Carl W. Gottschalk’s Contributions to Elucidating the Urinary Concentrating Mechanism

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Like all of the participants in this symposium, I am very pleased to contribute to a scientific gathering that honors Carl W. Gottschalk—esteemed colleague, superb scientist, good friend, and admirable human being. Carl contributed importantly to our knowledge in several areas of renal function; today, however, I will limit myself to the topic in which he made perhaps his major contribution, namely, the countercurrent mechanism by which urine is rendered hypertonic to plasma.

In preparing this review, I was acutely aware of Carl’s love for history and his insistence on historical accuracy. His remarkable, beloved collection of books on the kidney, which he bequeathed to the University of North Carolina at Chapel Hill, is probably the best compilation of its kind. And it was no coincidence that Carl served as archivist of the International Society of Nephrology, that he chaired its Commission on the History of Nephrology, and that he authored a number of chapters and articles about its history. As just one example: In 1993, Carl participated in a conference on the History of Nephrology, held in Naples, Italy, where he spoke on “Alex- un the annual meetings of the American Society of Nephrology, Philadelphia, October 26, 1998.

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To fully appreciate Carl Gottschalk’s role in proving the countercurrent hypothesis for concentrating urine (6,7), one must consider the setting in which he performed this work. It took courage, prescience, and perseverance, as well as independence as a young (as yet, largely unrecognized) investigator, to pursue renal micropuncture in the 1950s. In fact, some major personages in renal physiology advised against such a project. Homer Smith, for example, when asked by Carl what he thought of the countercurrent hypothesis, told Carl: “The smart boys don’t believe in it.” (5).

Erich Windhager divides the history of renal micropuncture into pre- and post-World War II phases (2). The method had been developed in 1921 by A. Newton Richards, a remarkable scientist (8) and for many years chair of pharmacology at the University of Pennsylvania, and Joseph T. Wearn, a young physician who had joined Dr. Richards’ laboratory in 1921. We have to emphasize the adjective renal at this point, for micropuncture of other structures had been developed earlier (for example, see reference (9)) and had been observed by Richards. In Richards’ own words, quoted by Carl Gottschalk (10): “In April of that year [1921] I saw Robert Chambers puncture erythrocytes and imagined that I might, with one of his pipettes, apply a little adrenalin to an exposed efferent vessel. In September of that year, Joseph T. Wearn came to work in my laboratory. After he had learned how to expose a living frog’s kidney for direct microscopic study and had come to realize its possibilities, I suggested that we make some capillary pipettes and try to apply adrenalin to an efferent vessel. He countered with the suggestion, Why not puncture a glomerular capsule with the pipette, get some glomerular fluid and find out what was in it.” Although the strategy was clear—again, in the words of Richards (10): “Many days in 1921–22 elapsed before Wearn succeeded in withdrawing uncontaminated glomerular urine [sic] from a frog...”

For the next 20 years—until 1941 (the year, that is, when the United States entered World War II)—Dr. Richards’ group constituted virtually the only laboratory in the world using the technique of renal micropuncture. They produced a series of famous reports, notable for their reliability and cautious interpretations (see reference (8), pp 38–40), that established beyond doubt and in great detail the nature of glomerular filtration and of selective tubular reabsorption. (Richards was reluctant to accept the existence of tubular secretion, although...
he did so ultimately, but on the basis of work by others.) Two people in Dr. Richards’ group, Arthur M. Walker and Phyllis A. Bott, working in collaboration with Dr. Jean Oliver and his able assistant, Muriel C. MacDowell, extended this work to mammals (11,12). These fruitful investigations came to a close when Dr. Richards and most of his coworkers entered national service during World War II.

By the time, after the war, that Carl Gottschalk wanted to utilize renal micropuncture, the technique had lain dormant for at least a decade. Carl has written (10): “Dr. Richards did not encourage the revival of the micropuncture technic after World War II and advised me, and I suspect others, against entering the field.” But lest Dr. Richards be misunderstood, Carl added in the next sentence, “I am certain he had no selfish or proprietary motivations for doing this; rather he was concerned that a field to which he devoted so much of his life would be sullied by less competent workers.” That makes Dr. Richards sound arrogant, which I think he did not mean to be. When one reads about the almost unbelievable care that Richards and his colleagues took to assure the correctness of their results (see, for example, p 31 of reference (10)), then one is convinced that Dr. Richards’ reluctance arose—just as Carl Gottschalk said—from the fear that others, less meticulous, would publish misleading results. This more charitable view of Dr. Richards is supported by ample testimony from his many coworkers (8), and especially by the fact that Richards had offered sole authorship to Wearn when their results of the first renal micropunctures were about to be published (15).

Why, then, did Carl Gottschalk pursue the very difficult project of investigating the countercurrent system? Partly, perhaps, because he had recently mastered micropunctures of surface nephrons and peritubular capillaries (see pp 112–113 of reference (2)), and especially by the fact that Richards had offered sole authorship to Wearn when their results of the first renal micropunctures were about to be published (15).

Table from the initial report by Gottschalk and Mylle, of micropunctures of loops of Henle. Slightly adapted from reference (17).

<table>
<thead>
<tr>
<th>Hamster No.</th>
<th>Loop of Henle</th>
<th>Collecting Duct</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1391</td>
<td>1402</td>
<td>308</td>
</tr>
<tr>
<td>2</td>
<td>725</td>
<td>720</td>
<td>336</td>
</tr>
<tr>
<td>3</td>
<td>1270</td>
<td>1206</td>
<td>325</td>
</tr>
<tr>
<td>4</td>
<td>453</td>
<td>453</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.

What is striking is the remarkable amount of important data loops of Henle (micropunctured at their bend) was clearly hypertonic to blood (plasma) collected from the inferior vena cava. And then, with characteristic caution, Carl stated his conclusion: “These results are highly consistent with the hypothesis of Hargitay and Kuhn . . . that the mammalian nephron functions as a countercurrent multiplier system to concentrate the urine.” (17).

With this successful and convincing micropuncture of loops of Henle, Carl Gottschalk provided what was probably the final proof needed for universal acceptance of the countercurrent hypothesis. This hypothesis had been proposed by Werner Kuhn, a brilliant Swiss physical chemist who had studied with some giants, such as Niels Bohr and Ernest Rutherford. With his colleague Kaspar Ryffel, Kuhn published a 34-page treatise describing and actually testing several arrangements by which concentrated solutions could be produced from dilute ones if semipermeable membranes separated the compartments that contained these solutions (18). The very title of their classic paper told the story, for not only did they describe and test the model, “Production of concentrated solutions from dilute ones purely through a membrane effect” (that is, without the application of pressure), but they even subtitled it, “A trial model for renal function.” In fact, the impetus for their work arose from the puzzle of how the kidneys could concentrate urine; and they cited the countercurrent arrangement of the loops of Henle in arguing for the feasibility of their model in vivo.

Note, however, not only that this paper was published in German but, more importantly, that it appeared in 1942, at the very height of World War II. It was fortunate for renal physiologist that a very able experimentalist, Heinrich Wirz (Figure 2), in the Department of Physiology at the University of Basel, where Kuhn chaired the Department of Physical Chemistry, began an extensive collaboration with Kuhn. Wirz, like Carl Gottschalk, saw clearly what experimental evidence was required to prove the countercurrent hypothesis. These requirements are outlined in Table 1, which shows, on the left, predictions that must hold if the theory is correct, and on the right the experimental proof for each prediction.

What is striking is the remarkable amount of important data in support of the countercurrent hypothesis, which was published by Heinrich Wirz and Werner Kuhn and their associates in just a 5-year span, between 1951 and 1956 (19–21)—and

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6As this passage shows, Carl Gottschalk remained a great admirer of Dr. Richards (10). Carl wrote of the 1924 report by Wearn and Richards (13): “I believe this to be the most significant single publication in all of renal physiology” (14).
before Carl Gottschalk’s first report on the subject in *Science* in 1958 (17). These were truly collaborative efforts, as emphasized by Wirz in footnotes to his 1953 and 1956 papers. Wirz had applied micropuncture on his own for his 1953 report on vasa recta, and then learned micropuncture of tubular segments from Phyllis Bott in Philadelphia. In addition, he ingeniously utilized a number of unusual features, such as the accessibility of the renal papilla to micropuncture in the golden hamster, where the papilla reaches into the renal pelvis (20), as well as a method for maintaining water diuresis in an anesthetized, laparotomized rat (21). The important findings included the following (Table 1): (1) demonstration of a corticopapillary osmotic gradient (with this *sine qua non*, Hargitay and Kuhn reiterated the applicability of countercurrent multiplication to renal function—albeit still in German and in a journal of electrochemistry [reference (22)]); (2) hypertonicity of vasa recta plasma, reflecting the hypertonicity of the papillary interstitium, and equality of the osmolality of vasa recta plasma with the osmolality of simultaneously sampled urine; these findings were complemented by Karl Ullrich and his coworkers (23,24), who showed that the gradient consisted mainly of sodium chloride and urea in antidiuresis, and mainly of sodium chloride in water diuresis; (3) isosmotic reabsorption from proximal tubules, in water diuresis as well as in antidiuresis (shown earlier for antidiuresis by Walker, Bott, Oliver, and MacDowell, reference (12)); (4) hypo-osmolality of early distal fluid, also in both water diuresis and antidiuresis (the latter shown provisionally by Walker et al., reference (12)); (5) distal fluid becoming isosmotic (but never higher) by the end of the distal tubule in antidiuresis, but (6) remaining dilute in water diuresis; and (7) hyperosmolality at the end of collecting ducts.

These landmark papers by Wirz are characterized by concise writing and admirable logic. Not only did his reports clearly describe countercurrent multiplication, but they even contained some predictions, such as an influence of vasopressin on solute transport out of ascending limbs of Henle and a role for the vasa recta as countercurrent exchangers. Note, however (Table 1), that experimental proof for one critically important prediction remained wanting despite Wirz’s extensive experiments, and that was that fluid at the bend of the loops of Henle must be hyperosmotic, not only in antidiuresis but also in water diuresis. Wirz pointed to this need in both his 1953 (20) and 1956 (21) papers. In the former he wrote, “Attempts to visualize the loops [of Henle] through the excretion of dye have so far failed.” And in the latter he stated, “Thus far, the loop of Henle has been inaccessible to micropuncture.” This major void was filled by Carl Gottschalk.

So difficult was it at that time to distinguish loops of Henle from vasa recta in a micropuncture experiment that Carl took special pains to describe his method for doing so, even in his preliminary report in *Science* (17): He and his able coworker, Margaret Mylle, demonstrated that their loop samples contained little or no protein, and they showed, through injection of dye and subsequent microdissection, that the punctured loop could be followed to the proximal and distal convolutions of a juxtamedullary nephron.

Hypertonicity of loop of Henle fluid was the “smoking gun” in support of the countercurrent hypothesis. Some highly respected authorities had remained in doubt about the theory, which to many seemed unbelievably complicated. They continued to search for other mechanisms by which the kidneys might elaborate concentrated urine; some, in fact, had speculated that fluid at the bend of loops of Henle might be hypo-osmotic (for detailed discussion, see reference (3)).

Margaret Mylle (Figure 3) was a mainstay in most of Carl Gottschalk’s early experiments. For a span of 12 years, from 1956 to 1968, her name appeared as coauthor on virtually all original reports coming out of Carl Gottschalk’s laboratory (or out of the “Chapel Hill Micropuncture Laboratory,” as Carl preferred to call it). Having been taught the technique of renal micropuncture by Carl, Margaret Mylle prepared the animals and performed the micropunctures. It is noteworthy that Carl played an active role in all of these experiments: He personally prepared the Ramsey/Brown micro-osmometer (25), which had been built especially for the Chapel Hill laboratory, by filling the cooler with dry ice, and he himself measured the osmolalities of the samples. Accurate determination of osmolality in nanoliter specimens was crucial to the work; in fact, it is interesting that except for a few flow rates, osmolality was the only variable reported in the 1959 paper (4).

Two striking characteristics of that classic report (4) are its thoroughness and the extraordinary care with which the experiments were conducted. No doubt Carl Gottschalk bore in mind the warning and concern of Richards about the danger of unintentional errors in the performance of micropuncture (see above).

Four species of mammals were used: (1) Wistar rats, for sampling of surface structures belonging to short-looped nephrons; (2) golden hamsters and one kangaroo rat, for sam-
pling of papillary structures, which in these species extend into the renal pelvis; and (3) *Psammomys obesus*, whose kidneys were at that time thought to consist entirely of long-looped nephrons, and thus could exclude any possible misinterpretation in having sampled surface structures belonging only to short-looped nephrons and papillary structures belonging only to long-looped nephrons. (It was discovered subsequently that approximately 66% of nephrons in *Psammomys obesus* are short-looped nephrons [see p R647 of reference (26)].)

Experimental states included: hydropenia; water diuresis; and osmotic diuresis. All sites of micropuncture were marked with nigrosine and then checked by macerating the kidneys, isolating the punctured tubular segment by microdissection, and determining the proportional distance along the segment.

<table>
<thead>
<tr>
<th>Predicted Condition</th>
<th>Experimental Proof</th>
</tr>
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<tbody>
<tr>
<td>Osmolality of tubular fluid during antidiuresis and water diuresis: isosmotic in proximal tubule</td>
<td>Walker, Bott, Oliver, MacDowell, 1941 (12) (antidiuresis only) Wirz, 1956 (21) a</td>
</tr>
<tr>
<td>hyperosmotic at bend of loop of Henle hypo-osmotic in early distal tubule</td>
<td>Gottschalk and Mylle, 1958 (17), 1959 (4) b Walker, Bott, Oliver, MacDowell, 1941 (12) (provisional; antidiuresis only)</td>
</tr>
<tr>
<td>isosmotic at end of distal tubule (antidiuresis only) hyperosmotic at end of collecting duct</td>
<td>Wirz, 1956 (21) a</td>
</tr>
</tbody>
</table>

a Findings of Wirz that were, importantly, confirmed by Gottschalk and Mylle in their 1959 report (4).
b Indicates the wholly new and critical data contributed by Gottschalk and Mylle (4,17).

*Figure 3. Margaret Mylle and Carl W. Gottschalk in their laboratory at the University of North Carolina, Chapel Hill, circa 1958.*
where the puncture had occurred. In this task, Carl Gottschalk relied on the meticulous studies and methods of Jean Oliver (11, 12). The next four figures summarize the major results of the study; the graphs have become classics, recognized instantly by anyone working in the field. Some of the important findings had been reported earlier or simultaneously, especially, as I have mentioned, by Heinrich Wirz. Carl dealt forthrightly with this possible repetition in the introduction to the 1959 report: “Due to the fundamental importance of these data and their limited number, we have reinvestigated this subject, and have confirmed and extended the previous micropuncture experiments.” Most important among the extensions were the data on loops of Henle, and these were wholly new.

The first figure (reproduced here as Figure 4) showed isosmotic reabsorption from the proximal tubules, no matter what the diuretic state. As in most illustrations of the report, the ratio of the osmolality of tubular fluid to that of systemic plasma was plotted on the left ordinate, and the concurrent osmolality ratio for urine to systemic plasma on the right. Each of the points obtained by micropuncture was placed on the abscissa as the relative distance along the proximal tubule where the puncture was made. The black dots represent hydropenia with a mean U/P osmolality ratio of around six, equivalent to a urine osmolality of approximately 1900 mosmol/kg H2O; the black squares are from animals that were in water diuresis, with urine osmolalities ranging from approximately 140 to 280 mosmol/kg H2O; and the remaining symbols represent four types of osmotic diuresis. Thus, reabsorption from proximal tubules appeared to be isosmotic, whether the urine was strongly hypertonic or dilute, or values in between. I say, “appeared to be,” because Carl Gottschalk was able to measure the osmolality of these minute samples within an accuracy of only ±5 mosmol/kg H2O, and we have since discovered that fluid reabsorbed from proximal tubules is actually very slightly hypertonic, so that what remains is very slightly hypotonic.

Next, Gottschalk and Mylle confirmed the very important finding of Wirz (21), which had been reported provisionally by Walker, Bott, Oliver, and MacDowell (12), namely (Figure 5), that fluid obtained from early distal tubules was always hypotonic to systemic plasma and that it reached isotonicity toward the end of distal convolutions—but never higher—even while the urine being excreted was very hypertonic. This was the very important finding that showed conclusively that the final concentration of urine occurs in collecting ducts, not in loops of Henle, as had been surmised initially (and logically) when it was found that only animals with loops of Henle could render urine hypertonic to plasma. Wirz had shown, in addition, that during water diuresis, fluid remains hypotonic throughout the length of the accessible distal convolution (21).

To be certain that the hypotonicity of early distal fluid was not limited to short loops of Henle (from which the data shown in Figure 5 were obtained), Gottschalk and Mylle produced similar results in micropunctures from *Psammomys obesus*, a desert rodent that, at that time, was thought to have only long loops of Henle.

To explore the mechanism for the early distal hypotonicity, Gottschalk and Mylle performed distal micropunctures during osmotic diuresis. Theoretically, the hypotonicity could have resulted from either reabsorption of solute from loops of Henle or secretion of water into them, or a combination of the two; the process had to occur in the loops because fluid at the end of proximal tubules was isosmotic under all circumstances (Figure 4). If it were the secretion of water, the transport would have to be active, and by the mid-1950s that possibility had been excluded pretty conclusively. If it were reabsorption of solute, then NaCl was judged to be the likely candidate because it was the only solute present in sufficient amounts to cause the measured degree of hypotonicity. Gottschalk and Mylle distinguished among these possibilities by testing the degree of early distal hypotonicity in three forms of osmotic diuresis: 25%
mannitol, 25% glucose, and 5% or 7% NaCl. The results (Figure 6) strongly suggested that the early distal hypotonicity was due to reabsorption of NaCl because the degree of hypotonicity was much greater with NaCl (down to an osmolality ratio of 0.3) than with mannitol or glucose, two solutes that are not reabsorbed from loops of Henle. Furthermore, the possibility of active secretion of water was pretty much excluded by these data because that process could be expected to be independent of the solute used—which, clearly, it was not.

Probably the most important result of the study—and the one that was wholly new—was the demonstration of hypertonicity of loop of Henle fluid (Figure 7). The data from nine hamsters, one kangaroo rat, and one Psammomys obesus showed virtual equality, at any one moment, of osmolality in collecting ducts and at the bend of thin loops of Henle. This finding was an absolute requirement if countercurrent multiplication in the loops of Henle was the mechanism by which urine is concentrated. We should not underestimate the achievement of obtaining these results. Mark Knepper, in his commentary to Carl Gottschalk’s “Milestones” paper (5), referred to them as a “technical triumph,” and Carl himself, in his commentary, spoke of them as the “greatest technical challenge,” and stated:

“Nothing I had ever done before or have done subsequently was as thrilling as obtaining these data. We had demonstrated that the tubular fluid is first concentrated in the descending limb and then diluted in the ascending limb of the loop of Henle before undergoing final concentration in the collecting ducts.”

The additional fact shown in Figure 7, that vasa recta plasma shares this equality of osmolality with collecting ducts at the same level in the renal papilla (the open circles), presumably reflected the osmotic equilibration between collecting duct fluid and the surrounding interstitium. This result not only constituted further support for the countercurrent hypothesis...
but also suggested that the vasa recta act as counter-current exchangers.

Thus, it was the combined efforts of Werner Kuhn, Heinrich Wirz, and Carl Gottschalk—abetted, as Carl would have been the first to acknowledge, by important results contributed by others (reviewed in reference (3))—that solved the long-standing mystery of how urine is concentrated. In addition to the new and absolutely crucial data from the loops of Henle, Carl contributed enormously to this effort by producing and assembling so much reliable data in a single, concise report that reflected his methodical, cautious, accurate scholarship.

Carl Gottschalk and Heinrich Wirz met for the first time at a symposium held in Göttingen, Germany, in August 1959—just 4 months after the publication of Carl’s definitive report. They were introduced to one another by Klaus Thurau, who recalls that each said, in effect, “Oh, you are Dr. Wirz!” and “Oh, you are Dr. Gottschalk!” (29). It was, according to Thurau, the beginning of a long and close friendship.6

It is interesting that despite further ingenious ideas and technical achievements during the ensuing 40 years, we remain in the dark about what brings about the continuing interstitial osmotic gradient in the inner medulla. The current status on this question was summarized succinctly by Mark Knepper in the last paragraph of his commentary on Carl Gottschalk’s “Milestones” paper (5). Knepper may well be right when he suggests that history may have to repeat itself before the problem is finally solved.

I would like to close this tribute with a photograph of Carl Gottschalk that speaks volumes about the person (Figure 8). It shows Carl in his office in 1993 and reflects, I think, his modesty (the old desk and chair); his care for accuracy, as evidenced by the two dictionaries constantly on his desk; and even, I think, his mild manner.7 These qualities are abundantly evident in Carl Gottschalk’s scientific contributions. He was a paragon.

Acknowledgments

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References

2. Windhager EE: Micropuncture and microperfusion. In: Renal

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6Carl Gottschalk had used those very words when, around the same time, I introduced him to Dr. David Rytand, whose paper on the number and size of glomeruli in different mammalian species (30) Carl had long admired: “Oh,” Carl had said, “so you are Dr. Rytand.”

7 Also shown, above his left shoulder, is the major three-volume work, “Diseases of the Kidney,” which Carl Gottschalk coedited, at first with Laurence E. Earley and then with Robert W. Schrier.