial voltage (Vt) across the isolated renal tubules perfused in vitro. From the deflection of the Vt when the composition of the bathing fluid was varied, the permeabilities of sodium and of potassium relative to chloride ($P_{Na}/P_{Cl}$ and $P_K/P_{Cl}$, respectively) were determined in either the descending limbs of the short-loop nephron (SDL) or the segments of the upper protion of the long-loop nephron (LDLu). In hamsters and rats, the values of $P_{Na}/P_{Cl}$ of the LDLu (3.98 ± 0.66 and 5.03 ± 0.79) were higher than those of the SDL (0.68 ± 0.03 and 0.61 ± 0.00). In contrast, in rabbits the value of $P_{Na}/P_{Cl}$ of the LDLu (0.96 ± 0.05) was only slightly higher than that of the SDLu (0.75 ± 0.03). The similar tendency was also noted in the values of $P_K/P_{Cl}$. In hamsters and rats, the $P_K/P_{Cl}$ ratios were 4.90 ± 0.82 and 6.44 ± 0.90, respectively, in the LDLu and 1.09 ± 0.04 and 1.02 ± 0.0, respectively in the SDL. When a transepithelial osmotic gradient was imposed by adding raffinose to the bath, a lumen-negative streaming voltage of about −8 mV was generated in the hamster and the rat LDLu. Taken together with the findings in the preceding paper, these observations support the view that the descending limbs of rabbits are different from those of hamsters and rats in that internephron heterogeneity is less remarkable, and that the LDLu of hamsters and rats is highly permeable to sodium and to potassium as well as to water.

**Key words:** Concentration of urine – Renal medulla – Countercurrent systems – Na transport – K transport

### Introduction

In the preceding article [15] we reported that functional internephron heterogeneity exists in the descending limbs of Henle's loop of the hamster. The upper portion of the descending limb of the long-loop nephron (LDLu) is highly permeable to sodium, moderately permeable to chloride and less permeable to urea. By contrast, the descending limb of the short-loop nephron (SDL) is less permeable to sodium and to chloride and moderately permeable to urea. Differences in salt permeability between these segments correlated well with the morphological characteristics of the intercellular junctions [1]. On the basis of these observations we suggested that internephron heterogeneity of the descending limbs of Henle's loop must be taken into consideration for constructing a countercurrent model to explain the mechanism of formation of concentrated urine.

However, it remains uncertain whether the functional internephron heterogeneity found in the descending limbs of the hamster kidney can be generalized to all mammalian kidneys. Morphological studies [1–4, 10, 13, 23, 28, 29, 32, 41, 42] strongly suggest that interspecies differences may exist in the function of the descending limbs of Henle's loop. In vivo micropuncture and in vitro microperfusion studies also suggest this view. An extreme case is found in the rabbit descending limbs perfused in vitro [26, 27], which are highly permeable to water but less permeable to urea and sodium. Although no in vivo data are available about the function of the rabbit descending limbs, it is assumed that about 96% of osmotic equilibration along this segment occurs by water extraction [26, 27]. At the other extreme are the descending limbs of the kidney of *Psammomys obesus* [17, 19, 33, 34], in which transepithelial addition of sodium appears to contribute in a major way to osmotic equilibration. The functions of the descending limbs of the rat [19, 31, 33] seem to represent intermediate characteristics between these two extreme cases.

These functional studies, however, preclude a firm conclusion for the existence of interspecies differences, since these data have been obtained by different techniques. Since internephron heterogeneity was clearly demonstrated in the hamster descending limb [15], one must compare function of the descending limb derived from comparable nephrons (long or short-loop nephron) among various species of mammals in order to obtain direct evidence for interspecies differences. Therefore, in the present study, I have compared the function of the descending limbs of Henle's loop derived either from long-loop nephrons (LDLu) or from short-loop nephrons (SDL) among rabbits, hamsters, and rats by measuring the diffusion potential and streaming potential across the segments perfused in vitro. The results indicate that the LDLu of both hamsters and rats are highly permeable to sodium and to potassium relative to chloride but that the rabbit LDLu is less permeable to sodium and to potassium relative to chloride. In contrast, the permeability characteristics of the SDL are not different among these species.

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## Materials and methods

Female Japanese white rabbits (1.5–2.5 kg body weight), female golden hamsters (*Mesocricetus aureatus,* 50–100 g body weight) and female Wistar rats (150–300 g body weight) were maintained on regular laboratory chows for the respective animals. After animals were anesthetized with pentobarbital (35 mg/kg IV in rabbits and 50 mg/kg IP in rats and hamsters), both kidneys were removed. Thin kidney slices were made and placed in a cooled dish containing modified bicarbonate Krebs-Ringer solution (BKR). The descending limbs of Henle's loop were isolated with forceps (Dumond, Inox 5) under a stereomicroscope. Isolated renal tubules were perfused according to the method of Burg et al. [7] with slight modifications. Transepithelial voltage (Vt) was measured as previously described [14].

The descending limbs of Henle's loop of hamsters were identified as reported in the preceding paper [15]. The segments of rats were similar in appearance to that of hamster, and therefore were easily identified. In the rabbit, it was difficult to distinguish the LDLu from the SDL. Although the former is comprised of relatively thick cells and has a large diameter, I was not confident enough to discriminate it from the latter even under an inverted microscope. Kawamura et al. [24] reported that the rabbit proximal straight tubule of the long-loop nephron is more permeable to sodium than to chloride but that the converse holds true for the proximal straight tubule of the short-loop nephron. This characteristic is so reliable that one can exactly distinguish the long-loop segment from the short-loop one simply by measuring the diffusion potential across the proximal straight tubule in the presence of a sodium chloride gradient. These characteristics of the proximal straight tubules were used as a tool to identify the origin of the rabbit descending limbs. Segments of the descending limb were always dissected in conjunction with the terminal portion of the proximal straight tubules. Initially the portion of the proximal straight tubule was hooked up with a holding pipette of the perfusion site and both the proximal straight tubule and the descending limb of Henle's loop were perfused with the 154 Na solution (Table 1), while the tip of the perfusion pipette was located in the lumen of the proximal straight tubule. Initially, the bathing solution was BKR solution. After the Vt was stabilized, the bathing fluid was changed to 154 Na solution (Table 1). Then the bath was changed to 78 Na solution (Table 1) and deflections in the Vt were observed to determine the permeability of the proximal straight tubule for sodium relative to chloride. The perfusion pipette was then advanced so that its tip was located in the lumen of the descending limb, or the portion of the proximal tubule was further aspirated into the holding pipette so that only the descending limb portion was perfused. The bathing solution was then successively changed to 154 Na, 50 K, and BKR, and changes in the Vt were observed.

**Table 1.** Composition of artificial solutions used in this study

| | BKR | 154 Na | 78 Na | 50 K | Raff |
|---|---|---|---|---|---|
| NaCl | 110 | 140 | 64 | 95 | 110 |
| $NaHCO_3$ | 25 | 5 | 5 | 5 | 25 |
| $Na_2HPO_4$ | 1.6 | 2 | 2 | 2 | 1.6 |
| $NaH_2PO_4$ | 0.4 | 0 | 0 | 0 | 0.4 |
| Na acetate | 10 | 5 | 5 | 5 | 10 |
| KCl | 5 | 5 | 5 | 50 | 5 |
| $MgCl_2$ | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| CaCl | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| D-glucose | 8.3 | 0 | 0 | 0 | 8.3 |
| L-alanine | 5.0 | 0 | 0 | 0 | 5.0 |
| Raffinose | | | 140 | | 200 |
| $V_a$[a] | | 0 | + 1.1 | + 0.6 | |

[a] $V_a$ is the circuit asymmetry voltage with reference to 154 Na solution measured in the absence of the tubule

The following protocol was used for studying either hamster or rat descending limbs of Henle's loop. In most cases the SDL was isolated in conjuction with the terminal portion of the proximal straight tubules for convenience of fixation to the holding pipette. On the other hand, the LDLu could be easily hooked up with the holding pipette in the absence of the proximal straight tubule. Initially the tubules were perfused with 154 Na solution and bathed in BKR solution. After 10–15 min the bathing fluid was changed successively to 154 Na, 78 Na, 50 K, raffinose BKR, and BKR, and changes in the Vt were observed. In some of the initial experiments, the bathing solution was returned to 154 Na solution before examining the effects of other solutions. Since in these studies it was found that the Vt was stable throughout the experiment, such control periods were not interposed between different experimental conditions. The medullary thick ascending limb of Henle's loop (MAL) of rats and hamsters and the thin ascending limb (TAL) of hamsters were also perfused under a similar protocol for comparison.

The composition of solutions used in this study is shown in Table 1. When the Vt was measured while the composition of the perfusate and that of the bathing medium were different, the observed voltage ($V_0$), was corrected for a liquid junction potential ($V_{lj}$). Henderson's equation as modified by Barry and Diamond [5] was used for calculation of the $V_{lj}$ between two different solutions:

$$V_{lj} = \frac{RT}{F} \frac{\Sigma u_i(A_i^p - A_i^b) - \Sigma u_j(C_j^p - C_j^b)}{\Sigma u_i(A_i^p - A_i^b) + \Sigma u_j(C_j^p - C_j^b)} \ln \frac{\Sigma u_i A_i^p + \Sigma u_j C_j^p}{\Sigma u_i A_i^b + \Sigma u_j C_j^b} \quad (1)$$

where $u_i$ and $u_j$ denote the mobility of the $i$th cation and the $j$th anion, $A$ and $C$ the activities of the anion and the cation, and $p$ and $b$ perfusate and bathing fluid, respectively. However, as pointed out by Berry, Warnock, and Rector [6], the electrical circuit to measure the Vt is complicated by the existence of asymmetry between salt agar bridges and solutions in addition to liquid junction potential when the composition of the perfusate is different from that of the bathing solution. Therefore, the observed $V_0$ must be corrected not only by the theoretical value for $V_{lj}$ but also by the circuit asymmetry potential ($V_a$), that is, the value observed in the absence of renal tubules. Since renal tubules were perfused always with 154 Na solution when there was an asymmetry of solutions, the calculated $V_{lj}$ and the experimentally measured $V_a$ in each solution are indicated with reference to 154 Na solution in Table 1.

The permeabilities of sodium and potassium relative to chloride ($P_{Na}/P_{Cl}$, $P_K/P_{Cl}$) was calculated from the deflection of voltage when the bathing solution was changed to 78 Na solution ($V_{Na}$) and to 50 K solution ($V_K$), respectively. According to Goldman's constant field assumption, $V_{Na}$ and $V_K$ are expressed as follows:

$$V_{Na} = \frac{RT}{F} \ln \left[ \frac{(P_{Na}/P_{Cl})A^p_{Na} + (P_K/P_{Cl})A^p_K + A^b_{Cl}}{(P_{Na}/P_{Cl})A^b_{Na} + (P_K/P_{Cl})A^b_K + A^p_{Cl}} \right] \quad (2)$$

$$V_K = \frac{RT}{F} \ln \left[ \frac{(P_{Na}/P_{Cl})A^p_{Na} + (P_K/P_{Cl})A^p_K + A^b_{Cl}}{(P_{Na}/P_{Cl})A^{b'}_{Na} + (P_K/P_{Cl})A^{b'}_K + A^p_{Cl}} \right] \quad (3)$$

where $A_{Na}$, $A_K$, and $A_{Cl}$ indicate activity of Na, K, and Cl, respectively. The superscript $p$ denotes perfusate (154 Na solution); $b$ is bathing solution 78 Na and $b'$ is bathing solution 50 K. From these Eqs. (2) and (3) we can solve for $(P_{Na}/P_{Cl})$ and $(P_K/P_{Cl})$. For these calculations, the contributions of ions other than Na, Cl, and K were assumed to be negligible since their concentrations were low.

For calculations of the liquid junction potential (Eq. 1) and the relative permeabilities (Eqs. 2 and 3), ion activity instead of concentration was used. Ion activity coefficient $(\gamma_m)$ was calculated according to the Debye-Hückel equation [36] as:

$$-\log \gamma_m = \frac{A z_i^2 \sqrt{I_m}}{1 + B\mathring{a}\sqrt{I_m}} \quad (4)$$

where $A$ and $B$ are temperature-dependent constants of 0.5215 and 0.3305, respectively, at 310° K, $z_i$ is the valence of the ion, and $\mathring{a}$ is the ion size parameter obtained from Kielland [24], and $I_m$ is the ionic strength.

The data are expressed as means ± SEM. When comparison of data was made among more than three groups, variant analysis was performed and the significance of difference was evaluated by the method of Sheffé [11], the most strict criteria.

## Results

### *Light microscopic observations*

Figure 1 compares the light microscopic appearance of the segments of the MAL, the LDLu, and the SDL of a rabbit, a hamster and a rat while the tubules were perfused in vitro. In the hamster it should be noted that the LDLu is the largest in diameter and in thickness of epithelia even as compared to the MAL, showing a marked contrast to the SDL which is lined by very thin epithelia. In the rabbit, the LDLu tends to be larger in diameter and the numbers of epithelia per unit tubular length appear to be higher than those of the SDL, but these differences are not so remarkable as are the differences between these segments in the hamster. The epithelia composing the LDLu of rat seem to be intermediate between those of the rabbit and the hamster. There are no apparent differences in the appearance of the SDL among these species. These findings are essentially in good agreement with more detailed morphological observations reported from other laboratories [1, 23, 28, 42].

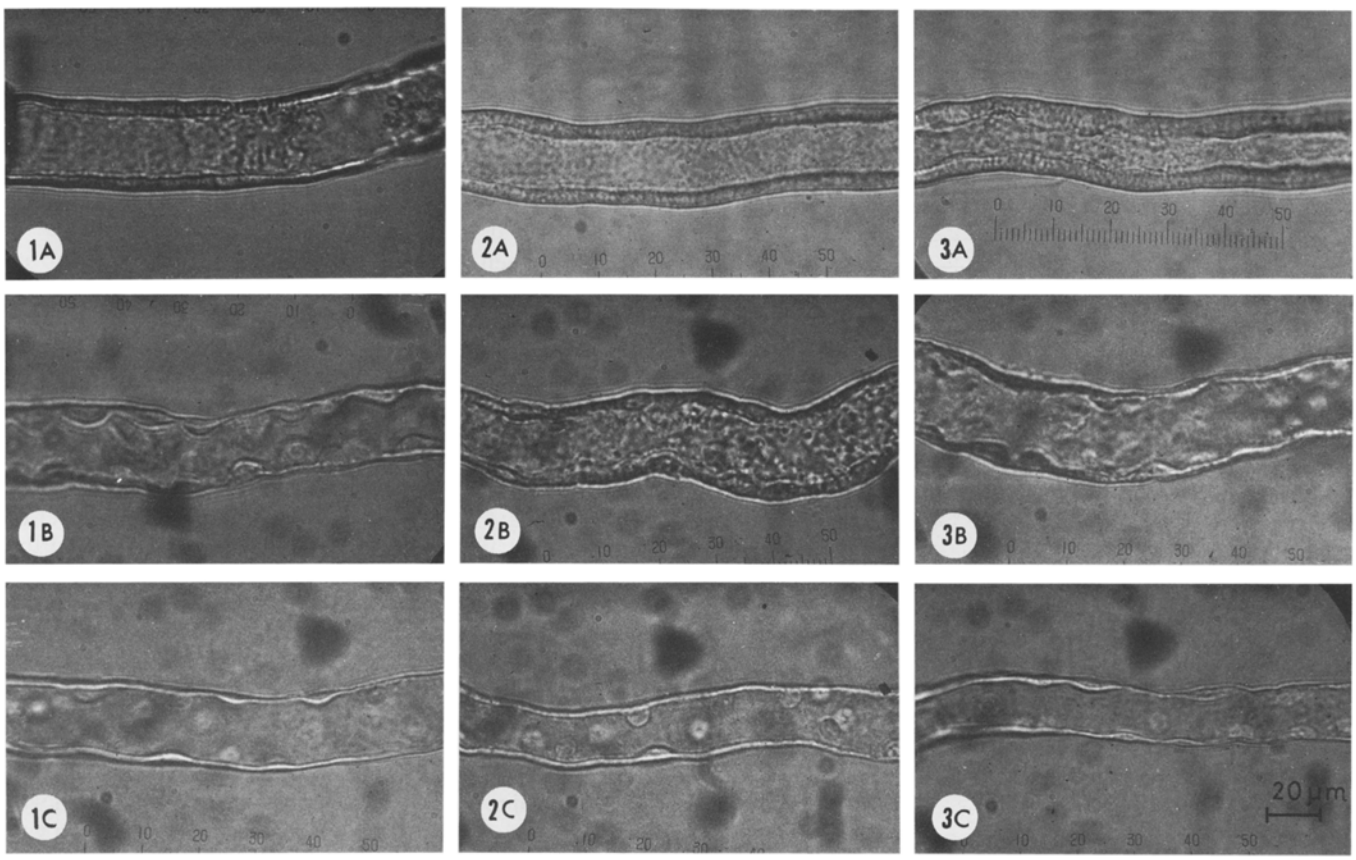



**Fig. 1.** Light microscopic appearance of the MAL *(upper row)*, the LDLu *(middle row)*, and the SDL *(lower row)* of a rabbit, a hamster and a rabbit while tubules were perfused in vitro at 37° C

*Internephron heterogeneity of the rabbit descending limbs*

Figure 2 summarizes the results of the experiments in which permeabilities to sodium and potassium relative to chloride ($P_{Na}/P_{Cl}$ and $P_K/P_{Cl}$) were examined in the descending limbs of Henle's loop of the rabbit. Distinction between the short-loop and the long-loop nephron was made by an electrophysiological mean on the basis of the established fact [24] that the proximal straight tubule of the long-loop nephron is more permeable to sodium than to chloride whereas the segment of the short-loop nephron is more permeable to chloride than to sodium. When the portions of both the proximal straight tubule and the descending limb were perfused with 154 Na solution, bathed in the BKR and the tip of the perfusion pipette was located in the lumen of the former, the Vt of the short-loop nephron was more positive than that of the long-loop nephron. This difference may be due to diffusion potential, because the Vt of the both nephrons became identical when the bathing medium was changed to 154 Na. When the bathing solution was changed to 78 Na, the orientation of the deflection of Vt was completely opposite between the long-loop and the short-loop nephron.

Under the same conditions (perfusate 154 Na, bath 78 Na), the tip of the perfusion pipette was advanced or the portion of the proximal straight tubule was aspirated into the holding pipette so that the tip of the perfusion pipette was located in the lumen of the descending limb. By this procedure, the difference in voltage deflection between the LDLu and the SDL became less but was still statistically significant ($P < 0.01$). When the bathing fluid was changed to 154 Na solution, no appreciable Vt was demonstrated in either segments. Exchanging the bathing medium with 50 K solution caused modest negative deflections in the Vt in the DLH of both nephron types.

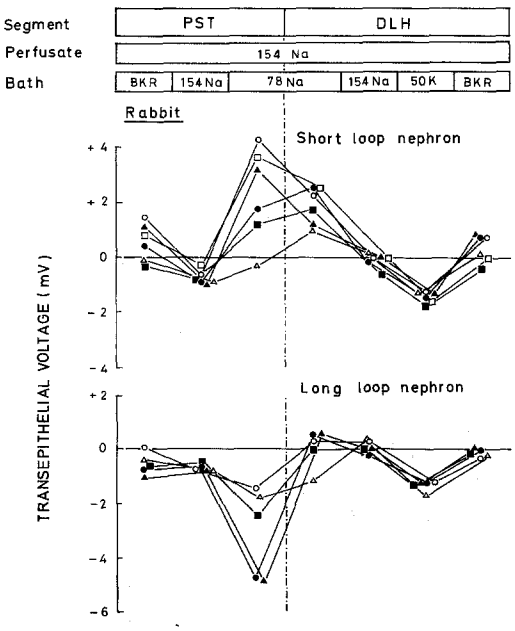



**Fig. 2.** Changes in transepithelial voltage of the proximal straight tubule *(PST)* and descending limb of Henle's loop *(DLH)* of rabbits when composition of the bathing fluid was varied. See text for detailed explanation

From these data, both $P_{Na}/P_{Cl}$ and $P_K/P_{Cl}$ were calculated for each segments. Table 2 summarizes the results. The ratio of ($P_{Na}/P_{Cl}$) in the proximal straight tubule of the long-loop nephron exceeded that of the short-loop nephron. The segment of the long-loop nephron was more permeable to sodium than to chloride, whereas the converse was true for the segment of the short-loop nephron. These values are in good agreement with those reported in the literatures [24, 44]. The ($P_{Na}/P_{Cl}$) ratios of the LDLu and the SDL were 0.96 and 0.75, respectively. These values were significantly different ($P < 0.01$). The ($P_K/P_{Cl}$) ratios of the two segments were 1.42 for the LDLu and 1.19 for the SDL. They were also significantly different ($P < 0.01$).

*Internephron heterogeneity of the hamster descending limbs*

When the LDLu or the SDL of the hamster were perfused with 154 Na solution, changes in Vt was observed by sequentially changing bathing media from BKR to 154 Na, 78 Na, 50 K, Raff, and BKR. The results are summarized on the upper panel of Fig. 3. When the bathing solution was BKR, the SDL showed a slight lumen positive Vt, whereas the Vt of the LDLu tended to be negative in the lumen. These values are significantly different ($P < 0.01$). When the bathing medium was changed to 154 Na solution which is identical to the perfusate, neither segment showed appreciable Vt. When the concentration of sodium chloride in the bath was reduced, a marked lumen negative Vt ($-9.6 \pm 1.1$ mV) was observed in the LDLu, whereas a lumen positive Vt ($3.0 \pm 0.40$ mV) was observed in the SDL. When the bathing medium was changed to Raff solution, the changes in Vt must be compared with the value for the BKR periods, since composition of electrolytes in the Raff solution is identical to that the BKR solution. It is clear that addition of raffinose to the bath generated a marked lumen negative Vt in the LDL ($-8.3 \pm 1.4$ mV) but not in the SDL ($1.2 \pm 0.1$ mV).

It has been reported that the thin ascending limb of Henle's loop of the hamster is more permeable to chloride than to sodium [14, 17]. On the other hand, the medullary thick ascending limb of Henle's loop of the hamster is assumed to be more permeable to sodium than to chloride if the membrane characteristics of this segment in the hamster are similar to those observed in the thick ascending limbs the rabbit [8, 34], the rat [39] and the mouse [12]. It is also known that both the thin [14, 16] and the thick ascending limb [8, 34] are virtually impermeable to water. In order to confirm the validity of our method, both the thin and the medullary thick ascending limbs were also studied under the same protocol to see if the established facts could be confirmed. The results are summarized on the lower panel of Fig. 3. When the hamster TAL was perfused with 154 Na and bathed in BKR solution, a lumen positive Vt ($2.8 \pm 0.3$ mV) was observed. This may be a diffusion potential caused by differences in permeability to chloride and to bicarbonate. When the composition of the bathing medium was identical to that of the perfusate, no appreciable Vt was observed ($0.1 \pm 0.04$ mV). When the bathing medium was changed to the 78 Na solution, a marked lumen-positive Vt ($7.2 \pm 0.5$ mV) was generated. The Vt returned toward zero when the bathing medium was changed to the 50 K solution ($0.0 \pm 0.23$ mV). When Raff solution was applied

**Table 2.** Deflections in transepithelial voltage ($\Delta$ Vt) while the composition of the bathing fluid was varied, and permeabilities of sodium and potassium relative to chloride in various nephron segments of rabbits, hamsters, and rats

| Bathing fluid | $\Delta$ Vt (mV) | | $P_{Na}/P_{Cl}$ | $P_K/P_{Cl}$ |
|---|---|---|---|---|
| | 78 Na | 50 K | | |
| Rabbit | | | | |
| Short-loop nephton | | | | |
| PST (6) | 3.1 ± 0.7 | | 0.66 ± 0.06 | |
| SDL (6) | 1.9 ± 0.3 | 1.3 ± 0.1 | 0.75 ± 0.03 | 1.19 ± 0.04 |
| Long-loop nephron | | | | |
| PST (5) | − 2.3 ± 0.7 | | 1.30 ± 0.12 | |
| LDLu (5) | 0.0 ± 0.4 | 1.2 ± 0.2 | 0.96 ± 0.05 | 1.42 ± 0.02* |
| Hamster | | | | |
| Short-loop nephron | | | | |
| SDL (8) | 2.9 ± 0.4 | 1.2 ± 0.0 | 0.68 ± 0.03 | 1.09 ± 0.04 |
| Long-loop nephron | | | | |
| LDLu (8) | − 9.4 ± 1.0 | 1.0 ± 0.2 | 3.98 ± 0.66* ** | 4.90 ± 0.82* ** |
| MAL (6) | − 5.3 ± 1.1 | 1.0 ± 0.7 | 2.30 ± 0.27 | 2.64 ± 0.40 |
| TAL (5) | 7.1 ± 0.5 | −0.1 ± 0.2 | 0.38 ± 0.03 | 0.49 ± 0.04 |
| Rat | | | | |
| Short-loop nephron | | | | |
| SDL (3) | 3.6 ± 0.1 | 1.3 ± 0.0 | 0.61 ± 0.0 | 1.02 ± 0.0 |
| Long-loop nephron | | | | |
| LDLu (5) | −10.7 ± 0.7 | 1.3 ± 0.1 | 5.03 ± 0.79* ** | 6.44 ± 0.90* ** |
| MAL (6) | − 4.7 ± 1.0 | 2.7 ± 1.0 | 1.86 ± 0.25 | 3.27 ± 0.73 |

Numerals in parentheses indicate numbers of perfused tubules
* Significantly different ($P < 0.05$) from the values of comparable segments of the short-loop nephron
** Significantly different ($P < 0.05$) from the values of comparable segments of rabbits

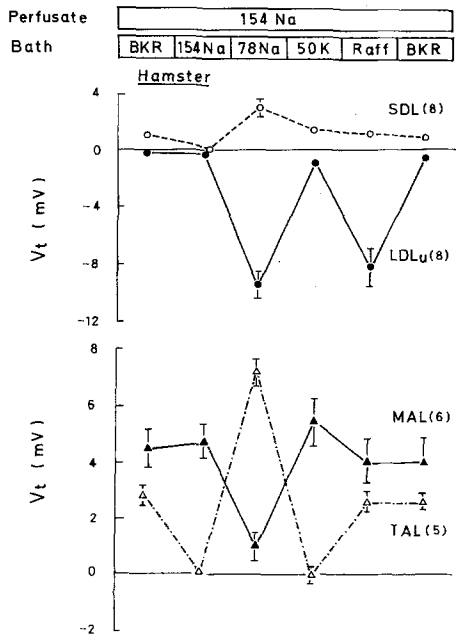



**Fig. 3.** Changes in transepithelial voltage *(Vt)* of the *SDL*, the *LDLu*, the *MAL* and the *TAL* isolated from hamster kidney when composition of the bathing fluid was varied. See text for detailed explanation

to the bath, the Vt was not different from that observed when the bathing medium was BKR solution (2.7 ± 0.4 vs 2.8 ± 0.3 mV). These findings are in accord with the view that the TAL is more permeable to chloride than to sodium, but that it is virtually impermeable to water.

When the hamster MAL was perfused with 154 Na solution a lumen positive Vt was observed (lower panel of Fig. 3). The magnitude of the Vt was 4.5 ± 0.7, or 4.7 ± 0.7 mV when the bathing medium was BKR or 154 Na, respectively. When the bathing medium was changed to 78 Na solution, the Vt deflected more negatively (−1.0 ± 0.7 mV) suggesting that this segment is more permeable to sodium than to chloride. When the bathing medium was changed to 50 K solution, the Vt became slightly more positive (5.4 ± 0.9 mV) than in the control. Addition of raffinose to the bath did not cause any changes in the Vt as in case of the TAL (3.9 ± 0.9 vs 4.1 ± 1.0 mV).

Based on these observations, both ($P_{Na}/P_{Cl}$) and ($P_K/P_{Cl}$) were calculated for each segment. Table 2 summarizes the results. The ($P_{Na}/P_{Cl}$) ratio in the MAL was 2.30, a value comparable to those observed in this segment of rabbit [34] and mouse [12]. The ($P_{Na}/P_{Cl}$) ratio in the TAL was 0.38, a value also comparable to that reported in the previous papers [14]. The $P_{Na}/P_{Cl}$ ratio in the LDLu was extremely large (3.98), whereas the value in the SDL was very small (0.68). Thus it is concluded that permeability of

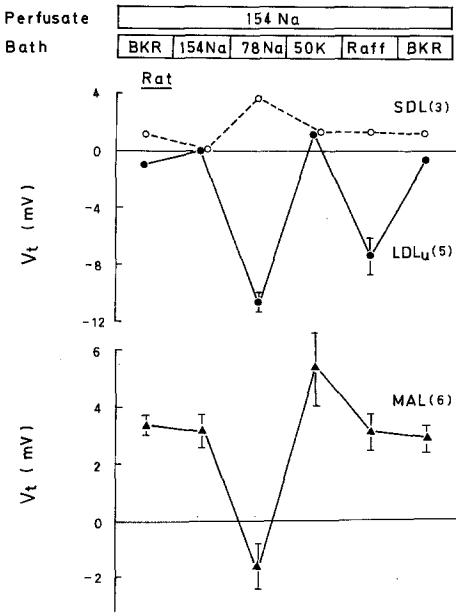



**Fig. 4.** Changes in transepithelial voltage *(Vt)* of the *SDL*, the *LDLu*, and the *MAL* isolated from rat kidney when composition of the bathing fluid was varied. See text for detailed explanation

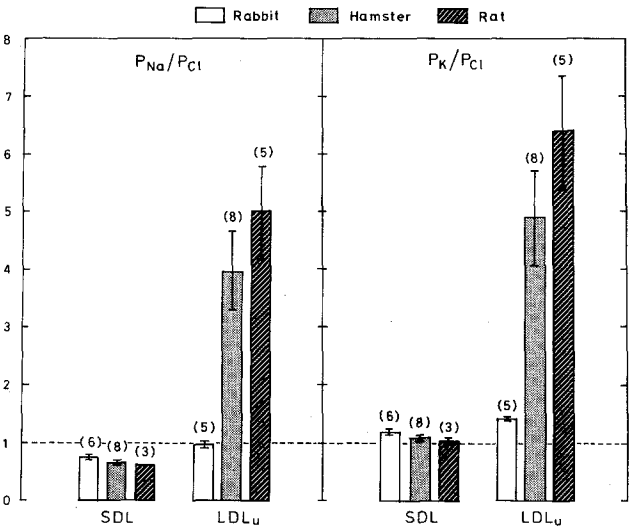



**Fig. 5.** Comparison among rabbits, hamsters, and rats of the permeabilities for sodium and potassium relative to chloride across the descending limbs of Henle-s loop

the LDLu is highly selective for sodium, whereas that of the SDL is more selective for chloride. The $(P_K/P_{Cl})$ ratios of these nephron segments showed almost identical values to $(P_{Na}/P_{Cl})$ ratios except that in the SDL the $(P_K/P_{Cl})$ ratio was slightly higher than the $(P_{Na}/P_{Cl})$ ratio.

*Internephron heterogeneity of the rat descending limb*

The characteristics of membrane permeability of the LDLu and the SDL of the rat nephron were also examined according to the same protocol as used in the cases of hamsters. The results are summarized in Fig. 4 and Table 2. As shown in Fig. 4, the orientations and magnitudes of changes in the Vt under the same experimental protocol are strikingly similar to those observed in hamsters, although data on the TAL are lacking.

The $P_{Na}/P_{Cl}$ ratios as well as the $P_K/P_{Cl}$ ratios calculated from these data were, therefore, similar to those in the hamster (Table 2). Differences in these ratios between the SDL and the LDLu were much more prominent than those observed in hamster kidneys.

*Interspecies difference of the descending limb*

Figure 5 summarizes the permeabilities for sodium and potassium relative to chloride in the descending limbs of Henle's loop of three different species based on the data presented in Table 2. It is clear that the permeability properties of the SDL are almost identical among these species of animals. By contrast, a marked interspecies difference is noted in the LDLu. Both $(P_{Na}/P_{Cl})$ and $(P_K/P_{Cl})$ are significantly greater in the hamster and the rat than in the rabbit ($P < 0.01$).

## Discussion

By simple measurements of diffusion potential and streaming potential the present study clearly demonstrates that there are interspecies differences in the membrane permeability of the descending limb of Henle's loop. In addition, we also confirmed that there is internephron heterogeneity in the descending limb in the rat and hamster but not in the rabbit.

A possible error in the measurement of diffusion potential may be incorporated into the correction for liquid junction potential. The observed potential differences were corrected by the experimentally determined asymmetry of voltage in the absence of the renal tubule in addition to the theoretical liquid junction voltage correction as reported by Berry et al. [5]. This correction method may not always be perfect since it was impossible to measure the voltage asymmetry in the absence of renal tubule under a condition with an ideal liquid interface. Because of these shortcomings, the absolute values of $(P_{Na}/P_{Cl})$ and $(P_K/P_{Cl})$ might not be completely accurate.

Because of this uncertainty in the measurement of the diffusion potential, the MAL of hamsters and rats and the TAL of hamsters were perfused under a similar protocol for comparison with the previously published values. The finding that the hamster TAL is more permeable to chloride than to sodium is in good agreement with the results of a more detailed study reported previously [14]. Thus, relatively speaking, the differences of the values for descending limbs observed among species or between nephron segments are clearly unequivocal.

In general, there was an internephron heterogeneity in the membrane properties between the LDLu and the SDL in all species tested in this study. The membrane characteristics of the LDLu of hamsters and rats were very similar and quite different from those of rabbits. In both hamsters and rats, the LDLu was more permeable to sodium than to chloride. Although the ratios of $(P_{Na}/P_{Cl})$ were extremely high in both species, the present study did not provide absolute values of permeability coefficients. However, it is not unreasonable to assume that the absolute permeability of the rat LDLu to sodium is also high if one accepts the similarity of membrane characteristics of rats LDLu with

those of hamsters. In the rabbit, the ($P_{Na}/P_{Cl}$) ratio of the LDLu was much lower than those observed in the rat and the hamster. An unpublished observation from this laboratory demonstrated that the permeability to sodium was low in the rabbit LDLu. Thus, it is safe to conclude that the rabbit LDLu is less permeable to sodium chloride compared to the rat or hamster LDLu.

In marked contrast to the interspecies difference of the LDLu, the membrane characteristics of the SDL seem to be similar among these species. Although the similarity of the values of ($P_{Na}/P_{Cl}$) ratio among species does not necessarily mean that absolute values of permeability coefficients are similar. I would suggest that absolute values are also similar because of the morphological similarity as will be discussed later. In other word, the SDL may be less permeable to sodium chloride in all these species of animals.

Although I did not measure water permeability of the nephron segments in this study, the observation that a streaming potential was generated when an osmotic gradient was imposed in the LDLu of hamsters and rats suggests that these segments are highly permeable to water. It is generally accepted that the presence of unstirred layer in the vicinity of a membrane causes to underestimate the volume flux in the presence of an osmotic gradient. Therefore, it is possible that the streaming potential observed in the LDLu of the hamster and rat may be underestimated if a sizable unstirred layer exists in this preparation. However, as we discussed in the previous paper [30], the contribution of an unstirred layer to the net water flux is less than 5% of net water flux in the LDLu of the rabbit since tubular diameter is very small. Actually we did not observe any phenomenon of osmotic transient after imposing a transepithelial osmotic gradient across the LDLu of the hamster and rat.

It is well established that the luminal fluid in the descending limbs becomes hypertonic as it flows into the medulla. As mentioned in the introduction, it is possible that osmotic equilibration processes along the descending limb may vary among species of animals. Although the present study clearly demonstrates that interspecies differences exist in the function of the descending limb, the processes of osmotic equilibration along the descending limb predicted from the present data are not always in good agreement with the observations by in vivo micropuncture studies. Jamison and his associates [19, 33] have reported that in rats both water abstraction and urea addition are responsible for an increase in osmolality of the fluid in the descending limbs. However, if the rat LDLu is highly permeable to sodium chloride, the entry of sodium chloride rather than urea would be expected. In this regard, it is of interest to note that in *Psammomys* a significant entry of sodium into the descending limb was found [36]. Pennell et al. [33] calculated that in the normal antidiuretic rat the solute entry accounted for 35% and water abstraction 65% of the rise in osmolality at the end of the descending limb. Since this calculation is based on an assumption that net entry of sodium in the descending limb is negligible, the amount of solute entry may be underestimated. Although we do not know about membrane characteristics of the lower part of the descending limb of the long-loop nephron (LDLl), it is not unreasonable to assume that urea permeability of this segment is higher than that of the LDLu, since the morphology of the LDLl is very similar to that of the SDL in rats and hamsters. Morgan and Berliner [31] has reported that in rat papillary slices perfused in vitro the descending limb is permeable to sodium and to urea. Thus it is possible that the entry of urea occurs mainly in the LDL, since the in vivo entry of urea into the rat papillary descending limb is unequivocal [33].

Another unique finding in this study is the internephron and interspecies differences in potassium permeability relative to chloride. It has been shown in the rat that a considerable fraction of potassium is secreted into somewhere along the proximal straight tubule of descending limbs of Henle's loop [21]. Although it has been demonstrated in the rabbit that potassium secretion occurs in the proximal straight tubule [45, 46, unpublished observation of Miwa, Tabei, and Imai], the amount of potassium calculated to be secreted from the proximal straight tubule seems to be insufficient to account for the amount of potassium secretion observed at the tip of the loop in the rat provided that the rat proximal straight tubule has a similar capacity to secrete potassium. The present observation that the LDLu is highly permeable to potassium strongly suggests that potassium may enter into the LDLu probably mainly by a passive mechanism. Thus the LDLu may play a critical role in the mechanism of medullary recycling of potassium. A more detailed study on potassium transport across the hamster LDLu will be reported elsewhere (Tabei and Imai, unpublished).

It is highly possible that the diffusion potential mainly represents the permselectivity of the paracellular pathway, since Tabei and Imai [43] reported that the bi-ionic diffusion potential in the LDLu of the hamster was completely symmetrical. However, this would not necessarily exclude the possible contribution of a cellular route for permselectivity in addition to the paracellular route. In fact, the ratio of sodium to chloride flux determined by isotopes reported in the preceding paper [15] was twice as much as the value determined from diffusion potential.

It is of importance to compare the results of the present study with morphological observations. Both LDLu of hamsters [1] and rats [42] are characterized by the presence of a complicated interdigitation between adjacent cells and shallow tight junctions with few junctional strands. From these morphological features, it has been suggested that this segment may be highly permeable to solutes. On the other hand, the SDL of rabbits [23], rats [42] and hamsters [1] are characterized by the absence of the intercellular interdigitation and the presence of the deep tight junctions with several junctional strands. The LDLu of rabbits shows a similar morphological appearance, although the epithelia are taller than those of the SDL. From these morphological features, it has been suggested that these segments are less permeable to solutes. The results of the present study are quite in good agreement with these notions.

It is also interesting to compare the morphological features with membrane characteristics of the thin ascending limb of these species. I have previously reported that the membrane characteristics of the thin ascending limb are qualitatively same among these species [14, 16]; i.e., this segment is impermeable to water, highly permeable to sodium and to chloride, but less permeable to urea. It has been reported that there are no essential differences in the morphology of the thin ascending limbs among these species [1, 23, 42]. The slightly complicated interdigitation and shallow tight junction may account for the membrane characteristics of high salt permeability.

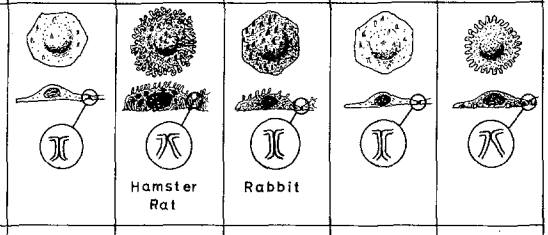
| Nephron | Short loop | Long loop | | | |
|---|---|---|---|---|---|
| Segment | SDL | LDLu | | LDLl | TAL |
| Cell type | I | II a | II b | III | IV |
| Morphology |  | | | | |
| | | Hamster Rat | Rabbit | | |
| Function | | | | | |
| $P_{water}$ | ++ | ++ | ++ | ++ ? | 0 |
| $P_{Na}/P_{Cl}$ | < 1 | ≫ | = | < ? | ≪ |
| $P_{Na}$ | ± | ++ | ± | ± ? | ++ |
| $P_{urea}$ | + | ± | ± | + ? | + |

**Fig. 6.** Correlation between morphology and function of the thin segments of Henle's loop. ++ highly permeable; + moderately permeable + less permeable; 0 impermeable to water

Thus it is clear that morphology of the intercellular junction is closely related with salt permeability of the segments of the Henle's loop. These relationships are schematically illustrated in Fig. 6. On the other hand, it is difficult to define those morphological features which represent permeability to water. Freeze fracture studies in the toad urinary bladder demonstrated that the number of intramembrane particles in the urinary surface is closely related to the water permeability stimulated by vasopressin [9, 22]. A number of intramembrane particles have been found in the luminal membrane of the rat and rabbit LDLu [40, 41] But these particles might not represent water permeability, since they have not been demonstrated in the SDL which is also highly permeable to water. Moreover, intramembrane particles have been also demonstrated in the cell membranes of the thin ascending limbs which are impermeable to water.

*In summary,* the present study demonstrates that there are species differences in the function of the descending limbs of Henle's loop among rabbits, rats, and hamsters. Membrane characteristics of the SDL were similar among these species, whereas those of the LDLu in the rabbit were quite different from those in the rat and the hamster. Taken together with the results of the preceding paper [15], these findings suggest that the detailed mechanism of formation of concentrated urine must be different among species. It is possible that these differences may account for species differences in the maximum capacity to concentrate urine. To construct computer simulations of the renal countercurrent system, these interspecies differences and internephron heterogeneity of the DLH must be taken into consideration.

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