Effect of Prostaglandin E₂ on Chloride Transport Across the Rabbit Thick Ascending Limb of Henle

SELECTIVE INHIBITION OF THE MEDULLARY PORTION

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ABSTRACT Prostaglandins are present in large quantities in the kidney and have been shown to directly affect transepithelial transport. The present studies were designed to examine whether prostaglandin E₂ could affect chloride transport across the thick ascending limb of Henle. Isolated segments of the cortical and medullary thick ascending limb of Henle were perfused in vitro and the transepithelial voltage and net chloride flux were measured. Exposure of the medullary thick ascending limb to 2 µM prostaglandin E₂ resulted in a fall in net chloride transport of 40–50% with a concomitant fall in voltage. In contrast, net chloride transport in the cortical thick ascending limb was not affected by prostaglandin E₂. Under similar conditions, the medullary thick ascending limb possessed twice the capacity to transport chloride than did the cortical thick ascending limb. The results suggest that endogenous renal prostaglandins may play a modulating role in the addition of salt to the renal medullary interstitium and may, under some circumstances, by chloruretic.

INTRODUCTION

The thick ascending limb of Henle transports substantial quantities of salt out of its lumen into the interstitial space. This process is presumed to be accomplished by active extrusion of chloride with passive movement of sodium in the absence of significant net water movement (1, 2). This nephron segment is thus able to render its luminal contents dilute with respect to the interstitium.

During a portion of these studies, Dr. Stokes was a recipient of National Institutes of Health research service award 5 F32-AM05318. Dr. Stokes' current address is Department of Internal Medicine, University of Iowa School of Medicine, Iowa City, Iowa 52242.

Received for publication 21 August 1978 and in revised form 16 April 1979.

The factors that control chloride transport across the thick ascending limb of Henle are largely unknown. There is good evidence to indicate that chloride reabsorption is load dependent (1, 2). In addition, some diuretics produce their major effects by inhibiting chloride transport in the thick ascending limb (3). To date, there is no evidence that an endogenous substance can affect the intrinsic capacity of this nephron segment to transport chloride.

The medullary portion of the thick ascending limb of Henle traverses the outer medulla, a region rich in prostaglandins (4, 5). Prostaglandins have been shown to inhibit sodium transport by direct action on the epithelium of the rabbit cortical collecting tubule (6, 7). They have also been shown to alter active chloride transport in other epithelia (8, 9). The present study was designed to examine whether prostaglandin E₂ (PGE₂)¹ could modulate chloride transport directly across the in vitro isolated, perfused rabbit thick ascending limb of Henle. The results show that PGE₂ inhibits net chloride transport across the medullary thick ascending limb of Henle but has no effect on chloride transport across the cortical thick ascending limb of Henle. This finding is consistent with the hypothesis that endogenous renal prostaglandins may play a role in the regulation of renal medullary tonicity, and in the regulation of chloride excretion.

METHODS

Isolated segments of rabbit nephrons were perfused in vitro by the methods described (6, 10). Briefly, New Zealand white rabbits were killed by decapitation and kidney slices were dissected in a chilled solution the composition of which was identical to the solution used to bathe the tubule throughout

¹Abbreviations used in this paper: CTALH, cortical thick ascending limb of Henle; MTALH, medullary thick ascending limb of Henle; JCl, net chloride flux; Vₜ, transepithelial voltage.
the experiment. Segments of either medullary thick ascending limb of Henle (MTALH) or cortical thick ascending limb of Henle (CTALH) were perfused at 37°C using concentric glass pipettes. Transepithelial voltage ($V_T$) was continuously measured as described (6).

Three different solutions were used to bathe the tubules: (a) an artificial isotonic solution designed to simulate ultrafiltrate; (b) an artificial hypertonic solution designed to simulate the content of outer medullary interstitial fluid (11); and (c) rabbit serum (Irvine Scientific, Santa Ana, Calif.). The artificial isotonic solution contained (in millimolar) NaCl, 105; NaHCO$_3$, 25; KCl, 5; Na$_2$HPO$_4$, 2.3; MgSO$_4$, 1; CaCl$_2$, 1.8; Na acetate, 10; L-alanine, 5; and glucose, 8.3. The hypertonic solution contained in addition 45 mM NaCl and 25 mM urea. The osmolalities of these solutions were 297±3 and 400±3 mosmol/kg H$_2$O respectively. To each of these solutions was added fetal calf serum 5% vol/vol. The perfusion fluid was identical to the bathing solution except that no fetal calf serum was added to the artificial solutions and the perfusate for tubules bathed with rabbit serum was an ultrafiltrate prepared through an ultrafiltration membrane (PM-30, Amicon Corp. Scientific Sys. Div., Lexington, Mass.). All solutions were gassed with 95% O$_2$ and 5% CO$_2$ so that the pH was 7.4.

The collected fluid was analyzed for chloride concentration according to the method of Ramsay et al. (12). A volume marker (6-Methallovate, Abbott Diagnostics, North Chicago, Ill.) was used throughout all experiments where the concentration of chloride in the collected fluid was measured. Though the present experiments confirm that the thick ascending limb of Henle is relatively impermeable to water (1, 2), a negative value for net water movement > -0.1 nl/min occurred in two experiments. These experiments were discarded because this finding is most compatible with a leak in the seal at the collecting pipette and would therefore create an error in the calculation of net chloride flux ($J_{Cl}$). The $J_{Cl}$ was thus calculated by the following expression that assumes no water movement:

$$J_{Cl} = \frac{V_T}{L} (|Cl|_{o} - |Cl|_{i}),$$

where $|Cl|_{o}$ and $|Cl|_{i}$ represent the concentration of chloride in the perfused and collected fluid respectively (pico-equivalent per nanoliter), $V_T$ is the rate of fluid collection (and is equal to the perfusion rate nanoliter per second), and $L$ is the length of the tubule (centimeter).

Segments 0.5–1.0 mm in length were perfused at rates of 2.3–5.0 nl/min. This range was chosen because it is probably close to the physiologic rate and because it allowed a sufficient decrease in the chloride concentration of the collected fluid in the control period (at least 15 meq/liter in the MTALH) so that a decrease in $J_{Cl}$ and thus a rise in the concentration of chloride in the collected fluid would be easily discernible.

The voltage in both the CTALH and MTALH tended to decrease gradually over the first 40–60 min after an immediate increase upon warming to 37°C. Though not all tubules exhibited this behavior, all studies were initiated at least 60 min after warming or when the voltage became stable, whichever was longer. The flow rate was adjusted by hydrostatic pressure and three to five timed collections were made so that an accurate flow rate could be determined. Then three to five control samples were collected for chloride microanalysis. The flow rate was again measured at the end of the period by timed collection. This protocol was followed for each period (control, experimental, and recovery) and tubules that exhibited variations in flow rate of >0.5 nl/min within a period were discarded. Each chloride sample was analyzed two to five times with frequent standardization to be certain no machine drift occurred.

Pilot studies indicated that PGE$_2$ in doses of 2 μM would cause a fall in voltage. The dose of 2 μM was chosen because in pilot studies, this dose gave a reduction in $V_T$ and $J_{Cl}$ that was consistently measurable. Because of potential difficulties in distinguishing spontaneous changes with time from that of PGE$_2$, a series of 12 experiments was conducted in a randomized, blinded fashion where the (unknown) bathing solution contained either 2 μM PGE$_2$ or the identical amount of carrier (ethanol, 0.008% vol/vol). The unknown solution was prepared by someone not connected with the studies.

PGE$_2$ was obtained from Dr. John Pike, Upjohn Co., Kalamazoo, Mich. The stock solution was prepared in ethanol (10 mg/ml) and stored at -20°C as described (6). PGE$_2$ stored in this way is stable (13). The final solution was prepared immediately before use. The bath was continuously changed by the use of a delta micrometering pump (Lab-line Instruments, Inc., Melrose Park, Ill.) at 0.5 ml/min. Changing the bath to the PGE$_2$-containing solution simply required changing the reservoir. With this system it is not necessary to gas the bathing solution while in the perfusing chamber since the flow rates are sufficiently fast to obviate any changes in pH because of CO$_2$ loss. Also, there is no significant change in osmolality using this technique.

Special attention was paid to the specific location of the tubules as they were dissected from the kidney. Because of the possibility of heterogeneity of function along the thick ascending limb of Henle, segments labeled MTALH were all dissected from the inner stripe of the outer medulla and segments labeled CTALH were all dissected from the midcortical region.

Statistical analysis was done using the paired or unpaired Student’s t test as appropriate.

RESULTS

Effect of PGE$_2$ on the function of the MTALH. Table I displays the results of the randomized, blinded studies conducted on the in vitro isolated, perfused MTALH. All tubules in this series were exposed only to the artificial hypertonic solution. The mean $V_T$ in the control period for all tubules was 2.5±0.3 mV and the mean $J_{Cl}$ was 15.6±1.4 peq·cm$^{-1}$·s$^{-1}$. Though the carrier-treated group had a lower-than-average control $V_T$ and $J_{Cl}$ (2.1±0.3 and 13.5±2.4) and the PGE$_2$-treated group had a higher-than-average control $V_T$ and $J_{Cl}$ (2.8±0.4 and 17.0±1.5); the differences between the two groups were not significant. Of note is the wide range in $J_{Cl}$ demonstrable in these segments with values ranging from 7.4 to 22.5 peq·cm$^{-1}$·s$^{-1}$.

The addition of 2 μM PGE$_2$ to the bathing solution resulted in a fall in $V_T$ that began in ≈ 5–10 min and stabilized in 20 min. The $V_T$ fell from 2.8±0.4 to 1.6 ±0.2 mV and concomitantly the $J_{Cl}$ fell from 17.0±1.5 to 10.9±1.0 peq·cm$^{-1}$·s$^{-1}$. This fall in $J_{Cl}$ of 36% is in marked contrast to the group receiving only carrier (ethanol, 0.008% vol/vol), which showed no significant change in $J_{Cl}$. After exposure to PGE$_2$ for 50 min the tubules demonstrated no significant recovery of either $V_T$ or $J_{Cl}$, a situation that confirmed the results of the pilot studies. Interestingly, however, the control group receiving only the carrier displayed a tendency to increase both $V_T$ and $J_{Cl}$ with time and though it was a
TABLE I
Effect of Carrier (Ethanol) or PGE₂ on \( V_T \) and \( J_{cl} \) Across the MTALH when Bath and Perfusate were Artificial Hypertonic Solutions

<table>
<thead>
<tr>
<th>Experiment</th>
<th>L (mm)</th>
<th>V (nl/min)</th>
<th>Control</th>
<th>Experiment</th>
<th>Recovery</th>
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<td>NS</td>
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<td>&lt;0.02</td>
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Effect of 2 \( \mu \)M PGE₂*

<table>
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<tr>
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<th>V (nl/min)</th>
<th>Control</th>
<th>PGE₂</th>
<th>Recovery</th>
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</table>

Abbreviations used in this table: L, length; V, perfusion rate (equal to collection rate).
* Carrier or PGE₂ was added to the bathing solution in a randomized, blinded fashion in the sequence given by the experiment number.
1 \( P > 0.05 \).

Gradual change, during the recovery period the difference was statistically significant (Table I).

Because these results are in apparent conflict with those reported by Fine and Trizna (14) using a similar protocol, experiments were conducted with rabbit serum as the bathing solution and an ultrafiltrate of rabbit serum as the perfusate as they reported. In four experiments, displayed in Table II, PGE₂ when added to the serum bathing the tubule produced no change in \( V_T \) or \( J_{cl} \). This finding is consistent with their report.

TABLE II
Effect of 2 \( \mu \)M PGE₂ on \( V_T \) and \( J_{cl} \) Across the MTALH when Bathed with Rabbit Serum and Perfused with Ultrafiltrate

<table>
<thead>
<tr>
<th>Experiment</th>
<th>L (mm)</th>
<th>V (nl/min)</th>
<th>Control</th>
<th>PGE₂</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_T )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
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<td>0.2</td>
<td>0.6</td>
<td>0.6</td>
<td>0.7</td>
</tr>
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</table>

For abbreviations see Table I.

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sults, and is in sharp contrast to the results obtained with the artificial hypertonic solution. There was, however, one significant difference between the group exposed to rabbit serum and the group exposed to the artificial hypertonic solution. The former group displayed a more positive \( V_T \) (4.3±0.6 vs. 2.5±0.3), a difference that is statistically significant \( (P < 0.01) \). Though the \( V_T \) was larger the mean \( J_Cl \) was different.

The explanation as to why PGE\(_2\) would diminish \( J_Cl \) in artificial hypertonic solution and not in rabbit serum is not immediately apparent. One explanation would be that this segment is sensitive to PGE\(_2\) only in a hypertonic environment. To test this hypothesis the MTALH was bathed and perfused in an artificial solution designed to simulate an ultrafilterate of plasma. The results of five experiments are displayed in Table III. The \( V_T \) and \( J_Cl \) exhibited the same response in this environment as they did in the hypertonic environment. The control \( V_T \) and \( J_Cl \) were not different from those observed in the hypertonic environment, and the fall in \( J_Cl \) of 48% after exposure to 2 \( \mu \)M PGE\(_2\) was similar to the results in the former experiments. Thus, it appears that the difference in osmotic pressure does not affect the tubule’s capacity to respond to PGE\(_2\). Rather, it must be that a component of rabbit serum interferes with the interaction of PGE\(_2\) and the cells of the MTALH.

Substances capable of modulating transport often demonstrate specificity for either the luminal or peritubular surface. When the luminal surface was exposed to 2 \( \mu \)M PGE\(_2\), the \( V_T \) fell in a fashion similar to that described for peritubular exposure (Fig. 1). The perfusion solution was sham changed to insure that the act of changing perfusate did not alter \( V_T \). The resultant decrease in \( V_T \) was stable within 20 min. When PGE\(_2\) was washed out, a significant partial recovery was observed \( (P < 0.05) \).

**Effect of PGE\(_2\) on the function of the CTALH.** CTALH also transports chloride against an electrochemical gradient (2), and it was of some interest to determine whether PGE\(_2\) might affect transport similarly in this segment. Table IV displays the results of five experiments where the CTALH was perfused and bathed with the artificial isotonic solution. The tubules were treated in an identical fashion to that of the MTALH. Several differences, however, were apparent. First, as noted (15), the CTALH had a much thinner epithelium than did the MTALH. Second, though the \( V_T \) was not different in the two segments exposed to identical isotonic solutions (2.6±0.5 for CTALH and 3.1±0.5 for MTALH), the \( J_Cl \) was much lower across the CTALH (6.5±0.7 peq cm\(^{-1}\cdot s\(^{-1}\)) than across the MTALH (13.3±1.4 peq cm\(^{-1}\cdot s\(^{-1}\) \( (P < 0.002) \) (Fig. 2, Tables III and IV). Third, the \( V_T \) and \( J_Cl \) were not affected by 2 \( \mu \)M PGE\(_2\) when applied to the peritubular surface of the CTALH. Although some tubules had a tendency to display a gradual reduction in \( V_T \) and/or \( J_Cl \) with time, a finding alluded to by Burg and Green (2), the change in the present experiments was not significant. Four additional experiments were conducted to test the effect of 2 \( \mu \)M PGE\(_2\) in the lumen. The \( V_T \) showed no significant change (control, 3.3±0.1; sham, 3.2±0.2; PGE\(_2\), 3.1±0.2). The \( V_T \) was likewise unaffected by 10 \( \mu \)M PGE\(_2\) applied from the bath (control, 3.6±0.2; PGE\(_2\), 3.5±0.2 \( [n = 4] \)). Thus, it appears that the CTALH differs functionally from the MTALH in at least two ways: the capacity to transport chloride and the ability of the transport process to respond to PGE\(_2\).

**Effect of various doses of PGE\(_2\) on MTALH.** A series of experiments was conducted to test the effective dose range on MTAHL bathed and perfused with hypertonic solution. In these experiments, only \( V_T \) was measured since it correlates closely with changes in chloride transport (Tables I–IV) and is considerably

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**TABLE III**

*Effect of 2 \( \mu \)M PGE\(_2\) on \( V_T \) and \( J_Cl \) Across the MTALH when Bath and Perfusate were Artificial Isotonic Solution*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>L (mm)</th>
<th>( V_T ) (mV)</th>
<th>( J_Cl ) (peq cm(^{-1}\cdot s(^{-1}))</th>
<th>Recovery</th>
<th>Control</th>
<th>PGE(_2)</th>
<th>Control</th>
<th>PGE(_2)</th>
<th>Recovery</th>
</tr>
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<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

*P* <0.01  NS | <0.02  NS

For abbreviations see Table I.
FIGURE 1 Response of the $V_T$ across the MTALH to the addition of 2 $\mu$M PGE$_2$ to the lumenal contents. Sham values demonstrate no change by simply changing the perfusate. Inhibition and recovery are significant ($P < 0.05$).

PGE$_2$, by reducing the transepithelial voltage (Fig. 1; Tables I and II) and the net efflux of chloride (Tables I and III). The voltage is sensitive to concentrations as low as 10 nM (Fig. 3). In contrast, the CTALH has a lower capacity to transport chloride and is not sensitive to PGE$_2$ (Table IV).

DISCUSSION

The results of the present study demonstrate that the in vitro isolated, perfused MTALH responds to 2 $\mu$M PGE$_2$, by reducing the transepithelial voltage (Fig. 1; Tables I and II) and the net efflux of chloride (Tables I and III). The voltage is sensitive to concentrations as low as 10 nM (Fig. 3). In contrast, the CTALH has a lower capacity to transport chloride and is not sensitive to PGE$_2$ (Table IV).

Throughout the thick ascending limb of Henle, chloride is transported out of the lumen against an electrochemical gradient. In both CTALH and MTALH,
the chloride transport process has been shown to be sensitive to ouabain (1, 2) and furosemide (16, 17). The present study is the first to demonstrate modulation of chloride transport in the thick ascending limb of Henle by an endogenously produced substance.

The reduction of $J_{Cl}$ across the MTALH by PGE$_2$ appears to be in conflict with the reported results of Fine and Trizna (14). Though there may be many reasons why the results differ, the most likely reason is that the activity of PGE$_2$ in rabbit serum appears to be quite different from that in an artificial solution.

The reason why PGE$_2$ should inhibit chloride transport in the MTALH and not the CTALH is not obvious. However, at least three possible explanations exist. First, prostaglandin dehydrogenase (the enzyme responsible for the major portion of the degradation of PGE$_2$) has been shown to be present in the CTALH but apparently not in the MTALH (18). If these biochemical findings are relevant to the effect of PGE$_2$ on chloride transport, then one must postulate that PGE$_2$ is inactivated by the cells of the CTALH before it can exert its effect on the chloride transport process. A second possible explanation involves the concept of functional heterogeneity between MTALH and CTALH. It is possible that the mechanism(s) responsible for the higher transport rate in the MTALH are sensitive to PGE$_2$ but that both MTALH and CTALH segments possess a basolateral transport process that cannot be inhibited by PGE$_2$. A third possibility is that the CTALH has a higher threshold for PGE$_2$. The lack of effect of 10 $\mu$M PGE$_2$ on the $V_r$ of the CTALH is against this hypothesis. Also, if higher doses were to cause an inhibition, the physiologic significance would be questionable since the concentration of PGE$_2$ in the cortex is $<$5% of that in the medulla (5).

The possibility that the thick ascending limb of Henle contains a functionally heterogeneous epithelium has been suggested by four studies. (a) Allen and Tisher (15) have found anatomical differences between the CTALH and MTALH in Sprague-Dawley rats. (b) Imbert et al. (19) have demonstrated that the rabbit MTALH generates more cyclic AMP in response to a given dose of vasopressin than does the CTALH. (c) Schmidt and Horster (20) have demonstrated greater activity of Na-K-ATPase in the MTALH than in the CTALH. Their findings are consistent with the present study if one postulates a role for Na-K-ATPase in “active” chloride transport (21). (d) Suki et al. (22) have demonstrated that calcium efflux in inhibited by furosemide in the MTALH but not in the CTALH. Thus, the evidence for structural and functional heterogeneity of the thick ascending limb of Henle is beginning to emerge in a consistent pattern.

The demonstration that a substance can inhibit chloride transport in the MTALH and have no effect in the CTALH is without precedent. The translation of this phenomenon into whole kidney function suggests that alteration of endogenous renal prostaglandin production would affect the ability to concentrate the urine maximally but would not affect the ability to generate and excrete solute-free water. It is interesting to note that Work et al. (23) have shown no impairment of free water generation in conscious dogs treated with inhibitors of prostaglandin synthesis. This finding would thus support the lack of effect of PGE$_2$ on chloride transport across the CTALH.

It is tempting to speculate on the physiological and clinical relevance of the role of PGE$_2$ in modulating active chloride transport out of the MTALH. Four interesting possibilities arise: (a) That PGE$_2$ is active from the luminal surface as well as the peritubular surface of the MTALH raises the possibility that secretion of PGE$_2$ by the pars recta (24-26) as well as medullary production (4, 5) may play a role in the regulation of chloride reabsorption. In support of an in vivo effect of PGE$_2$ from the luminal surface, Kauker (27) has found increased 22Na recovery in the urine when late proximal tubular microinjections contained PGE$_2$. (b) Endogenous prostaglandins may be participating in the diuretic response to furosemide (28). The reason why furosemide-induced diuresis is blunted by prior administration of inhibitors of prostaglandin synthesis is not clear (29, 30). It is possible, however, that furosemide increases renal prostaglandin concentration either by increasing its production (28) or by inhibiting its degradation (31), or both. (c) PGE$_2$ may play a role in the modulation of medullary hypertonicity through its effect on chloride transport as well as its effect on medullary blood flow. In support of this thesis, Ganguli et al. (32) have demonstrated a sharp rise in medullary sodium chloride content after indomethacin therapy. Danon et al. (33) have demonstrated a stimulation of prostaglandin synthesis by hypertonic media. A primary increase in medullary prostaglandin production would thus reduce the interstitial salt concentration of the medulla as well as render the collecting tubule epithelium less sensitive to vasopressin (34). These processes would be synergistic and would result in less water backdiffusion across the collecting tubule and ultimately in the excretion of a less concentrated urine. The in vivo experiments demonstrating an increase in the concentration of the urine after administration of inhibitors of prostaglandin synthesis (35, 36) support the idea that one or both of these mechanisms may be operative. (d) Finally, endogenous prostaglandins may play a role in the regulation of NaCl excretion under certain circumstances. Though the exact role of endogenous renal prostaglandins on salt excretion is difficult to assess, the recent data of Higashihara et al. (37) suggest that the chloruresis of
isotonic volume expansion may be due in part to a disproportionate reduction of chloride reabsorption from juxtamedullary nephrons and that this reduction can be reversed by treatment with inhibitors of prostaglandin synthesis.

In summary, the results of the present study demonstrate that PGE₂ can inhibit the transport of chloride out of the in vitro isolated, perfused rabbit MTALH. It is effective from either the peritubular or luminal surface. In contrast there is no effect on the CTALH. These results support the idea that endogenous PGE₂ can modulate the salt content of the renal medulla and may modulate salt excretion under appropriate circumstances.

ACKNOWLEDGMENTS

I appreciate the technical assistance of Rebecca Himmel and Allen Williams, and the encouragement of Dr. Juha P. Kokko and Dr. Donald W. Seldin. This work was supported in part by National Institutes of Health Research grants 5-R01-AM14677 and 1 R01-AM25231.

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