Significance of urea transport: the pioneering studies of Bodil Schmidt-Nielsen

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This essay looks at the historical significance of five APS classic papers that are freely available online:


UREA WAS FIRST DISCOVERED as an isolate from evaporated urine in the early 18th century by Boerhaave. However, renal handling of urea has been a major subject of interest only since the early 20th century, when techniques were developed to assay urea concentrations in both blood and urine. While many leading physiologists have worked in this area and made important contributions, perhaps no investigator has contributed more to this field than Dr. Bodil Schmidt-Nielsen (Fig. 1). When she started her seminal series of comparative physiological studies, it was generally accepted that urea was filtered and excreted without membrane interactive processes. Indeed, it was felt that urea was reabsorbed from the tubule by purely passive mechanisms, and as such, the kidney did not participate in the regulation of urea excretion. Dr. Schmidt-Nielsen quickly recognized the homeostatic dilemma that this conviction brought. She argued that urea clearance and glomerular filtration rate (GFR) must be independently regulated to maintain constancy of blood urea concentration under widely varying intakes of fluid and protein (one of the primary sources of urea synthesis) intakes. However, this view was not easily accepted, even though her data were compelling. Thus the purpose of this editorial is to review some of her works in the light of what currently is understood with respect to the mechanisms of urea transport and the significance that these transport mechanisms might have in overall fluid homeostatic mechanisms.

The understanding of renal physiology has matured with evolution of progressively more and more sophisticated techniques. Table 1 catalogs these techniques in roughly chronological order. As one progresses from clearance techniques to the more recent techniques, there is a danger that one gets away from integrated physiology and applicability to overall physiological significance. Bodil Schmidt-Nielsen used the first three of these techniques, with clearance techniques being the backbone of her studies. The advantages of clearance techniques are quite many: easy to learn, easy to perform, does not alter the physiological state of an animal, and is applicable to evaluation of kidney function as an integrated whole. It re-
mains the most important way to evaluate renal function in humans and, furthermore, has opened many areas of investigation for techniques that have been developed later. It is remarkable how many examples can be given where the suggestions and conclusions of clearance studies have predicted segmental tubular and cellular events that subsequently have been proven to be correct using more sophisticated newer techniques. Certainly Bodil Schmidt-Nielsen’s studies fall into this category.

Bodil Schmidt-Nielsen’s first study on renal urea handling emanated from her studies in southern Arizona on desert kangaroo rats and was published in 1948 (24). This publication was followed by 12 more publications that had a central theme of renal function in desert rodents with a special focus on urea and led to the landmark publication in the American Journal of Physiology (AJP) in 1952, where it was definitely shown that urea clearance in the kangaroo rat can exceed the filtered load when the kangaroo rats were fed a high-protein diet (18). In this study she suggested the presence of an active urea transport mechanism somewhere along the renal tubule. These studies were extended to include white laboratory rats as well as kangaroo rats and demonstrated that urea excretion can vary independently from the GFR and that this clearance was markedly affected by dietary intake of protein and the animal’s level of “excitement” (19). Animals fed high-protein diets showed a much higher urea/creatinine clearance ratio than did animals on low-protein diets, whereas animals that were caused to be excited lowered their urea excretion rates. This led her to conclude “the results reported do not conform with the filtration-rediffusion theory for urea excretion” and provided further support to her previously suggested active secretion of urea (19). She then published another 15 papers in various mammals including camel, dog, human, and sheep, leading to another seminal AJP publication in sheep in 1958 (22). This was a very complex clearance study during normal and low-protein intakes having a wide range of urine outputs from extreme osmotic diuresis to minimal flow rates. Although Dr. Schmidt-Nielsen had been thinking about active reabsorption and secretion of urea for some time, we feel this was the manuscript in mammals that demonstrated regulation of urea excretion as a function of intake of protein both by active secretory and reabsorptive mechanisms. She further argued that the quantitative measurements she made probably were magnified by the operation of a countercurrent multiplication system (22). The data were clear and conclusive, but for reasons that are not clear, the conclusions were not accepted by the renal community at large since the prevailing view then was that such a small molecule as urea must be permeant through all cellular barriers. It was then not recognized that urea, being a highly polar molecule, should not freely permeate cellular lipid membranes.

Bodil Schmidt-Nielsen, however, persisted with vigor in pursuing how the kidney handled urea. Some of her most interesting findings have come from her comparative physiological approaches that were in part conducted in parallel with her mammalian studies. These nonmammalian studies were largely conducted at the Mount Desert Island Biological Laboratories where she was highly stimulated by such greats as Homer Smith, E. K. Marshall, and Roy Forster. It is to be noted that Bodil Schmidt-Nielsen spent most of her summers working at Mount Desert Island and still is a Trustee of that great organization. In her first paper from Mount Desert Island in 1954 (21), she showed in bullfrogs that urea clearances were consistently much higher than GFR. The mean urea/creatinine ratio of 6.9 lead to the unequivocal conclusion that urea is actively secreted by the bullfrog tubules. These findings supported the previous conclusions of Marshall (13) and Walker and Hudson (31). In 1966, she and Rabinowitz (23) studied four different species of sharks, and in each case they found that the urinary urea that ranged from 72 to 202 mM was significantly below plasma urea concentration that ranged from 285 to 387 mM. This occurred at a time when urine-to-plasma inulin concentration varied between 2 and 5, thus demonstrating that urea was reabsorbed against a concentration gradient, which led them to conclude that an active transport mechanism must exist for urea reabsorption. In a follow-up in vivo micropuncture study of another species of shark, she was able to show that the shark proximal tubule did not actively reabsorb urea and concluded that the active reabsorptive process must occur somewhere along the distal tubule (25). She further characterized this transport process to transport acetamide and methylurea, but not thiourea. She appropriately concluded that there exists an active reabsorptive mechanism out of the shark distal tubule. This active outward pump is qualitatively different from the frog inward pump that transports thiourea, but not acetamide or methylurea (23). In 2003, Dr. Schmidt-Nielsen wrote a review with Bankir (20) in which they concluded that the lowering of the urine urea concentration to values below plasma could occur as a consequence of tubular fluid secretion and was not necessarily attributed to active urea reabsorption. To date this controversy is not settled, since segmental tubular to plasma inulin and urea concentrations are not available. However, what is clear is that Dr. Schmidt-Nielsen continues to think about urea transport in elasmobranchs and is a leader in reconsidering her own conclusions as new data evolves.

Thus the conclusion that can be drawn from her studies to this point is that her studies not only challenged the then widely held view that urea excretion occurred primarily by passive mechanisms according to filtration-passive back-diffusion principles, but showed that urea transport was highly regulated by tubular membrane interactive processes. Her studies clearly demonstrated both qualitative and quantitative variation of urea transport among the various species she had studied and also that the state and direction of active transport of urea is dependent on the physiological state of the animal. Perhaps the most important of these variables was the intake of protein. Species on high intake of protein had much higher urea clearance rates than the same animals on low-protein diets. Modern day pressures are such that it is highly desirable to demonstrate that the findings are applicable to humans, either

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by performing similar studies in humans or having reasonable
data to suggest that the findings can be extended to humans.
This thought was not lost on Dr. Bodil Schmidt-Nielsen and
she was ahead of her time by carrying out parallel studies in
humans. She was familiar with the earlier experiments of
Kempner (8), published in 1945 (8), which were designed
primarily to examine the effect of rice diet on blood pressure
and progression of renal failure. In these studies it was noted
that urea clearance was reduced significantly when subjects
were placed on low-protein diets. Murdaugh, Schmidt-Nielsen,
Doyle, and O’Dell (15) extended these studies in adult male
subjects who were mainly volunteer house staff physicians on
normal and low-protein diets. Simultaneous urea and inulin
clearances were determined. A low-protein diet did not alter
the subjects’ GFR but did significantly decrease the fraction
of filtered urea at any given rate of urine flow. Thus they con-
cluded that urea excretion in men is qualitatively similar to
other mammals studied and that their animal studies most
likely were applicable to urea transport in the human kidney
(15).

We have highlighted only few of Dr. Bodil Schmidt-Niel-
sen’s some 250+ manuscripts, the vast majority being pub-
lished in AJP, that have the central theme of urea transport.
Clearly these defined the importance of urea transport to fluid
homeostasis and the urine concentrating mechanism. These
studies have been a springboard of thought for many studies
using newer techniques (Table 1), only some of which we have
highlighted in this essay.

Several of the conclusions that Dr. Schmidt-Nielsen drew
from her clearance and comparative studies were proven in
studies using isolated perfused kidney tubules. As discussed
above, Dr. Bodil Schmidt-Nielsen’s data suggested that urea
must be actively secreted (18, 19, 22, 24), although her studies
could not determine the precise nephron segment. Our subse-
quent tubule perfusion studies conclusively established that
urea was actively secreted in two different nephron segments:
first, in the straight segment of the rabbit proximal tubule (7);
and second, in the terminal portion of the rat inner medullary
collecting duct (6). Thus tubule perfusion studies conclusively
established that urea was actively secreted, as Dr. Bodil
Schmidt-Nielsen had concluded from her clearance studies.

Dr. Schmidt-Nielsen also found evidence for active urea
reabsorption somewhere along the distal tubule (21, 23, 25).
Our subsequent tubule perfusion studies again confirmed Dr.
Schmidt-Nielsen’s conclusion and conclusively established
that urea was actively reabsorbed in the initial portion of the rat
inner medullary collecting duct (5). One of the challenges in
demonstrating active urea reabsorption is that it only occurs in
the initial inner medullary collecting duct. In addition, it occurs
in rats fed a low-protein diet but not in rats fed a normal protein
diet. This was first suggested by Dr. Schmidt-Nielsen’s mi-
cropuncture studies (30) and then proved by our studies of
perfused tubules (5).

Dr. Schmidt-Nielsen’s studies of the effects of low-protein
diets on urea clearance in rats and sheep (18, 19, 22, 28) also
showed an interesting change in the distribution of urea within
the kidney. In animals fed a normal protein diet, the urea
concentration increases from the cortex to the papillary tip,
with the highest concentration at the tip. However, in animals
fed a low-protein diet, the highest urea concentration occurs in
the base of the inner medulla, and decreases toward the
papillary tip. Her observation inspired us to study the effect of
low-protein diets on facilitated urea transport in rat inner
medullary collecting ducts, and we found that vasopressin
(antidiuretic hormone) stimulated urea reabsorption in perfused
initial inner medullary collecting ducts from low-protein fed
rats, but not from rats fed a normal protein diet (5). Thus there
were two changes in urea transport in the initial inner medul-
lar collecting duct that result in the changes in tissue urea
distribution and urea clearance measured by Dr. Schmidt-
Nielsen: the appearance of active urea reabsorption and vas-
opressin-stimulation of facilitated urea reabsorption.

Dr. Schmidt-Nielsen’s many studies of urea in the kidney,
only some of which are summarized above, inspired many
studies of urea transport in perfused tubules, which in turn
resulted in the definition of the functional properties of a
facilitated urea transporter. This functional definition permitted
Hediger’s laboratory to expression clone the first urea trans-
porter, now named UT-A2 (33). At present, two urea trans-
porter genes have been cloned, UT-A and UT-B (reviewed in
Refs. 16 and 17). There are currently six protein isoforms of
UT-A, named UT-A1 through UT-A6, and a single isoform on
UT-B. UT-A1 and UT-A3 are expressed in the inner medullary
collecting duct, UT-A2 is expressed in the descending thin
limb, UT-A4 is expressed in low abundance in the kidney
medulla, and UT-B is expressed in red blood cells and de-
sending vasa recta (reviewed in Refs. 16 and 17). Neither
UT-A5 nor UT-A6 is expressed in kidney (3, 26). Another
facilitated urea transporter, UT-C, is present in eel proximal
tubule (14), and many marine species express facilitated urea
transporters that are highly homologous to UT-A (reviewed in
Refs. 16 and 17). Thus, as predicted by Dr. Schmidt-Nielsen’s
studies of marine species (25), they do express facilitated urea
transporters. A goal for future studies will be to clone the
active urea transporters.

Lastly, Dr. Schmidt-Nielsen’s studies of urea, along with the
demonstration by several investigators that a low-protein diet
results in a urine concentrating defect (4, 12), led to the idea
that urea played a critical role in urinary concentration. Dr.
Schmidt-Nielsen recognized the role of urea in the urine
concentrating mechanism, but did not explicitly propose a
model that incorporated urea into the concentrating mecha-
nism. However, her clearance and micropuncture studies sug-
gested the importance of studying urea transport in the kidney.

Dr. Schmidt-Nielsen’s studies inspired other investigators
to study urea transport in nephron segments that participated
in the operation of the countercurrent multiplication system by
perfusing these nephron segments in vitro (10). The results of
these tubule perfusion studies (10) led to the formulation of the
passive mechanism hypothesis in 1972 by Kokko and Rector
(11) and by Stephenson (27). The key components of the
passive mechanism hypothesis include: urea being transported
down its concentration gradient from the highly urea-perme-
able terminal inner medullary collecting duct (via UT-A1 and
UT-A3) into the inner medullary interstitium; urea being
trapped in the inner medulla by the descending thin limb (via
UT-A2) and by countercurrent exchange in the vasa recta (via
UT-B); and the higher urea concentration in the interstitium,
along with the higher NaCl concentration in the ascending thin
limb, providing a concentration gradient for passive NaCl
absorption into the interstitium. Recent studies of mice lacking
1) UT-A1 and UT-A3; 2) UT-A2; or 3) UT-B, all have urine
concentrating defects (1, 2, 9, 29, 32) and further support the vital role that urea transport plays in the urine concentrating mechanism. Thus the passive mechanism remains the best accepted hypothesis to explain how the inner medulla contributes to the production of a concentrated urine in the absence of active NaCl absorption.

Juha Kokko first met Dr. Bodil Schmidt-Nielsen in 1968 at the Southern Salt and Water Club meeting, which is annually held in Sarasota, FL. It was late fall, and the water temperature had already fallen to uncomfortably low levels where no one was swimming. However, Kokko’s attention was drawn to a pretty blonde woman who ran into the water not being the least bit bothered by its coolness. She stayed there for some time, and being curious, Kokko went and introduced himself to her and found out that she was Dr. Bodil Schmidt-Nielsen. Later he heard her talk on urea transport and thus started a long-term professional association of which Juha Kokko has grown deeply fond. She has participated actively at these meetings, and it was at these Sarasota meetings that Jeff Sands also first met her in the late 1980s. Together we have admired her work and have been stimulated by her findings and thus have written this editorial to pay homage to one of the greats in renal physiology.

Dr. Bodil Schmidt-Nielsen was born into an eminent Danish family. Her father, Dr. August Krogh, was awarded the Nobel Prize in Physiology or Medicine in 1920 for his work on capillary physiology. Her mother, Dr. Marie Krogh, was a physician and respiratory physiologist. Bodil herself is a 1937 graduate from a Danish gymnasium. In 1939 she married Knut Schmidt-Nielsen, and together they moved to the United States in 1946, and shortly thereafter she began her focus on urea transport (she originally was trained as a dentist, but later became fascinated by physiology). Together with her husband, they published many of the early papers that we have referenced in this essay. She has had many milestones in her career, including being the 48th president of the American Physiological Society (1975–1976). Today at age 88 she remains intellectually very active, and recently we have had many stimulating discussions with her. She has graciously given us much of the material in this essay and has read the material and given her approval to us to publish this contribution honoring her career.

REFERENCES