Antenatal Bartter’s syndrome: why is this not a lethal condition?


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Summary

There are four themes in this teaching exercise for Professor McCance. The first challenge was to explain how a premature infant with Bartter’s syndrome could survive despite having such a severe degree of renal salt wasting. Second, the medical team wanted to know why there was such a dramatic decrease in the natriuresis in response to therapy, despite the presence of a permanent molecular defect that affected the loop of Henle. Third, Professor McCance was asked why this patient seemed to have a second rare disease, AQP2 deficiency type of nephrogenic diabetes insipidus. The fourth challenge was to develop a diagnostic test to help the parents of this baby titrate the dose of indomethacin to ensure an effective dose while minimizing the likelihood of developing nephrotoxicity. The missing links in this interesting story emerge during a discussion between the medical team and its mentor.

In this clinical teaching exercise, the focus is on a newborn with a severely compromised ability to retain sodium (Na⁺) and chloride (Cl⁻) in his body. Once again, the central figure in this clinical detective story is the imaginary consultant, Professor McCance. Readers will know that his overall objective is to apply principles of integrative physiology at the bedside, together with a quantitative analysis, to reach a more accurate clinical diagnosis, reveal the underlying pathophysiology and help design better options for therapy.

The consultation

The medical team was eager to present a very interesting patient to Professor McCance, because they hoped he could improve their understanding of a complex series of findings in the salt and water area. The patient is now a 2-year-old male, the first child of consanguineous parents (first cousins). The pregnancy was complicated by severe polyhydramnios and the patient was born after a 26-week gestation with a mass of 1 kg. The neonatal team
noticed that his initial urine flow rate was large and this urine had a very high concentration of Na⁺ (≈100 mmol/l, Table 1). Over the first month of life, he required large supplements of NaCl (≈15 mmol/kg/day). Based on this information, the diagnosis was obvious—the patient had antenatal Bartter’s syndrome (Figure 1). This diagnosis was supported by molecular studies: microsatellite markers were assessed at each of the loci encoding the transport steps that may cause Bartter’s syndrome (Figure 1)—the only locus where the patient was homozygous, but his related parents were not. Around the gene encoding the main transporter to reabsorb Na⁺ and Cl⁻ in the thick ascending limb of the loop of Henle (LOH), NKCC2 (SLC12A1). Sequencing of this gene for identification of the actual mutation had not been performed yet. The consultant added, ‘It is not surprising that when there is a major lesion in the thick ascending limb of the loop of Henle, there is an enormous risk for the patient and an extreme challenge for the physicians caring for that patient after birth. Added to this risk are the difficulties of a very “fragile” premature infant and issues related to the normal physiology of these infants’.

Table 1  Values in the first month of life

<table>
<thead>
<tr>
<th>Day of life</th>
<th>1</th>
<th>4</th>
<th>6</th>
<th>13</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>962</td>
<td>746</td>
<td>764</td>
<td>823</td>
<td>964</td>
</tr>
<tr>
<td>Na⁺ intake (mmol/kg/day)</td>
<td>1</td>
<td>13</td>
<td>16</td>
<td>12</td>
<td>17</td>
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<tr>
<td>Water intake (ml/kg/day)</td>
<td>123</td>
<td>259</td>
<td>243</td>
<td>216</td>
<td>234</td>
</tr>
<tr>
<td>Urine output (mmol/kg/day)</td>
<td>140</td>
<td>138</td>
<td>42</td>
<td>125</td>
<td>164</td>
</tr>
<tr>
<td>Plasma (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺ (mmol/l)</td>
<td>136</td>
<td>123</td>
<td>137</td>
<td>137</td>
<td>132</td>
</tr>
<tr>
<td>K⁺ (mmol/l)</td>
<td>5.7</td>
<td>4.0</td>
<td>2.8</td>
<td>5.4</td>
<td>4.7</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>91</td>
<td>167</td>
<td>238</td>
<td>155</td>
<td>90</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>30</td>
<td>44</td>
<td>32</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>Urine Na⁺ (mmol/l)</td>
<td>96</td>
<td>94</td>
<td>111</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

I: Survival despite a severe degree of renal salt wasting

Before appearing on the ward, Professor McCance wished to be brought up to date on how the LOH carried out its functions. The medical team and the nephrology consultant were delighted to participate in this role reversal; they were certain that this would be a wise investment. ‘Before you begin the refresher course, I cannot let the requirement for 15 mmol Na⁺ per kg body weight per day pass without placing it in a quantitative perspective. Let me compare these values to ones in a 50-kg adult who has 30 l of water with 10 l in the ECF compartment for illustrative purposes’ said Professor McCance. ‘The daily excretion of Na⁺ is simply astounding’. With a Na⁺/water ratio of 150 mmol/l of water in the ECF compartment, the total content of Na⁺ in the ECF compartment in a 50-kg subject is 1500 mmol (150/l × 10 l) — this is equivalent to 30 mmol Na⁺/kg of body weight (1500 mmol/50 kg). Since the vast majority of Na⁺ is in the ECF compartment, ‘an excretion of 15 mmol/kg each day represents the elimination of half of the content of Na⁺ in the ECF compartment in just one day. Hence the nephron site responsible for such a large natriuresis is an upstream one, the proximal convoluted tubule or the loop of Henle’, the medical team added—since there was no evidence of defective reabsorption of all the valuable nutrients and ions that occur in the proximal convoluted tubule (PCT), the most likely site of the defect was in the LOH.

‘Refresher course’ for Professor McCance

The nephrology fellow was pleased to be asked to discuss how the LOH operates. There are
two sequential mechanisms to reabsorb Na+ and Cl− from the thick ascending limb of the LOH and each is responsible for reabsorbing half of the Na+. The linkage between these two mechanisms is the result of entry of K+ into its lumen via the rat outer medulla K+ ion channel (ROMK) (the name merely reflects the site where it was first isolated) (Figure 2).

(i) Active reabsorption of half of the Na+ occurs by the Na-K-2-Cl cotransporter (NKCC2)—the supply of K+ via ROMK appears to limit ion flux through NKCC2.

(ii) The other half of the Na+ is reabsorbed passively between cells of the medullary thick ascending limb, driven by the lumen-positive voltage, which is also dependent on K+ entry into the lumen via ROMK.

Professor McCance thanked the nephrology fellow for providing this new and exciting information. ‘I guess that when regulation of the loop of Henle is considered, the emphasis will be on control of ROMK’, he mused. He then asked with some humility, ‘What is antenatal Bartter’s syndrome?’

**Question 1. What is antenatal Bartter’s syndrome?**

The registrar provided the following information. Bartter’s syndrome is an inherited disorder in which there is a defect in the reabsorption of Na+ and Cl− in the thick ascending limb of the LOH. The medullary part of this nephron segment is responsible for increasing the osmolality in the renal medullary interstitial compartment because it reabsorbs solutes (Na+ and Cl−) ‘without water’ (i.e. it is impermeable to water). At present, there are five different molecular defects that can cause this renal salt wasting syndrome (Figure 1). The molecular basis for antenatal Bartter’s syndrome is usually due to a defect in the ROMK channel or a mutation that compromises the function of NKCC2; rarely the defect is in the basolateral Cl− channel or its associated subunit, Barttin in this setting.

When tubular function is severely affected in utero, one can be alerted to this diagnosis prior to birth because polyhydramnios will occur very early in pregnancy, as in our patient. Polyhydramnios is the result of the formation of more amniotic fluid by the infant (due to the very large production of urine) than can be absorbed by the mother in utero. As a result of the markedly enlarged uterus, there is often an early end to the pregnancy and the birth of a premature newborn as occurred in our case. Professor McCance wished to add one more observation concerning polyhydramnios. ‘Since the urine volume should be just as high in nephrogenic diabetes insipidus as in antenatal Bartter’s syndrome, “Why is polyhydramnios not present in all pregnancies as nephrogenic diabetes insipidus is always present in utero?”’

**Question 2: Why is polyhydramnios not present in all pregnancies as nephrogenic diabetes insipidus is always present in utero?**

*Physiology principle 1:* The composition of the urine is literally pure water in a patient with nephrogenic diabetes insipidus, whereas it is salt and water in a patient with Bartter’s syndrome. In utero, the electrolyte load must be cleared by the placenta.

*Return to the bedside:* In the setting of antenatal Bartter’s syndrome there is a very high electrolyte load delivered to the placenta. Perhaps the placenta cannot reabsorb all of the extra Na+ and Cl−. Accordingly, the retained Na+ and Cl− in the amniotic fluid lead to the retention of water, and thereby, polyhydramnios develops.

The massive renal salt wasting should make itself known immediately after birth and it poses a severe challenge to the treating physicians. After the neonatal period, the patient will manifest abnormalities secondary to an excessive delivery of Na+ and Cl− to the late cortical distal nephron [i.e. the presence of hypokalaemia due to renal potassium (K+) wasting]. When the basis for antenatal Bartter’s syndrome is a defective ROMK, there will be
a different early postnatal complication, the development of hyperkalaemia, but this is usually transient and the patients will develop life-long hypokalaemia after this interlude. Since the concentration of K⁺ in plasma (P_K) was consistently low in this patient, this suggested that the molecular lesion was a defect in NKCC2 rather than ROMK; this suspicion was verified by the results of the molecular studies.

The nephrology consultant wished to make an additional comment. ‘The hyperkalaemia you described is due to a lack of ROMK in its second important location—in the luminal membrane of the principal cells in the late cortical distal nephron, the sites where K⁺ secretion occurs. In fact, there is a different K⁺ channel (the maxi-K⁺ channel) that has a developmental delay,⁵ and it can become the K⁺ channel that mediates the secretion of K⁺ to remove the threat of hyperkalaemia and induce the development of hypokalaemia after the first month of life (reviewed in reference⁶),’ she said.

II. Review of the micropuncture data in rats

Since Professor McCance wished to examine data in quantitative terms, he asked, ‘How much Na⁺ is reabsorbed in the loop of Henle in an adult in a day?’

Question 3. How much Na⁺ is reabsorbed in the LOH in an adult in a day?

Physiology principle 2: The amount of Na⁺ reabsorbed is the difference between how much Na⁺ is delivered to the LOH minus the amount of Na⁺ delivered to the distal convoluted tubule (DCT). Because an invasive technique (micropuncture) must be used to obtain the data in vivo, experiments were carried out in fed rats. In more detail, the key measurements are the concentrations of Na⁺ in that tubular fluid and an estimate of the volume of fluid delivered to the site of micropuncture in the distal tubule based on the concentration of a substance that is freely filtered at the glomerulus, and not reabsorbed or secreted in the nephron—that substance is inulin.⁷

Return to the bedside: The results of these experiments will be strongly influenced by the diet the rat consumes. When expressed per kilogram body weight, the rat consumes close to 4-fold more NaCl as compared to a 70-kg human. Hence these data represent values when the ECF volume is expanded.⁸

The concentration of inulin in fluid obtained from the last accessible part of the proximal and the earliest portion of the DCT reveals how much filtrate was reabsorbed in intervening nephron segments (Table 2).

Proximal convoluted tubule: Since the concentration of inulin was 3-fold higher in the last accessible site of micropuncture in the PCT than in the plasma, 2/3 of filtered water (120/180 l) is reabsorbed between the glomerulus and this site. Since the concentration of Na⁺ was virtually identical in both of these sites, the quantity of Na⁺ reabsorbed in this nephron segment is ~18 000 mmol (120 l × 150 mmol/l) per day. Therefore ~9000 mmol of Na⁺ are delivered daily to the LOH (60 l × 150 mmol Na⁺/l).

Table 2 Quantitative analysis of events in the LOH

<table>
<thead>
<tr>
<th>Volume (L/day)</th>
<th>Na⁺ (mmol/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivered</td>
<td>Leaving</td>
</tr>
<tr>
<td>PCT 180</td>
<td>60</td>
</tr>
<tr>
<td>LOH 60</td>
<td>20</td>
</tr>
<tr>
<td>PCT 180</td>
<td>30</td>
</tr>
<tr>
<td>LOHmedulla 30</td>
<td>27</td>
</tr>
<tr>
<td>DCT 27</td>
<td>27</td>
</tr>
</tbody>
</table>

? The amount of Na⁺-leaving DCT is unknown and thus the amount reabsorbed cannot be calculated.

The concentration of Na⁺ in the earliest distal convoluted tubule is 50 mmol/l.

The concentration of Na⁺ in the earliest distal convoluted tubule was 50 mmol/l.

In the revised view, the events in the loop of Henle represent only reabsorption of water from those descending thin limbs that have AQP1 and of Na⁺ in the medullary thick ascending limb of the LOH.

There is much less Na⁺ delivered to the distal convoluted tubule than exitd from the medullary thick ascending limbs of the LOH because Na⁺ (and Cl⁻) were reabsorbed in the cortical thick ascending limb of the LOH.

For details, see text. The concentration of Na⁺ in the PCT is 150 mmol/l of water whereas it is ~50 mmol/l. DCT: distal convoluted tubule.
Distal convoluted tubule: Since the concentration of inulin was 2-fold higher in the fluid obtained in the early DCT, half of the filtrate was reabsorbed between these two micropuncture sites (30 of the 60 l each day). Since the concentration of Na\(^+\) in fluid obtained from the early DCT is ~50 mmol/l, 1500 mmol are delivered here (30 l x 50 mmol/l) (Table 2). Hence it appears that 7500 mmol of Na\(^+\) (i.e. 9000–1500 mmol) are reabsorbed in the only nephron segments that can reabsorb this Na\(^+\), the thick ascending limb of the LOH, in both its medullary and cortical components using numbers that reflect our 50-kg human equivalent of the rat (in nephron physiology terms, she added with a smile).

All eyes turned to their mentor, expecting a nod of approval, but Professor McCance appeared puzzled. He said, ‘If everything you said is true, antenatal Bartter’s syndrome should be a “lethal” disorder. Therefore, my next question is, “How large is the deficit of Na\(^+\) in your patient?”’

**Question 4. How large is the deficit of Na\(^+\) in your patient?**

**Physiology principle 3:** The content of Na\(^+\) in the body determines the ECF volume, and thereby the plasma volume. When the deficit of Na\(^+\) is very large, the haematocrit rises and there is a high concentration of proteins in plasma.\(^9\) Hence the heart cannot pump enough of this viscous blood to important organs in the body.

**Return to the bedside:** Normal adults can survive with a very large deficit of Na\(^+\) (i.e. almost 50%) with few symptoms at rest.\(^10\) With a 50% deficit in the quantity of Na\(^+\) in his ECF compartment, there should have been a 2-fold rise in the concentration of albumin in plasma (P\(_{\text{Albumin}}\)) and a haematocrit around 0.60, as we discussed previously.\(^11\) Notwithstanding, since there was only a modest rise in his P\(_{\text{Albumin}}\) (i.e. from 30 to 44 g/l; Table 1), we doubt that he had such a severe degree of contraction of his ECF volume. Therefore we must re-examine everything we have heard about the quantitative aspects of Na\(^+\) reabsorption in the LOH.

**The conundrum in antenatal Bartter’s syndrome**

Using the numbers in a 50-kg adult, the total quantity of Na\(^+\) in the ECF compartment is the product of the ECF volume (10 l) and the P\(_{\text{Na}}\) (150 mmol/l of water) or 1500 mmol in this imaginary person. This number is ~20% of the possible loss of 7500 mmoles of Na\(^+\) per day when there is a complete defect in Na\(^+\) reabsorption in the LOH (Table 2). Hence this patient should not have been able to survive for even 5 h if he excreted all the Na\(^+\) delivered to the LOH.

**Attempts to resolve the conundrum**

(i) Lower the delivery of Na\(^+\) reaching the LOH:

For this to occur, there must be both a much lower GFR and possibly adaptive changes in the PCT.

(a) A lower GFR in a newborn: This value is typically around 30 ml/min/1.73m\(^2\).\(^12\) Since the P\(_{\text{Creatinine}}\) at birth in this newborn was ~90 mmol/l (1.0 mg/dl) instead of the usual value of 30 mmol/l (0.3 mg/dl), his GFR was only 1/3 of normal. We note that this fell markedly (his P\(_{\text{Creatinine}}\) rose over a matter of several days to 238 mmol/l (2.7 mg/dl), which suggests that his GFR was now close to 10% of normal (i.e. 3–5 ml/min/1.73m\(^2\))). The most likely basis for this fall is a very marked decline in his ‘effective’ arterial blood volume. Parenthetically, this low GFR actually helped preserve his circulatory volume, as it decreased urinary loss of Na\(^+\).

(b) Enhanced reabsorption of Na\(^+\) in the PCT: When the ‘effective’ arterial blood volume is low, the reabsorption of Na\(^+\) in the PCT is stimulated. Taken together with the GFR, these could help the patient to survive, but we doubt that this is the entire answer.

(ii) Increase the reabsorptive capacity in downstream nephron sites:

While this will undoubtedly occur, some of these adaptive changes may require time to develop.

Conclusion: ‘I am not sure that I have a satisfactory, complete answer to the conundrum’, said Professor McCance. Therefore, we wish to ask the nephrology consultant if there is new information that may help me understand why this patient had a much smaller deficit of Na\(^+\) than we expected. She said, ‘As a matter of fact, there is something that was just published that troubles me too. Close to 85% of the nephrons do “not” have water channels [aquaporin 1 (AQP1)] in their descending thin limbs of the loop of Henle’.\(^13\)

‘Aha’, said Professor McCance, let us explore the consequences of this new discovery. ‘Although the following discussion will be more detailed than I usually present at rounds, it may help explain why antenatal Bartter’s syndrome is not a lethal disorder’. We shall do something different from our previous rounds and provide my thought processes during this discussion. Some readers may prefer to skip the next section or delay reading it until a later time to maintain the continuity of the clinical discussion; accordingly, they should proceed directly to Question 5.
Special tutorial

Professor began by stating, ‘I shall provide an in-depth look at the reabsorption of Na+ and water in the loop of Henle taking into consideration the new discovery of ‘a lack of AQP1 in its descending thin limb’.\textsuperscript{13} This may help to explain why antenatal Bartter’s syndrome is not a lethal disorder. For illustrative purposes, I shall present the ideas as we usually do, beginning with a question, state the physiology principle, and then discuss its impact on antenatal Bartter’s syndrome’.

Focus on water

1. How does a lack of AQP1 in the descending thin limb influence the volume of water delivered to the DCT?

**Physiology principle:** This lack of a water channel prevents the reabsorption of water in the LOH, as its medullary thick ascending limb also lacks AQP1.

**Interpretation:** Virtually all the water delivered to the LOH will be delivered to the DCT! The minor exception is the long descending thin limbs of the LOH that enter the inner medulla, as they do have AQP1 and thus they are permeable to water; they constitute only 15% of the nephrons.

2. Why did we believe that 2/3 of the GFR is reabsorbed in the PCT and that water was reabsorbed in the descending thin limb of the LOH?

**Physiology principle:** When water channels are present, water will move from a compartment with a lower osmolality to one with a higher osmolality (i.e. out of the lumen of the PCT). This osmotic force is enormous (19.2 mm Hg/mOsm/l).

**Interpretation:** The interpretation of a ($TF/P_{\text{Inulin}}$) of ~3 is valid, but it only applies to the ‘last accessible’ micropuncture site in the PCT, which is not the end of this nephron segment (Figure 3). In fact, there are portions of the PCT ‘after the site of micropuncture’, and they are capable of reabsorbing Na\textsuperscript{+}, Cl\textsuperscript{−} and water before fluid reaches the LOH. Hence the volume of filtrate reabsorbed in the PCT was underestimated.

There is another issue—only nephron segments of superficial nephrons can be sampled with the micropuncture technique, as they are the only ones that reach the kidney surface. Hence the data we are working with may not represent events in all the nephrons.

With respect to the suspicion that AQP1 was present in the descending thin limbs of the LOH, the techniques used in earlier times were not sensitive enough to be sure that AQP1 was absent.

3. If we cannot rely on the micropuncture data from the PCT to reveal the volume of filtrate delivered to the loop of Henle, are the micropuncture data from the early DCT truly similarly ‘tainted’?

**Physiology principle:** One ‘cannot discard’ data if the techniques used are sound. Hence the micropuncture data in the DCT are reliable.

**Interpretation:** If all the nephron segments between the beginning of the LOH and the early DCT are impermeable to water, the ($TF/P_{\text{Inulin}}$) in the early DCT reveals the volume of filtrate delivered from the proximal tubule to the LOH. Moreover, there is no change in this volume up to the distal site of micropuncture. Since the ($TF/P_{\text{Inulin}}$) in the early DCT is ~6 (summarized in reference \textsuperscript{2}), 1/6 of the GFR (180 l/day) in a normal human adult is delivered to the LOH (i.e. ~30 l/day).

**Caveat:** When one uses these data to understand human physiology, one equates a human to a 70-kg rat, but this may not be true in renal physiology terms. Of great importance, the diet of the rat may influence the results, as rat chow provides a Na\textsuperscript{+} load that is ~4-fold greater than that of an adult human when expressed in mmol/kg body weight terms.\textsuperscript{8} Since these data in the rat represent values...
when the ECF volume is expanded, a smaller proportion of the GFR is likely to be reabsorbed in the PCT of the rat than in the human. Hence the volume of filtrate delivered to the LOH may be somewhat smaller in the human than in the rat.

**Conclusion concerning water**

If 30 l/day are delivered to the LOH and 3 l are reabsorbed in 15% of the LOH, the volume delivered daily to the medullary thick ascending limb of the LOH of the human is ~27 l/day; this number will be very valuable for future calculations.

**Focus on Na⁺**

1. **Does this lack of AQP1 mean that the concentration of Na⁺ in the lumen of the descending thin limb of the LOH cannot rise as one proceeds deeper into the outer medulla?**
   
   **Physiology principle:** The Na⁺ concentration can rise due to a decreased volume of water or to an increased quantity of Na⁺.

   **Interpretation:** Although water re-absorption cannot occur due to the lack of AQP1 in the majority of descending thin limbs of the LOH, there is another way to raise in the Na⁺ concentration, the entry of Na⁺ into the descending thin limbs of the LOH if they have ion channels for Na⁺ and Cl⁻ and thus they are permeable to these ions. This luminal concentration of Na⁺ and Cl⁻ will rise as this nephron segment passes through regions with a high interstitial concentration of Na⁺. The nephrology consultant reminded the medical team that the descending thin limbs of the LOH are permeable to both Na⁺ and Cl⁻.

2. **Why is it critical for the concentration of Na⁺ to rise in the lumen of the descending thin limbs of the LOH to concentrate the urine?**
   
   **Physiology principle:** If Na⁺ diffuses into a compartment, positive voltage enters this region unless it is possible for Cl⁻ to enter or K⁺ to leave that compartment. In fact there is no known Cl⁻ channel that would permit Cl⁻ to enter and K⁺ must diffuse into and not out of the medullary thick ascending limb of the LOH.

   **Interpretation:** There is a marked danger for the LOH if the lumen had a much lower concentration of Na⁺ in the bend of the loop as compared to the interstitial compartment. In more detail, the ‘medullary thick’ ascending limb of the LOH is very permeable to Na⁺ via a paracellular route. Hence if a large quantity of Na⁺ were to enter its lumen, the luminal voltage would become very positive. As a result, K⁺ entry would be arrested, and there would be insufficient K⁺ to permit Na⁺ reabsorption by NKCC2 in this nephron segment (Figure 4).

   **Conclusion:** It is critically important that the concentration of Na⁺ in the medullary interstitial compartment to be equal to that in the lumen at the bend of each LOH to have a functioning renal concentrating process.

   **How high is the concentration of Na⁺ at the bend of the LOH?**

   **Physiology principle:** The loops of Henle constitute a family of nephrons where individuals in that family...
have their bends at progressively deeper levels in the outer (and inner) medulla.

**Interpretation:** There are no data for the composition of luminal fluid for each absolute depth in the medulla. We calculated the luminal Na⁺ concentration assuming that the luminal and interstitial Na⁺ concentrations would be equal and did so at 10 different equal divisions between 300 and 900 mOsm/kg H₂O (range of values in a normal human adult). This helped define an ‘average’ nephron with its bend occurring at ~700 mOsm/kg H₂O. Since the bulk of the osmoles are Na⁺ and Cl⁻, this resulted in a luminal Na⁺ concentration of 1/2 of 700 mOsm/kg H₂O or 350 mmol/l.

4. **How much Na⁺ diffuses into the descending thin limbs of the LOH in a normal adult?**

**Physiology principle:** The quantity of Na⁺ that would ‘diffuse into’ all the descending thin limbs of the LOH each day is the product of the total flow rate each day and the rise in the concentration of Na⁺ at a depth in the medulla of the ‘average’ nephron.

**Interpretation:** Professor McCance returned to his favorite pastime, a quantitative analysis.

(i) Since the volume delivered to the LOH from the GFR is 30 l/day and the luminal Na⁺/water ratio is 150 mmol/l, therefore 4500 mmol of Na⁺ are delivered to the LOH via the GFR.

(ii) The flow rate in the loops of Henle is 27 l/day and the diffusion of Na⁺ into the ‘average’ nephron is 200 mmol (350 mmol/l minus 150 mmol/l) into each of the litres delivered here. Hence 5400 mmol of Na⁺ gain access to the luminal fluid from the medullary interstitial compartment.

**Conclusion:** This movement of 5400 mmol of Na⁺ and Cl⁻ into the descending thin limbs each day decreases the osmolality in the medullary interstitial compartment. Accordingly, there must be another step to replace this lost 5400 mmol of Na⁺—this second step is the active reabsorption of this 5400 mmol of Na⁺ and Cl⁻ by the medullary thick ascending limb of the LOH with no net gain for the process of concentrating the urine. ‘In fact this whole process simply returns the interstitial composition back to its original Na⁺ concentration while the luminal fluid now has a Na⁺ concentration of 150 mmol/l’.

**How much Na⁺ must be reabsorbed in the medullary thick ascending limb of the LOH in a normal adult to concentrate the urine?**

**Physiology principle:** ‘By the term, concentrate the urine, I mean draw water out of the water permeable medullary collecting duct’, said Professor McCance. Every litre of water that is re-absorbed from all water-permeable nephron segments must exit the renal medulla in the ascending vasa recta—this means that the concentration of Na⁺ in these vessels at the junction of the cortex and the medulla must be virtually identical (i.e. ~150 mmol/l of water).

**Interpretation:** Based on the usual excretion of osmoles and the fact that 900 mmol of urea recycle, 5 l of water are reabsorbed daily from the medullary collecting ducts (see Appendix 1 for more details about this calculation). In addition, 15% of the thin descending thin limbs of the LOH are permeable to water (those that enter the inner medulla). This results in the re-absorption of another 3 l of water in the medulla, but this time the source is not the medullary collecting duct (discussed in Appendix 1 as well).

Since 150 mmol of Na⁺ must be re-absorbed from the medullary thick ascending limb of the LOH for each litre of water re-absorbed in the medulla, an additional 750 mmol of Na⁺ must be re-absorbed to abstract water from the medullary collecting duct (5 l x 150 mmol/l) and an additional 450 mmol of Na⁺ must be re-absorbed to abstract the 3 l of water from the thin descending thin limbs of the nephrons with very long loops of Henle. Therefore, 1200 mmol of Na⁺ and Cl⁻ must be reabsorbed daily in the medullary thick ascending limb of the LOH to make these 8 l of water into isotonic saline. Accordingly, the quantity of Na⁺ remaining in the lumen is the quantity that entered (4500 mmol) minus the 1200 mmol of Na⁺ re-absorbed from the medullary thick ascending limb of the LOH after it became isotonic saline is 3300 mmol. Since 27 l/day exited the medulla, the concentration of Na⁺ fell from 150 mmol/l at the end of the proximal tubule to 122 mmol/l (3300 mmol/27 l/day).

**Conclusions:** Approximately 5400 of the 6600 mmol of Na⁺ that will be re-absorbed in the medullary thick ascending limb of the LOH represents Na⁺ that is ‘recycled’. In fact, it is the re-absorption of an additional 1200 mmol of Na⁺ that is directly involved in the extraction of water from water-permeable nephron segments (i.e. for concentrating the urine).

**Implications for antenatal Bartter’s syndrome**

In this disorder, there is a complete absence of NaCl re-absorption in the medullary thick ascending limb of the LOH. Hence the entire amount of Na⁺ delivered to the early DCT depends on
the degree of fall in the GFR and the rise in the avidity for the re-absorption of Na⁺ in the PCT (Table 3).

The delivery of Na⁺ and Cl⁻ to the medullary thick ascending limb of the LOH in patients with Bartter’s syndrome is much less than half of that in the normal subjects (Figure 5). By the way, ‘How much Na⁺ did the patient require to maintain haemodynamic stability?’

**Question 5. How much Na⁺ did the patient require to maintain haemodynamic stability?**

**Physiology principle 4:** Balance must be achieved in steady state. Therefore, if we examine how much NaCl had to be given to keep him in steady state in the first month of life before specific therapy was given, we can have a rough estimate of his degree of renal wasting of Na⁺.

**Return to the bedside:** After looking again at the hospital record, the registrar said that the intake of NaCl in steady state was ~15 mmol/kg (Table 1). This is probably much higher than we would have guessed from the above discussion, but it likely reflects a much higher GFR (his PCreatinine fell from 238 μmol/l to the first value of 90 μmol/l) and less avid proximal re-absorption of Na⁺ when his ‘effective’ arterial blood volume was partially re-expanded.

‘In conclusion, this new understanding of Na⁺ reabsorption in the loop of Henle explains why antenatal Bartter’s syndrome is not a lethal disorder’, said Professor McCance. He concluded by apologizing to the medical team for this long description, but he had to admit that he was very excited about these new insights and that he was ‘carried away with this enthusiasm’. As he glanced around the room, there were far more smiles than frowns, which pleased him.

**Return to the clinical discussion**

Let us return to our patient and see how we may help him and his family through the second issue related to his illness. Hence my next question is, ‘Is there a therapy to diminish the excretion of Na⁺ and Cl⁻ and does it have any important side effects?’

**Question 6. Is there a therapy to diminish the excretion of Na⁺ and Cl⁻ and does it have important side effects?**

**Physiology principle 5:** The nephrology consultant said, ‘The site of feedback between renal tubule and glomerulus begins at the macula densa, which is part of the cortical thick ascending limb of the LOH. The amount of salt reabsorption here “sends a message” to the glomerulus and one component of this message is delivered via prostaglandins. In a normal kidney, low levels of salt reabsorption in the cortical thick ascending limb of the LOH would indicate that not enough blood is being filtered, and this leads to the secretion of prostaglandins, which in turn helps to increase the production of renin to raise the GFR. In patients with antenatal Bartter’s syndrome, however, because of the complete inability to reabsorb Na⁺ and Cl⁻ in this nephron segment, this feedback is short-circuited and there is massive production of prostaglandins, worsening the polyuria and contributing to the renal wasting of Na⁺ and Cl⁻. Indomethacin, which inhibits the synthesis of prostaglandins, therefore decreases the excretion of Na⁺ and Cl⁻ in certain patients with Bartter’s syndrome’.

**Return to the bedside:** The registrar took up the story: ‘We monitored the “effective” arterial blood volume very closely to know how much NaCl to administer to this patient. The dose of
indomethacin was selected based on published recommendations’.

Professor McCance had another question, ‘Is there a simple test that the mother could use at home that provides an “instant readout” for the concentration of Na⁺ or Cl⁻ in the urine each time her infant voids? The mother need only “squeeze” the diaper to obtain a few drops of urine to perform the test! If the urine contains an appreciable quantity of these electrolytes, the dose of indomethacin is too low and this is when more of the drug should be given’. The nephrology consultant was intrigued by this suggestion. She added, ‘While the dose of indomethacin that is effective in most patients with Bartter’s syndrome may be known, I am hesitant to apply this information directly to our newborn, as his ability to eliminate this drug may differ from these other patients’.

The medical team decided to take up this challenge. They asked, ‘May we help to develop this test to improve the dosing of indomethacin for our patient? Professor McCance was said, “Meet me in my laboratory this afternoon and we shall have some fun with this project. In the meantime, I will go to the library to gather some information’.

III. Why was nephrogenic diabetes insipidus present?

With this improved understanding of the critical role of the LOH in facilitating both concentration and dilution of the urine, the team was eager to discuss another challenging aspect of the patient’s course. ‘After the initial problems with low “effective” arterial blood volume were behind us and the diagnosis of Bartter’s syndrome was established, we gave the patient indomethacin’, the registrar said. There was another surprise, however, when we reviewed his biochemistries. ‘It suddenly looked like we’ve had the wrong diagnosis after all’, and she showed the results to her mentor (Table 3). ‘Clearly, this looks more like a case of nephrogenic diabetes insipidus!’ Professor McCance asked, ‘What are the diagnostic criteria for nephrogenic diabetes in this patient?’

Question 7. What are the diagnostic criteria for nephrogenic diabetes insipidus in this patient?

Physiology principle 6: Diabetes insipidus is defined as the inability to concentrate the urine (urine osmolality below plasma osmolality) when the plasma osmolality is raised. It is called

![Diagram of the renal outer medulla and the LOH with its thin and thick limbs.](https://academic.oup.com/qjmed/article-abstract/101/12/927/1564087/10.1093/qjmed/hcz179)
‘nephrogenic’, when urine osmolality cannot be increased after administration of vasopressin or a vasopressin analogue.

Return to the bedside: Newborn infants have a unique physiology in that they have an inability to insert aquaporin 2 (AQP2) water channels into the late distal nephron even when the hormone responsible for this effect, vasopressin [antidiuretic hormone (ADH)] is present in abundant amounts (this is a true or AQP2-deficient form of temporary nephrogenic diabetes insipidus of the newborn).21–23 Notwithstanding, newborn infants are not in danger of an enormous water deficit, as they have an equally large intake of electrolyte-free milk (the concentration of Na⁺ in milk is usually 10–15 mmol/l). The diagnosis of nephrogenic diabetes insipidus is clearly established, as he was unable to decrease his urine flow rate or raise his urine osmolality (U_{osm}) following the administration of a vasopressin analogue (dDAVP) (Table 2). The registrar added an additional fact. ‘The results of the molecular studies with respect to nephrogenic diabetes insipidus revealed that there were no mutations detected by direct sequencing of the genes encoding the V₂-vasopressin receptor (AVPR2) and aquaporin 2 (AQP2),’ she said.

Our premature infant also has an additional burden, the daily excretion of a large quantity of Na⁺ and Cl⁻ in the urine. Therefore you will need to monitor the daily input of water and salt as well as the urine volume and its concentration of Na⁺ and Cl⁻ very closely to keep this patient alive. Professor McCance asked, ‘What will limit the excretion of water in the urine in normal newborns when vasopressin fails to act?’

Question 8. What will limit the excretion of water in the urine in normal newborns when vasopressin fails to act?

Physiology principle 7: The limit for the excretion of water when vasopressin fails to act is the distal delivery of filtrate. This in turn is equal to the GFR minus the re-absorption of filtrate in the water-permeable upstream nephron site (i.e. the PCT). This proximal re-absorption is enhanced when the ‘effective’ arterial blood volume is decreased.

Return to the bedside: There is a lower GFR in the newborn.21,22 The lower ‘effective’ arterial blood volume leads to a diminished distal delivery of filtrate. Moreover, the newborn has additional water permeability in the inner medullary collecting duct that is independent of actions of vasopressin (called basal water permeability),24,25 which also limits the water diuresis in this population.

The fact that there was no response to dDAVP could mean that the newborn fails to make either the V₂-receptor or AQP2. This developmental delay in the vasopressin response system was noted in a variety of animals, even though both the V₂ receptor and AQP2 were present (reviewed in reference 26). ‘If this developmental delay occurs in so many mammalian species, what advantage might it have for their survival?’ Professor McCance asked.

Question 9. If this developmental delay occurs in so many mammalian species, what advantage might it have for survival?

Physiology principle 8: The major strategy for survival in the newborn is to regenerate ATP as quickly as it is used in its brain. Glucose is the main (almost exclusive) fuel for its brain. Therefore, hypoglycaemia poses a threat, as the metabolic requirements of the brain are large, the availability of alternate fuels (cyclic ketoacids) is low, and the size of storage pools of glucose is limited. Therefore, the newborn needs a frequent supply of sugar from mother’s milk to avoid neuroglycopenia.27

Return to the bedside: Once the sugar from milk is oxidized, the newborn will be faced with a large water load. While some of this water is used for evaporative heat dissipation, a large volume of water must still be excreted in the urine. Therefore, a neonatal renal concentrating system that does ‘not’ respond to vasopressin could provide several advantages. First, if the infant had a non-osmotic stimulus for the release of vasopressin (e.g. nausea, pain, distress), acute hyponatraemia would not develop. Second, perhaps this renal non-responsiveness to vasopressin helps minimize the risk of developing hypoglycaemia. Since the source of milk sugar is the mother, the excretion of a large volume of dilute urine could lead to both thirst and a ‘wet diaper’—the net effect could produce signals for early arousal and a ‘call’ for a source of sugar.27 Safety is ‘built into the system’ because the residual (basal) water permeability could limit the magnitude of this water diuresis by permitting a fall in the urine flow rate when the effects of a low extra-cellular fluid volume are superimposed on the low GFR of the newborn. Thus it could account for the high U_{osm} once enough water was lost, making this a safer signal system.28 Accordingly, if there were a failure to drink milk, the newborn is not at extreme risk of having sufficient brain volume shrinkage and thereby a risk of an intracranial haemorrhage because of the much lower distal delivery of filtrate.
as soon as the ‘effective’ arterial blood volume declines somewhat.

The team thanked Professor McCance for this breadth of interpretation. Nevertheless, the consultant wanted one more point clarified. ‘I now understand the temporary nephrogenic diabetes insipidus of neonates, but this boy, who is now 2 years old still has urine osmolalities consistently below serum, typically around 150 mOsm/kg H2O. We repeated the dDAVP test at 18 months of age and he still showed no appreciable response! Moreover, considering the critical role of the thick ascending loop of Henle in urinary dilution, I do not understand how a patient with a genetic knock-out of this segment is able to dilute his urine to well below 100 mOsm/kg H2O.’

Prof McCance addressed the issue of urinary dilution first, but he would comment on the persistence of the AQP2 deficiency type of nephrogenic diabetes insipidus only after he had additional information. ‘Clearly’, he said, ‘the patient achieved these urine osmolalities only when taking indomethacin. Since urine flow under indomethacin was essentially unchanged, the mechanism must have been enhanced reabsorption of NaCl!’ He proceeded to ask the group, ‘Which nephron segment is responsible for the increased ability to reabsorb Na+ and Cl− when indomethacin acts?’

**Question 10. Which nephron segment is responsible for the increased ability to reabsorb Na+ and Cl− when indomethacin acts?**

**Physiology principle 9:** When Na+ is re-absorbed in a nephron segment that is permeable to water, Na+ and water will be absorbed as an iso-osmotic solution. Accordingly, less water will be delivered downstream from that site. Therefore, let us return to the urine flow rate after indomethacin caused the marked decline in the rate of excretion of Na+ and Cl−.

**Return to the bedside:** The registrar asked if she could address this issue. Seeing the nod of assent from Professor McCance, she continued. She stated, ‘Since there was no decline in the flow rate when indomethacin acted (Table 3), I believe that we have ruled out the PCT as the major site of action of this drug. Just to be sure, I measured the concentration of creatinine in the urine before and after the administration of indomethacin. Both of these urines had similar concentrations of creatinine (i.e. 700 and 900 μmol/l), which I interpreted to go along with the failure to lower the urine output when the rate of excretion of Na+ and Cl− fell so markedly. There was one additional fact, which was not a surprise. When the patient was given indomethacin, there was a fall in the Pcreatine from 47 to 39 μmol/l; this is consistent with a positive balance of Na+ and Cl−, with re-expansion of his ECF volume. As discussed above, his molecular lesion should lead to a complete absence of the re-absorption of Na+ and Cl− in the loop of Henle. Therefore, the site of stimulation of Na+ and Cl− re-absorption when indomethacin was given should be in the distal nephron.’

Professor McCance agreed and stated that this had several implications. First, downstream nephron sites have the capacity to re-absorb much more Na+ and Cl− than he would have thought from data in intact animals. One reason for this is that intact animals do not have such a large delivery of Na+ and Cl− in a setting where there is a strong stimulus for the re-absorption of these electrolytes. A second reason is that the vast majority of experimental evidence was derived from the rat, which consumes as much Na+ and K+ each day as they have in their ‘entire’ ECF volume, so they should have a chronically expanded ECF volume to have a stimulus to excrete a huge amount of Na+.8

As to mechanism, if indomethacin leads to a decreased rate of synthesis of prostaglandins, it is possible that at least one member of this class of compounds acts as an inhibitor of the re-absorption of Na+ and Cl− in the distal nephron while the ‘effective’ arterial blood volume is very ‘contracted’. Indeed, in vitro experiments in a distal tubular cell line show that the Na+-K+-ATPase is inhibited by prostaglandin E2, an effect that can be suppressed by indomethacin.17 The nephrology consultant added another comment. ‘Consistent with this impression is the fact that non-steroidal anti-inflammatory drugs may cause certain patients to retain an excessive amount of dietary NaCl’.29

Professor McCance thanked the consultant for this interesting comment and summed up the findings to the potential site and mode of action of indomethacin. ‘Your detailed clinical data show an unchanged GFR and a decrease in the excretion of Na+, but not in water when indomethacin acts. These data suggest that indomethacin enhances tubular re-absorption of Na+ in patients with Bartter’s syndrome and the tubular site of action must have been a segment impermeable to water, otherwise Na+ and water handling could not have been affected independently. Since the proximal tubule is freely permeable to water and the thick ascending limb of the loop of Henle was affected by his molecular defect, the site of action must have been the late distal nephron’.

The registrar asked, ‘Could we test this further by administering drugs, that specifically inhibit
Na⁺ transport in the potential segments, such as a thiazide or amiloride? Professor McCance commended her on her aim to further define the site of action of this drug. Nevertheless, ‘I would strongly advise against this, as your patient already has a critical impairment of Na⁺ transport in one segment. Adding another one could be very dangerous’.

The nephrology consultant was still eager to understand the persistence of the nephrogenic diabetes insipidus in the patient well beyond the neonatal period. This is actually not an unusual observation in antenatal Bartter’s syndrome; some of these patients have persistent hypostenuria rather than isostenuria. She had found other reports in the literature, where the diagnosis of Bartter’s syndrome was first missed and rather a diagnosis of nephrogenic diabetes insipidus was proposed. Therefore Professor McCance asked: ‘What non-genetic factors could cause nephrogenic diabetes insipidus in Bartter’s syndrome?’

**Physiology principle 10:** The most common cause of non-genetic AQP2 deficiency type of nephrogenic diabetes insipidus is lithium, but this is not applicable here, as neither the patient nor his mother took lithium. Temporary nephrogenic diabetes insipidus can be observed with obstructive uropathy (especially after release of obstruction), hypokalaemia and hypercalcaemia (Table 4).³⁰

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**Table 4 Physiological Principles**

<table>
<thead>
<tr>
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<th>Physiological Principles</th>
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<tbody>
<tr>
<td>1</td>
<td>Bartter’s syndrome is an inherited defect of Na⁺ and Cl⁻ reabsorption in the thick ascending LOH</td>
</tr>
<tr>
<td>2</td>
<td>The mTAL is said to reabsorb nearly 1/3 of filtered sodium, or about 8000 mmol/day in a 50-kg adult. A new discovery (lack of AQP1 in the DTL) has altered this impression.</td>
</tr>
<tr>
<td>3</td>
<td>The tubulo-glomerular feedback is short-circuited in Bartter’s syndrome by the inability to reabsorb Na⁺ and Cl⁻, resulting in excessive prostaglandin production; this causes increased polyuria &amp; NaCl wasting.</td>
</tr>
<tr>
<td>4</td>
<td>Balance must be achieved in steady state</td>
</tr>
<tr>
<td>5</td>
<td>Micropuncture and assessment of inulin concentrations in tubular fluid (TF) versus plasma (P) have shed light on the fraction of filtrate reabsorbed at different nephron sites.</td>
</tr>
<tr>
<td>6</td>
<td>The newborn has temporary nephrogenic diabetes insipidus, with an inability to insert AQP2 into the luminal membranes of the late distal nephron, even in the presence of vasopressin.</td>
</tr>
<tr>
<td>7</td>
<td>The excretion of water when vasopressin fails to act is determined by the distal delivery of filtrate and the volume of water reabsorbed by residual water permeability in the inner medullary collecting duct.</td>
</tr>
<tr>
<td>8</td>
<td>Provision of a constant supply of fuel to regenerate ATP for the brain is the major concern for the newborn</td>
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<tr>
<td>9</td>
<td>When Na⁺ is reabsorbed in segments that are permeable to water, Na⁺ and water will be absorbed iso-osmotically</td>
</tr>
<tr>
<td>10</td>
<td>The most common non-genetic cause of nephrogenic diabetes insipidus is lithium therapy.</td>
</tr>
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</table>

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8000 mmol is many-fold > the entire content of Na⁺ in the ECF compartment. Hence a severe lesion of mTAL should be but it is not lethal. In fact, much less Na⁺ is delivered to the loop of Henle.

Indomethacin decreases prostaglandin synthesis and lessens Na⁺ and Cl⁻ losses in the urine in some patients. Its site of action cannot be in the loop of Henle due to the molecular lesion.

In this case it allows the degree of salt-wasting to be inferred from the amount of NaCl needed to maintain the steady state.

There is little danger of a large water deficit, as milk is a source of electrolyte-free water and the water diuresis stops when the ‘effective’ arterial blood volume declines somewhat.

Loses are limited by a lower ‘effective’ arterial volume causing a decreased GFR and enhancing upstream reabsorption in the PCT. In addition, there is water permeability in the inner medullary collecting duct that is independent of the actions of vasopressin.

Glucose is the main fuel and the size of the storage pool is small compared to demand, so the infant must have a frequent supply of mother’s milk to avoid neuroglycopenia. This will cause a reduction in urine volume, as less water will be delivered downstream from that site.

Temporary nephrogenic diabetes insipidus can be observed with obstructive uropathy, especially after release of obstruction.
Return to the bedside: ‘I have my doubts that hypokalaemia or hypercalcaemia causes nephrogenic diabetes insipidus— I think the problem is with the definition of this disorders. I think there are two separate types of lesion in this diagnostic category, and this ‘muddies the water’. I shall now present what I have learned’, said McCance.

(i) AQP2 deficiency type of nephrogenic diabetes insipidus: ‘I prefer to use the term AQP2 deficiency type of nephrogenic diabetes insipidus to describe only the group of disorders where the late distal nephron is unable to synthesize and/or insert active AQP2 water channels into the luminal membranes of principal cells’. Its hallmark is a failure of either vasopressin or dDAVP to cause an appreciable fall in the urine flow rate and a rise in the $U_{\text{osm}}$ to values greater than the $P_{\text{osm}}$. There are two types of lesion, mutations in the gene encoding the V2-receptor (X-linked) or that for AQP2 (autosomal recessive); second, acquired disorders—the commonest is the administration of lithium to treat a bipolar affective disorder.

(ii) Non-AQP2 deficiency type of nephrogenic diabetes insipidus: In this subgroup, the late cortical distal nephron and the medullary-collecting duct are permeable to water when vasopressin acts. Accordingly, the $U_{\text{osm}}$ is ‘not’ appreciably higher than the $P_{\text{osm}}$ because the primary disorder is a failure to raise the medullary interstitial osmolality. As a result, the daily osmole load must be excreted in a larger volume of urine as compared to settings with a higher $U_{\text{osm}}$. The urine volume in these patients is determined by the rate of excretion of ‘effective’ osmoles and the current medullary interstitial osmolality. Typically, an adult patient will have a $U_{\text{osm}}$ close to 300 mOsm/kg H2O and the volume of urine will be close to 3 l/day when the diet results in the excretion of 900 mOsmol/day. Because of thirst and the desire to prevent the development of thirst, patients in this category will not become hypernatraemic. Since your patient has a persistently low $U_{\text{osm}}$, this implies that he has an AQP2 deficiency type of nephrogenic diabetes insipidus.

Patients with obstructive uropathy: These patients do develop an acquired form of nephrogenic diabetes insipidus after relief of the obstruction. Moreover, patients with Bartter’s syndrome also have a high urine output, which may lead to increased flow and pressure in the collecting system. If I were to try and link these two observations, perhaps a persistent high urine flow rate and/or pressure, which is common to both of these settings, could be responsible for the acquired form of nephrogenic diabetes insipidus. There are other examples of having a high distal flow rate leading to a change in altered channel availability in the lumen of these nephron segments. For example, there is a flow activated maxi-K channel present in a high flow state. The signal system for this is related to a raised intra-cellular ionized calcium concentration, and it appears that the microcilia on these cells detect the flow rate. Of interest, there is also a delay in the expression of these maxi-K channels in the newborn, much like the nephrogenic diabetes insipidus. While this is only speculation, it is intriguing. There is also a flow-activated Na+ reabsorption, which increases Na+ influx through ENaC—this will lower the concentration of Na+ during a water diuresis. Therefore, I would guess for the moment that a failure to lower the flow rate in the late distal nephron may lead to a persistence of this nephrogenic diabetes insipidus.

IV. Results of the test to determine Na+ and/or Cl− in the urine

For the test to be valuable for the parents of the patient and not require expensive equipment, the outcome measure would have to be visible. This means that the product should make a colour change, form a precipitate or bubbles that are easy to see.

Search of the literature

There were no easy ways to analyse the urine for Na+ without special equipment; hence I placed my emphasis on detecting Cl−. The obvious choice was precipitation of Cl− with silver ions because silver chloride (AgCl) is so sparingly soluble in water. In addition, when exposed to light, the precipitate turns black, like in photographic paper. Finally another silver salt, silver nitrate (AgNO3) is very soluble in water.

Technique

The test was carried out in a clear plastic pipette tip with a very small, elongated end that was sealed shut by heat. We added 100 μl of AgNO3 to the tube and then added 50 μl of a series of NaCl standard solutions (0–50 mmol/l). We confirmed the concentrations of Na+ and Cl− in the urine using a flame photometer for Na+ and an electromimetic technique for Cl− as previously described. Our goal was to detect when the concentration of Cl− in the urine was ~15 mmol/l, as this would be an early warning to the mother that the dose of indomethacin might be too low. The sensitivity of the assay was surprisingly good, as we were able to easily recognize when the Cl− concentration in the urine first rose to 15 mmol/l (Figure 6). Further sensitivity could be achieved by diluting the urine.
The registrar asked if she could summarize what they had learnt from the very interesting discussion of this case. First, we were reminded of the molecular basis of Bartter’s syndrome, which is a genetic impairment of NaCl re-absorption in the thick ascending limb of LOH. Further, we discussed, how the re-absorption of Na⁺ without water in this segment generates the high interstitial osmolality for urinary concentration. We were surprised to find out that the majority of thin descending limbs of the LOH are water-impermeable. This means that entry of Na⁺ and Cl⁻ into the lumen of the descending thin limb of the LOH is critical for this process. In light of these data, we now believe that a large part of salt and water extraction previously attributed to the LOH must actually occur in the distal parts of the proximal tubule and that therefore the total contribution of the LOH to tubular salt re-absorption is only about a quarter of what was previously thought, or <5% of the filtered load of Na⁺.

After this detailed review of the physiology of the LOH, we then considered the effect of indomethacin on our patient. We noted that indomethacin did not affect GFR or urine flow, but decreased the excretion of Na⁺ and Cl⁻. We therefore concluded that indomethacin must act in a water-impermeable segment, which excluded the PCT. We also used this effect of indomethacin on NaCl excretion to develop a simple bedside test to detect Cl⁻ in the urine that could be used to adjust the frequency and dosage of indomethacin administration to minimize its side effects. Lastly, we discussed the perplexing co-existence of nephrogenic diabetes insipidus in some patients with Bartter’s syndrome and speculated whether it might be related to the large urine flow that could create increased pressures in the collecting duct. Most of all, we learnt that what we were taught in medical school was the best understanding of the physiology at that time and that we must be prepared to discard what look like ‘certainties’ when new data become available. Then the whole team thanked Professor McCance for this thought-provoking and detailed linking of clinical data with the underlying physiology.

Conflict of interest: None declared.

References


**Appendix: Calculations**

**A more detailed commentary on the micropuncture data in the rat**

Prior to the discovery of the fact that the majority of descending thin limbs of the LOH lack AQP1, the belief was that 60 l of filtrate were delivered to the LOH, and that this nephron segment was a major site of re-absorption of water. In addition, 40 of these litres would be re-absorbed in the descending thin limbs of the LOH because water moved to osmotic equilibrium and that the interstitial osmolality rose ~3-fold in the outer medulla of the human. Based on this interpretation, the distal delivery of filtrate should be 20 l/day, rather than the 27 l/day as judged from the micropuncture data. In fact the inaccuracy of these data were even greater in the rat, as the osmolality rose by 7-fold as one proceeded deeper in the outer medulla of the rat, the species that provided these data.

**Water re-absorption in the descending thin limbs of the LOH that do have AQP1**

Only those nephrons with descending thin limbs of the LOH that descend into the inner medulla have aqp1; they account for 15% of the total (i.e. a GFR of 15% of 180 l/day or 27 l/day). If 5/6 of this volume reaches the LOH (i.e. 4.5 l/day) and ~2/3 are re-absorbed, there will be a re-absorption of close to 3 l/day.

**Water re-absorption in the medullary collecting ducts**

This is a rather detailed calculation, and one with many assumptions. The volume delivered to the medullary collecting ducts is equal to the number of millimoles of urea that are in the DCT (recycled urea (~900 mmol/day, see reference 2 for more details) + 1/2 of the filtered urea = 1350 mmol/day) plus an estimated 450 mosmol of electrolytes per day, yielding a total of 1800–mosmol/day. Dividing this number by the plasma osmolality equals close to 6 l/day. If one were to subtract the usual urine volume when concentrated urine is being excreted, the net result is a re-absorbed volume of close 5 l/day.