The paradoxical urinary concentrating mechanism

Charles Soper

Mammals and some birds concentrate urine (and thus conserve water) by a compact mechanism composed of several necessary and interdependent properties. It is an excellent example of 'irreducible complexity', a system which fails if only one component is removed. Its genesis poses a serious problem for gradualists. This is well illustrated by the 8-year resistance to adopting the current model of urine concentration by the leading renal physiologist of the time. He was a celebrated evolutionist, and opposed the model chiefly on the grounds that it violated gradualist principles.

Our current understanding of how the kidney concentrates urine is founded on the countercurrent hypothesis proposed by Hargitay, Kuhn and Wirz in 1951.[1] However, the hypothesis was by no means readily accepted at first. On the contrary, the great renal physiologist Homer Smith, was opposed to the idea until eight years later, when, in the face of accumulating evidence, he conceded defeat. Darwinian evolution was of special interest to him, and he believed it to be foundational to explaining renal function.[2] As he recounts, it was his adherence to strict gradualism which led to his considerable resistance to the new theory.[3] Curiously, an examination of the evolution of renal function, marking the centenary of Homer Smith's birthday, bypasses this.[4]

Darwin's challenge

Darwin's theory of evolution requires each modification of structure or function to be slight, and for each change to be justified by an advantage for survival. He adhered strictly to Carolus Linnaeus's maxim *'Natura non facit saltum'* (nature doesn't make leaps).

> 'If it could be demonstrated that any complex organ existed, which could not possibly have been formed by numerous, successive, slight modifications, my theory would absolutely break down.'[5]

He carefully qualifies this statement with three conditions under which relatively abrupt modifications might be observed. These are: firstly, the specialization of an organ possessing two functions into one function only (citing Hydra's ability to respire and digest from the same surface); second, the modification of one of two organs both performing an identical function to a separate function (e.g. the simultaneous respiration of oxygen from water via the gills, or from air via the swimbladder, the latter putative converting to primitive lungs); and finally, the acceleration or retardation of the period of sexual reproduction in relation to ordinary maturation. Richard Dawkins restates this basic claim as the holy grail of Neo-Darwinian orthodoxy.[6] On this basis of gradual steps, he even aspires to account for the evolution of the eye.

The countercurrent* concentrating mechanism

Reptiles and amphibians are able to excrete nitrogen-based waste products via their kidneys, but are unable to concentrate urine. Concentration is the unique property of mammals and some birds by virtue of an extraordinary concentrating system. Its mechanism is counterintuitive and complex. Before examining its simplified essence, we review a more familiar, related device, the countercurrent exchanger. Consider, for example, the system of heat exchange in an arm or a leg on an icy day (fig. 1). Blood coursing from the heart into the arteries is at core temperature, but as it passes down the arm, it cools rapidly. By the time it reaches a gloveless hand, it may reach temperatures similar to the environment. As the blood passes back through the veins, it warms again rapidly, and by the time of its arrival at the shoulder, while still less than core temperature, it is much warmer than the air around. This conservation of valuable core heat is facilitated by an intimate relationship between the arteries and the vein network. Heat is exchanged from the arteries (leaving the heart) to the veins (as they return). The result is a sharp gradient in temperature down the arm. There is a hairpin loop, with flow running into, and out of it, and an exchange of energy between its two limbs. In this situation, all the transfer is passive, or 'downhill'.

Countercurrent exchangers* of a different kind form a vital part of the kidney's concentrating mechanism, but its driving force is a countercurrent concentrator* (originally, but less helpfully, described as a multiplier). Unlike an exchanger, which *preserves* an existing gradient by passive transport, the concentrator *generates* a gradient by active transport. The transport that concerns us in the kidney is not of heat, but of salt and water. The lining of the tubules of the kidneys is equipped with a remarkably varied array of ion pumps and channels, each with a specific function and location, some of which are still being discovered and defined.[7] The arrangement of these pumps and channels is complex, as are their interdependent functions, but to understand the countercurrent model, it is only necessary

* Items with an asterisk are defined in the glossary at the end of this article.

to grasp some fundamental principles. The transport characteristics are set out in figure 2. The descending limb of the loop is permeable to water and salt, which for our purposes means the electrolytes sodium and chloride. The lining of the ascending limb is largely impermeable to salt and water. However, it possesses a system of pumps which result in the active removal of salt from the tubule.

It is difficult at first to see how active salt transport out of an impermeable tube should lead ultimately to a higher concentration gradient. After all, what takes place within the lumen of the ascending tube is dilution, which is why this part of the nephron* is often called the diluting segment. However, the looped arrangement enables salt pumped from the ascending limb to pass into the permeable descending limb, which leads to an incremental increase in salt gradient as the fluid in the loop reaches the tip. To help picture this mentally, consider a loop with these characteristics filled with, and surrounded by, saline at a particular concentration. As the fluid is driven through the loop, the gradients slowly change, first in the ascending limb in response to the pumps and then in the descending limb in response to local increases in salt concentration. This series of events is illustrated in figure 3. In life, the two microscopic limbs of the loop are long and intimately intertwined; therefore the length of the axis of the loop is vastly greater than distance between its two limbs.

The driving force for the concentrator, or 'single effect' as the original paper describes it, is the energetic pumping of sodium and chloride from the ascending limb of the loop. In the figure, a hypothetical maximum gradient of 200 mmol/l is generated between the lumen of the loop and the surrounding fluid. As filtrate runs through the loop, the first event is a progressing dilution of fluid as it rises up the ascending limb. Progressively, salt pumped from the ascending limb accumulates in fluid around it and then by passive diffusion in the descending limb. Salt is passively concentrated in the fluid descending in the loop. Then as it flows past the hairpin bend, it, too, is progressively diluted inside the loop by the salt pumps in the ascending limb. This accumulation of salt in the interstitium* and in the descending limb gives rise to an axial salt gradient from the base to the tip of the loop. Eventually, as salt diffusion dissipating this gradient matches the pumping mechanism which generates it, a steady state is reached.

The loop is also coupled with the final pathway of urine (the collecting duct*) before it is excreted (fig. 4). By varying the water permeability of the wall of the collecting ducts, fluid running inside it, up the concentration gradient generated by the loop, can be concentrated. This water permeability is controlled by the action of a hormone called vasopressin (VP). If VP is present, permeability is switched on and water is drawn out of the duct by the concentration gradient generated by the adjacent loop. If VP is absent, permeability is not activated, water remains in the duct and dilute urine is excreted.

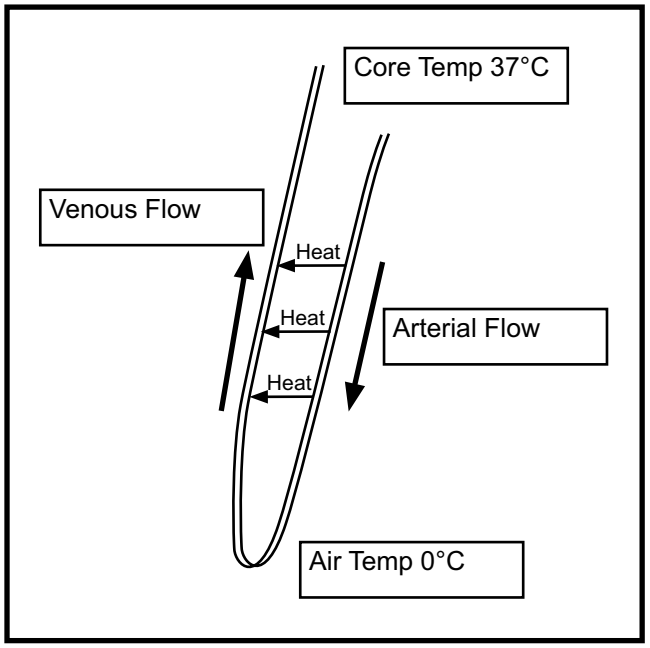

Figure 1. *Countercurrent exchanger. Passive heat flow in an arm or a leg preserves core temperature and a sharp temperature gradient from core to periphery.*

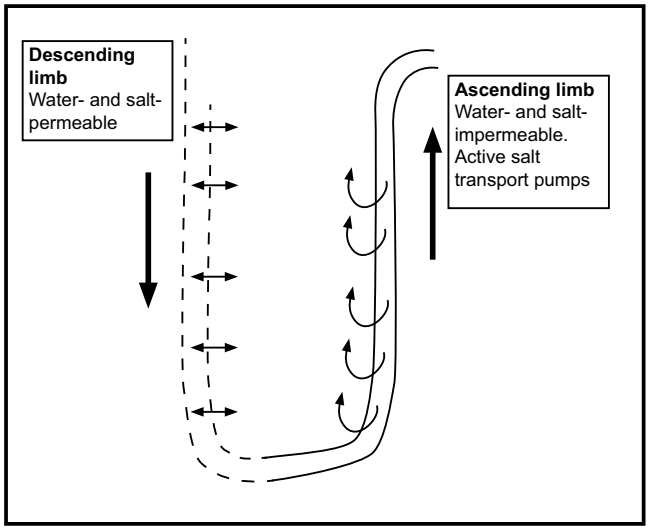

Figure 2. *Countercurrent concentrator, showing transport and permeability characteristics*

An obstacle for gradualism

It seems impossible to account for the urinary concentrating mechanism by 'numerous, successive, slight modifications', even after taking each of Darwin's qualifications into account. Urine concentration requires the simultaneous presence of several contrasting properties in different parts of the nephron loop. Can anything other than a large and precise leap be conceived to account for its existence? Four major contrasting properties, each essential to any utility of the whole, are evident: its biologically

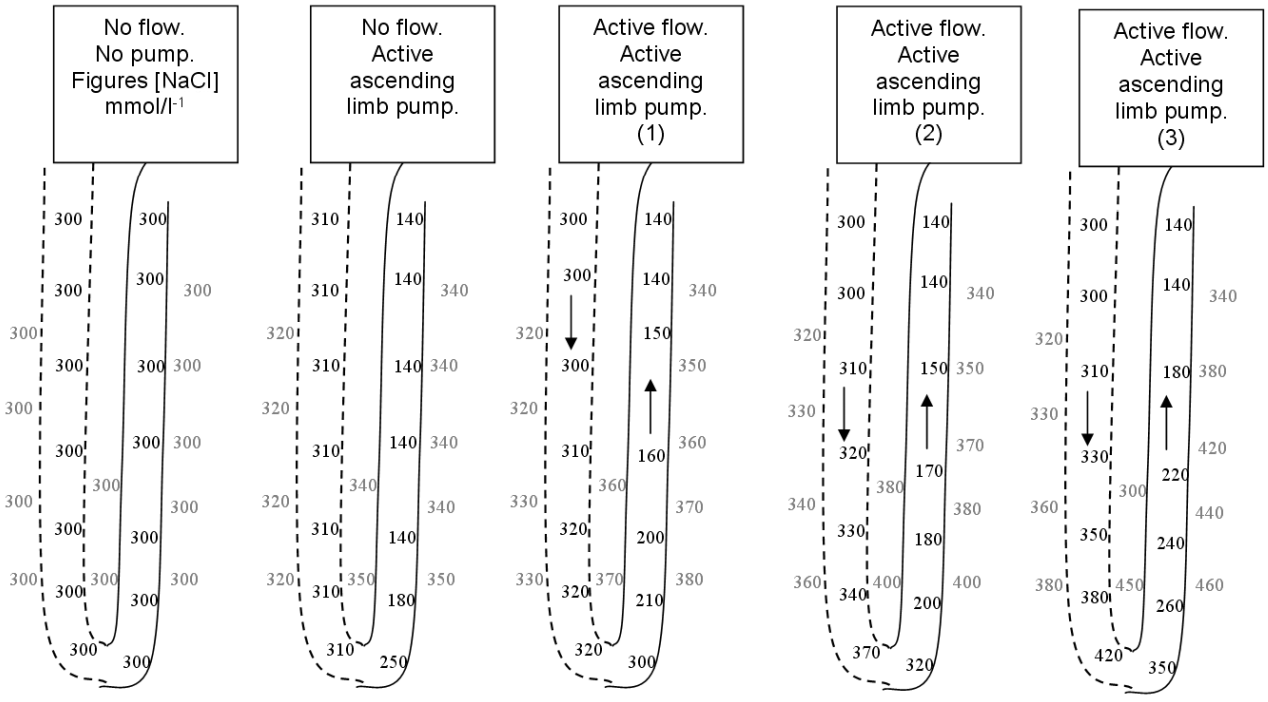

Figure 3. *Progressing axial concentration gradient with countercurrent flow and active transport*

eccentric hairpin loop structure, a salt- and water-permeable descending limb, a salt- and water-impermeable ascending limb, combined with 'uphill' active salt pumping, which is confined to the ascending limb.

How could a structure derived from straight reptilian nephrons gradually progress towards a long, hairpin-looped configuration, after a small-stepped Darwinian manner, unless there was an adaptive advantage in doing so? What use could this be if not to concentrate urine? Could urine even begin to be concentrated until this process had progressed to very near similarity of shape to a mammalian nephron? How could the descending and ascending limbs progressively acquire contrasting water permeability characteristics, despite the fact that such properties would be of no adaptive advantage until an axial concentration gradient had been established? What selection benefit is there if the ascending limb of the loop, as distinct from other portions of the nephron, progressively accumulated considerable potential for ionic transport until all the rest of the concentrating mechanism was in place? If the descending limb also shared this marked active ionic transport, then the necessity for a clear distinction between the two for both water and sodium permeability is only heightened. However, multiple nephron loops with all the other necessary properties but insubstantial active salt transport in the ascending limb would be completely futile for urine concentration. Nephrons with little difference in water permeability between the two limbs, despite every other necessary property, would again serve no purpose, particularly to the loop, other than to dissipate energy and thereby become a liability. A nephron of reptilian configuration with all the appropriate transport and characteristics, both active and passive, would achieve nothing other than generate valueless, transient ion fluxes, at the cost of its possessor.

The real difficulty is that none of these quite different and necessary properties appear to confer any distinguishing selective value unless all are found together simultaneously, and found to be substantially present; substantially enough, that is, to begin to subserve the concentration of urine, thus providing a selection advantage to its possessor. A slight tendency towards the demonstration of any, or all, of these properties by a reptilian nephron will not generate any axial gradient, until a discrete state of quite advanced similarity in all four aspects to the mammalian nephron is attained. If one aspect lacks, urine concentration will utterly fail.

Such a commitment to gradualism undergirded Homer Smith's considerable reluctance to adopt Kuhn and Hargitay's model. As he puts it:

> 'I still do not like it: it seems extravagant and physiologically complicated—though so is the whole glomerular filtration-tubular reabsorption pattern ... . Least of all however, do I like to see the squamous epithelium of the thin segment freely permeable to water (if not to sodium also) in the descending limb, only to acquire water impermeability and active sodium transport at the tip of the loop for no better reason, apparently, than

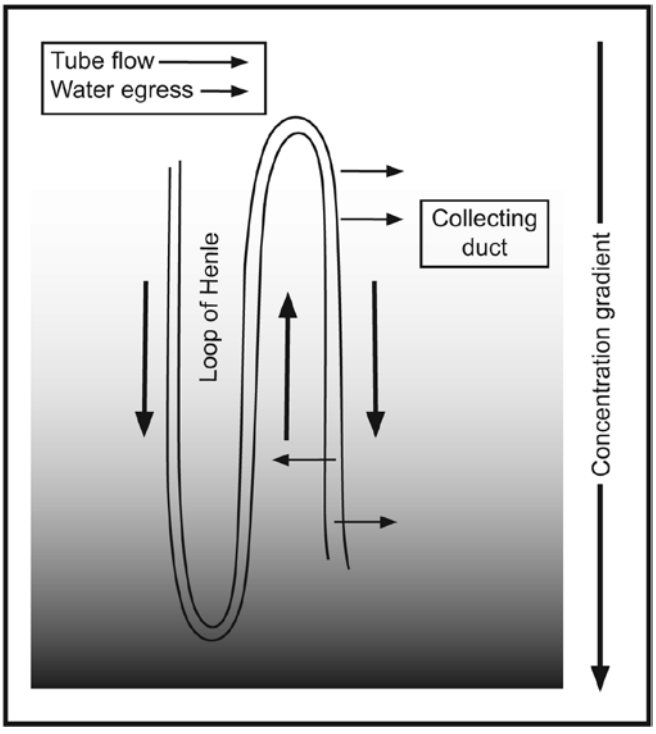

Figure 4. *The coupling of the collecting duct with the nephron loop enables removal of water from urine before excretion. The concentration gradient generated by the loop is denoted by shading. Water permeability in the collecting duct is under the control of the hormone vasopressin (VP).*

the circumstance that it has turned a corner.'[2]

This comment begs the question, is evolution such a valuable key to understanding nature, as we so often hear, or has it become a blinker, blinding even the brightest of minds from perceiving the intricacies of the Designer's handiwork? Has it become a presupposition to be defended in spite of the evidence?

'Has it [evolution] become a presupposition to be defended in spite of the evidence?'

Nor do these four principle characteristics constitute the only foundation of the mechanism. The coupling of the loop with the collecting duct is also essential to concentrating urine prior to its excretion, with its variable water permeability under the control of VP. Without this control mechanism, urine concentration would lack regulation, water balance regulation would become impossible, and the device would become a dangerous liability. Similarly, maintenance of the concentration gradient in the loop requires that the blood supply matches and follows the course of the loop exactly. The capillary network around the loop in this way acts as a countercurrent exchanger, similar to the arrangements of the blood supply in the arm for preserving core heat. This enables the capillary contents to match the osmolarity of the loop, in some desert rodents reaching levels of up to 35 times plasma levels. These arrangements in some species realize remarkable intricacy.[8,9] These blood vessel exchangers must also be sufficiently configured to allow for reasonable efficacy, right from the outset. Otherwise, any axial gradient would immediately disperse by downhill transport from isosmotic* blood.[10]

Gradualistic counterexamples examined

To defend the possibility that the looped nephron might have evolved gradually from mammals, two examples are sometimes cited. The first is the looped tubules found in the kidneys of two species of lamprey, *Lampetra fluviatilis* and *Petromyzon marinus*,[11,12] which have been claimed as evidence of a vertebrate antecedent for the loop of Henle. The claim is dubious. Briefly, micropuncture studies in the former showed no change in electrolyte concentration in the ascending limb of the loop, and although tubular fluid osmolarity falls by 13%, this appears mainly due to non-electrolytic osmolar transport,[13] more characteristic of an earlier portion of the nephron than the loop of Henle*. The ascending limb, in contrast to its descending partner, reabsorbs water, which destroys the possibility of generating a concentration gradient.[13] The length of the loop, at 1.1 mm seems too short compared even to simple avian nephrons[14] and the renal perfusion rate too slow to enable countercurrent concentration.[15] Therefore, these loops, and other looping structures akin to them, such as those found in the dogfish, *Triakis scyllia*, do not serve as a useful functional paradigm for Henle's loop,[16] and are not observed widely in kinds closer to birds and mammals.

The second example is the smooth transition of forms between the reptilian (straight) and mammalian (looped) nephrons found in the kidney of Gambel's quail, *Lophortyx gambii*.[14] This might be used to indicate that 'however the avian nephron did attain an advanced state, it most likely did so by small, discrete alterations'. Yet even its modest concentrating ability, at 2 to 3 times plasma osmolarity, is dependent not on the transitional nephrons, but on the longest-looped 'mammalian' nephrons (still short by mammalian standards). The situation has an analogy in mammals, in which nephron length varies considerably in the same kidney. Short-looped nephrons depend on, and augment, the concentrating work of longer-looped nephrons.[8] Without denying a contribution from intermediate 'reptile/mammal' nephrons in the quail, their small assistance is wholly dependent on a pre-existent osmotic* gradient, generated and maintained by the longer, 'more-advanced' nephrons. A kidney entirely composed of intermediate nephrons of an attainable kind would not concentrate, despite considerable energy expenditure. It is therefore no basis upon which to assert the gradual modification of structure, when adaptive utility to the whole organ, or rather whole creature, is obligated for every new investment. Evolutionary gradualism appears far too thrifty for this. It

is too short-sighted a workman to justify its reputation as a 'watchmaker', a visionary engineer capable of crafting improbable marvels.

Conclusion

Can any distinctive purpose for which Henle's loop exists be proposed, other than urinary concentration, which might obviate these difficulties? If not, here is another argument as to why the presuppositions of neo-Darwinism require profound revision.

Acknowledgments

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Glossary

Collecting duct: The final common pathway for filtered fluid before it's excreted as urine.

Countercurrent: A looped system in which two flows run side-by-side in opposite directions as they flow through the loop.

Countercurrent concentrator: A device which generates a solute concentration or energy gradient along the axis of a countercurrent loop, by a combination of loop properties, including active transport in the limb that exits the loop.

Countercurrent exchanger: A device which preserves an existing gradient by passive ('downhill') energy or mass exchange across the two limbs of the loop.

Interstitium: The extracellular tissue and space surrounding the loop.

Isosmotic: An equivalent solute concentration to mammalian plasma (about 280 mOsm).

Loop of Henle: The mammalian nephron loop, named after its first describer.

Nephron: A unit composed of the structures which filter and modify urine. A human kidney contains about one million of them.

Osmotic: The property of a solute concentrate arising from the tendency of solutes to flow down their concentration gradient. Osmosis is capable of generating considerable hydraulic pressure across a semipermeable membrane.

References

1. Hargitay, B. and Kuhn, W., Das Multiplikationsprinzip als Grundlage der Harnkonzentrierung in der Niere, *Zeitschrift fur Elektrochemie* **55**:539–558, 1951.
2. Smith, H.W., *From Fish to Philosopher*, Little, Brown and Co., Boston, MA, 1953.
3. Smith, H.W., The fate of sodium and water in the renal tubules, *Bull. NY Acad. Med.* **35**:293–316, 1959.
4. Natochin, Y.V., Evolutionary aspects of renal function, *Kidney Int*. **49**:1539–1542, 1996.
5. Darwin, C., *On the Origin of Species*, Watts & Co., London, 1859.
6. Dawkins, R., *The Blind Watchmaker*, Penguin, London, p. 91, 1986.
7. Wright, S.H. and Dantzler, W.H., Molecular and cellular physiology of renal organic cation and anion transport, *Physiol. Rev.* **84**:987–1049, 2004.
8. Jamison, R.L., Short and long loop nephrons, *Kidney Int*. **31**:597–605, 1987.
9. Bankir, L. and De Rouffignac, C., Urinary concentrating ability: insights from comparative anatomy, *Am. J. Physiol.* **249**:R643–666, 1985.
10. Stephenson, J.L., Models of the urinary concentrating mechanism, *Kidney IntI*. **31**:648–661, 1987.
11. Logan, A.G., Moriarty, R.J., Morris, R. and Rankin, R.C., The anatomy and blood system in the river lamprey, *Lampetra fluviatis*; *Anat. Embryol.* **158**:245–252, 1980.
12. Youson, J.H. and McMillan, D.B., The opisthonephric kidney of the sea lamprey of the Great Lakes, *Petromyzon marinus* L.II. Neck and proximal segments of the tubular nephron, *Am. J. Anat.* **127**:233–258, 1971.
13. Logan, A.G., Moriarty, R.J. and Rankin, R.C., A micropuncture study of kidney function in the river lamprey, *Lampetra fluviatis*, adapted to fresh water, *J. Exp. Biol.* **5**:137–147, 1985.
14. Braun, E.J. and Dantzler, W.H., Function of mammalian-type and reptilian-type nephrons in kidney of desert quail, *Am. J. Physiol.* **222**:617–629, 1972.
15. Natochin, Y.V., Filtration, reabsorption and secretion in the evolution of renal function, *J. Evol. Biochem. Physiol.* **13**:424–429, 1977.
16. Hyodo, S., Katoh, F., Kaneko, T. and Takei, Y., A facilitative urea transporter is localized in the renal collecting tubule dogfish *Triakis scyllia, J. Exp. Biol.* **207**:347–356, 2004.

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