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W. R. Chegwidden, N. D. Carter and Y. H. Edwards, **The Carbonic Anhydrases: New Horizons**

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Reviewed by Dr R. J. Schmidt, Barnoldwick, England

As every book publisher will recognise, there are some books that simply will never be bestsellers. This is one such book. Being very much a specialist text for researchers in the area, the market for the book will be limited to those who work in the carbonic anhydrase field, with some take up by those with interests in enzyme mechanisms and molecular biology in general. There will also be an inevitable take up by university libraries.

This is the sort of text that appears periodically in every field in which there is an ongoing research effort. It serves to consolidate what is known and may therefore be regarded as an essential introductory text for anyone entering the field. This particular text arose from a conference on the carbonic anhydrases held in Oxford in the summer of 1995. Fifty or so contributors have prepared 30 review papers. The book appears to cover the carbonic anhydrase field exhaustively ….

Look in any typical undergraduate level biochemistry textbook and you are likely to find only the briefest reference to carbonic anhydrase (CA), a zinc-containing metalloenzyme that hydrates carbon dioxide to form the bicarbonate ion, and vice versa. You are likely to learn only that it catalyses one of the fastest enzyme-mediated reactions known – one CA molecule can, it seems, hydrate 10^5 molecules of carbon dioxide in one second. Only catalase, the enzyme that converts hydrogen peroxide to water and oxygen, can boast a faster turnover number. As would be expected, the text here being reviewed explores the mechanism of the CA-mediated reaction in some detail. And, as always seems to be the case with such works, the mechanism described simply fails to provide a convincing explanation for the phenomenal increase in rate of reaction that the enzyme achieves. The authors of the papers in question might argue that it was beyond their remit to explain why enzymes in general are so good at doing what they do. However, I would say that those who study the mechanism of enzyme action all too often take the magic of enzyme action for granted. This is not a criticism of the book or the authors of the papers therein, but a jibe at the apostolic nature of scientific research and the peer review system in which it operates. A paradigm shift in this field is long overdue. Students of the history of science will recognise that there is a massive inertia in the scientific arena against paradigm shifts. Typically, those who have the temerity to propose alternative ways of thinking about a subject are ridiculed or their ideas are otherwise suppressed (through the peer review process, for example). Perhaps this review of a book on one of the most remarkable enzymes known to man is an appropriate forum in which to urge all those whose work involves a study of enzyme mechanisms to question just how the classical lock-and-key model of enzyme action can explain how one hundred thousand or more hydration events a second could possibly occur at the CA active site by the mechanism as currently understood bearing in mind that for each hydration event there must be a relaxation event as the lock resets itself to a state that can accept the next approach of the key. What about the vicinal water that surrounds all enzymes – bound and structured water that is believed to have an ice-like structure which may be 50 molecules deep. Why is this feature almost never contemplated in discussions of enzyme mechanisms, yet it underpins an hypothesis relating to enzyme action that dates back to the early 1950s when Gilbert Ling proposed that enzyme active sites exist in the structured water rather than on the enzyme surface itself. If this is the case, then a mechanism by which the enzyme-mediated chemistry can occur in such an environment remains to be described because this would render much of the postulated electrophile–nucleophile chemistry implausible, especially if the same mechanism in reverse has to be invoked to account for, for example, the dehydration reaction of CA to generate carbon dioxide from bicarbonate ion. Yet, an alternative general mechanism of enzyme action can be envisaged that may at once provide a role for vicinal water, and a plausible means by which

bond energies are very significantly reduced for both the forward and the reverse reactions in a structured water environment, and a means by which the product of the reaction is ejected by a conformational change of the whole enzyme, and possibly also a means by which the same active site will catalyse the reaction of several (and possibly, in some cases, several hundred) substrate molecules simultaneously! A mechanism of chemical reaction that is consistent with all of this is well known, but its formal description post-dates a massive bulk of literature that invokes electrophile–nucleophile mechanisms to explain enzyme action. I refer to a concerted pericyclic sigmatopic mechanism that involves macrocyclic rings of hydrogen bonded water molecules "conjugated" with functional groups (amide groups of peptide bonds, histidine residues; alcohol and amine groups, etc) in the enzyme molecule itself, which pass through the active site and in effect express the active site in the overlying vicinal water layer. This retains some of the characteristics of a "lock-and-key" mechanism but with a dynamic rather than static character in which HOMOs and LUMOs of the enzyme substrate and of the water molecules at the active site become perfectly matched both spatially and, crucially, in terms of their relative energy levels. The mechanism of the CA-catalysed reaction as currently understood and described in the book already invokes a "proton wire" component – what I am suggesting is that this "proton wire" is actually part of a macrocyclic ring involving both vicinal water molecules and functional groups on and in the enzyme.

What else do we find in the book? It was refreshing to find historical aspects of CA research described in some detail. The first paper in the book cites references dating back to the 1930s and earlier. It is so important to revisit the early literature because the currency of science is ultimately the experimental observation, not the story that is developed to account for the observation. Stories will change as new experimental data emerge but the story has to remain consistent with both old and new experimental observations. I also liked the personal stories provided in two papers as a postscript to the main body of the book – stories that explore the thoughts and aspirations of scientists involved in developing the field over a period of 40 years or so. It is also worth noting that the book contains a subject index at the end, a feature that is often omitted from similar such books. Unfortunately, it has missed at least one reference to acute mountain sickness on page 480 (probably because the word "acute" is missing from the phrase on that page).

As regards pharmacy and pharmacology, the book contains seven papers exploring clinically-related topics from the CA literature. On reading these, the impression is gained that clinical (and hence commercial) interest in CA is now focused mainly on the development of inhibitors for use in the topical management of glaucoma. The development of CA inhibitors as diuretics appears to have progressed as far as it is ever likely to progress, mainly because of the side-effects relating to inhibition of the enzyme at sites other than in the kidney, this in turn being a function of the high sequence homology of all the known mammalian isoforms of CA.

Because of the difficulties relating to unwanted and unavoidable side-effects alluded to above, discussions of the roles of CA in the nervous system, where there is the potential to develop CA inhibitors for use as anticonvulsants, are also mainly of only theoretical interest. The way in which the required specificity of action will be obtained, we may suppose, is through the development of an intelligent delivery system that targets only the required tissue(s). This may be an intractable problem.

On reading the book, I discovered that CAs are of almost ubiquitous distribution in nature ... but of course have rather different functions in different organisms. So, there is something in the book for those with an interest in plant, algal, and bacterial biochemistry. There are also papers on the genetics of CA, and on the physiological roles of CA, and even on the potential applications of CA (i.e. the apo-enzyme) in biosensors for detecting trace quantities of dissolved zinc.

Overall, this is an interesting book to read. It provides a fine example of the fractal structure of scientific knowledge. As new information is uncovered further layers of yet-to-be researched topics are revealed. This is reflected in the sub-title to the book: New Horizons. It is the nature of a fractal structure that there are new horizons beneath each visible layer, *ad infinitum*. It is also true that as we travel deeper and deeper, the information we reveal becomes technologically more and more difficult to address in the context of human disease management. This is the ultimate irony for pharmacy and pharmacology and is so wonderfully exposed by this book.

Dr Richard J. Schmidt spent almost 20 years teaching pharmacognosy, biochemistry and pharmaceutical chemistry at the Welsh School of Pharmacy in Cardiff. His research interests addressed redox processes in skin in the context of prohapten activation in allergic contact dermatitis and the development of new biomaterials for use as modulators of the redox environment in the treatment of chronic ulcers. After a couple of years with Johnson & Johnson Medical, he now works as an intellectual property realisation consultant and locum community pharmacist.