This Week's Citation Classic®

Maren T H. Carbonic anhydrase: chemistry, physiology, and inhibition. *Physiol. Rev.* **47**:595-781, 1967.
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In this review, the author attempted to integrate knowledge that had been gained in the 30 years following the discovery of carbonic anhydrase in 1933 so that physiological events might be understood in the light of the chemistry of CO_2 and the pharmacology of the sulfonamide inhibitors. [The *SCI*® indicates that this paper has been cited in over 1,035 publications.]

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In about 1961 I gave a paper at Harvard Medical School on the physiological roles of carbonic anhydrase and my early attempts to see how the chemical-reaction rates were related to the physiological. The enzyme had been discovered 28 years before by Meldrum and Roughton[1] at Cambridge, and the field was quite lively. Carbonic anhydrase was involved in renal acidification (Pitts,[2] at Cornell), in aqueous humor secretion (Friedenwald, Becker,[3] at Johns Hopkins), and, as Roughton worked out so beautifully, in respiration. Virtually all secretory systems were involved. Roblin,[4] at the American Cyanamid Company, had discovered inhibitors a thousand times more active than sulfanilamide, the compound that Roughton and Pitts had used. He gave me the job of developing these new sulfonamides as diuretics and as treatment for a bewildering but fascinating array of diseases, including epilepsy, ulcers, and renal calculi. By the time acetazolamide was ready for the market in 1954, its major medical use—treating glaucoma—became apparent, although this application was entirely unforeseen by its developers. In 1955 I left Cyanamid for Florida and turned to less practical aspects of the problem.

Major physiological questions about acetazolamide metabolism remained. Davies and Roughton[5] had been convinced that complete inhibition of the enzyme would lead to "speedy death," probably from CO_2 retention. But acetazolamide in the normal animal was no more toxic than NaCl! My host at Harvard was John Pappenheimer, who had been with Roughton at Cambridge, and who quite rightly demanded an answer to this paradox. In the course of this, he suggested that I review the entire field; perhaps the answer would pop out. Since he was chairman of the editorial board of *Physiological Reviews*, the whole matter seemed to fall together, and indeed, it fell on me. Five years later I submitted the manuscript.

In the review, I tried to put together the chemistry of CO_2 hydration and dehydration, the newly emerging knowledge of the active site and structure of the enzyme, the properties of the sulfonamide inhibitors, with the physiology of each of many vertebrate and invertebrate organ systems. The theme was reductionist in the extreme: reactions in the various organs were the same, and catalysis or inhibition could be mimicked or blocked by appropriate changes in acid-base balance. *In vitro* enzyme kinetics and inhibition *could* be applied to physiological events when certain realities of rate and properties of the sulfonamide drugs were taken into account. The respiration paradox was indeed answerable, in terms of new high gradients of pCO_2 that emerged when the enzyme was inhibited. In the case of the kidney, I took the view that 60 to 80 percent of HCO_3^- reabsorption occurs outside the carbonic anhydrase system. Now this appears true when the enzyme is inhibited, but not in the normal. In the absence of enzyme, new gradients (this time of HCO_3^-) provide a means for bypassing the enzymic reaction. I was trapped in a rough version of the Heisenberg Principle: can we judge the properties of a system as we change it? How much of pharmacology must be rewritten in this light?

Questions about other physiological systems emerged, one leading to a new and radical finding from our laboratory. As I wrote the chapter on cerebrospinal fluid (CSF), I realized I was getting nowhere in terms of the underlying electrolyte movement subserved by the enzyme. I was writing nonsense, and ended by weakly (and as it turned out, incorrectly) invoking H^+ and Cl^- co-transport, but did become committed to working on the problem. By the double good fortune of using fish for initial experiments and having Betty Vogh's mammalian studies,[6] we showed that CSF secretion depended on HCO_3^- formation, again analogous to many other systems (e.g., eye and pancreas).

This monograph has become a *Citation Classic* because it is hard to escape CO_2 in physiology and because it came at a time of great interest in so many of the varied aspects of the subject. Perhaps we should not be surprised that this time is still at hand.

1. **Meldrum N U & Roughton F J W.** Carbonic anhydrase: its preparation and properties. *J. Physiol.—London* **80**:113-42, 1933. (Cited 190 times since 1955.)
2. **Pitts R F & Alexander R F.** The nature of the renal tubular mechanism for acidifying the urine. *Amer. J. Physiol.* **144**:239-54, 1945. (Cited 250 times since 1955.)
3. **Becker B.** Carbonic anhydrase and the formation of aqueous humor. The Friedenwald memorial lecture. *Amer. J. Ophthalmol.* **47**:342-61, 1959. (Cited 70 times.)
4. **Miller W H, Dessert A M & Roblin R O, Jr.** Heterocyclic sulfonamides as carbonic anhydrase inhibitors. *J. Amer. Chem. Soc.* **72**:4893-6, 1950. (Cited 140 times since 1955.)
5. **Davies R E.** Hydrochloric acid production by isolated gastric mucosa. (With appendix by F.J.W. Roughton.) *Biochemical J.* **42**:509-21, 1948. (Cited 95 times since 1955.)
6. **Vogh B P & Maren T H.** Sodium, chloride, and bicarbonate movement from plasma to cerebrospinal fluid in cats. *Amer. J. Physiol.* **228**:673-83, 1975. (Cited 50 times.)

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