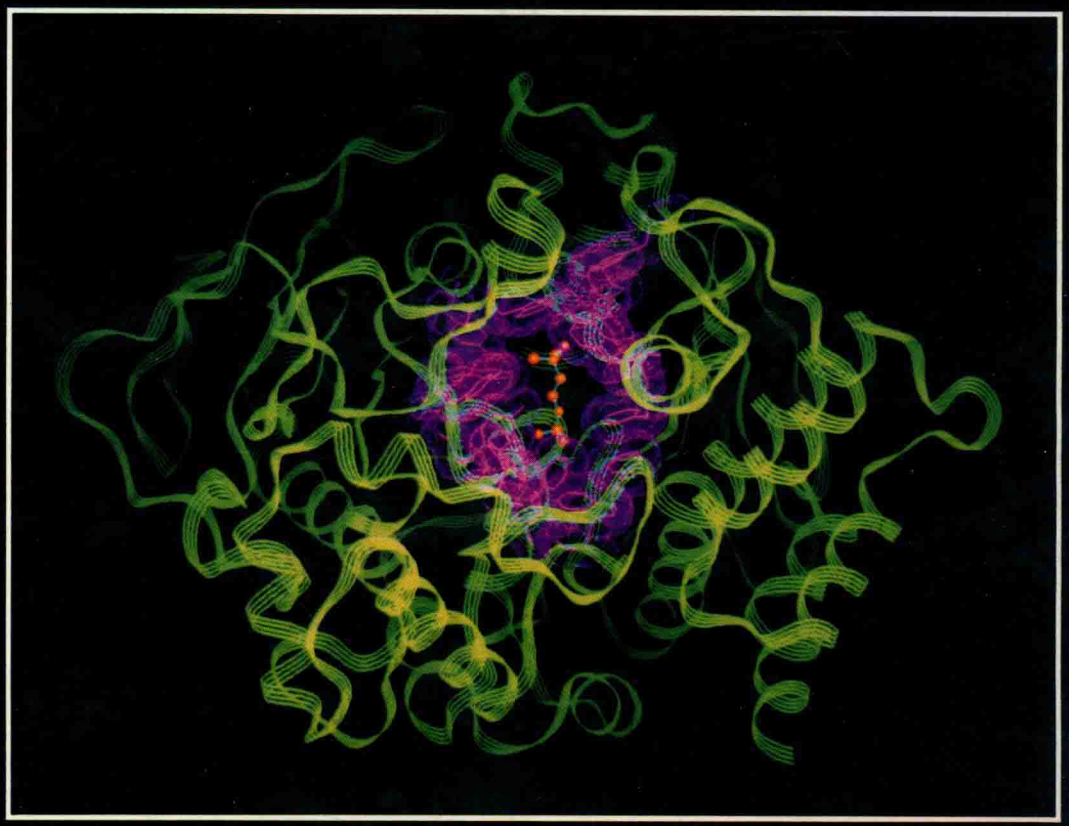
Multidisciplinary Approaches to Cholinesterase Functions



Edited by
Avigdor Shafferman
and
Baruch Velan

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Israel Institute for Biological Research
Ness-Ziona, Israel

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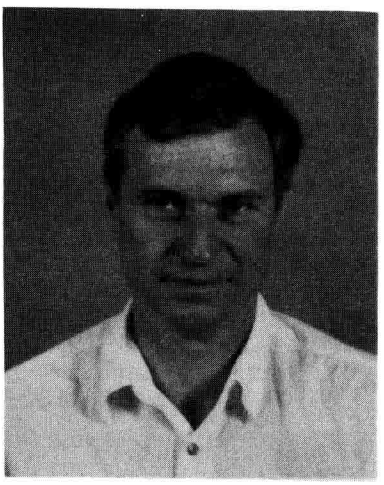
Christian Hirth 1944-1992



Christian Hirth succumbed to a sudden heart attack on May 28, 1992; he was only 48 years old at the time. He was a professor of organic chemistry at Université Louis Pasteur in Strasbourg, where he was director of a CNRS research unit.

Christian obtained his Ph.D. thesis at Strasbourg in the laboratory of Jean-François Biellmann in 1972 and was a postdoctoral fellow under Georges Cohen at the Pasteur Institute in Paris, where he extended his interests to biology and made many fruitful and long-lasting associations. On his return to Strasbourg in 1975, he set up, together with his friend and colleague, Maurice Goeldner, a research team specializing in bioorganic chemistry, in the laboratory of Professor Guy Ourisson.

It was in this framework that he carried out his most important research, which involved the design and utilization of photosuicide compounds as topographic probes of biological macromolecules, among which the acetylcholine receptor and acetylcholinesterase were particularly noteworthy subjects.

His achievements led to the establishment of the CNRS research unit which he directed. He had just recently started to organize a new research team in the Protein Engineering Laboratory of the CEA at Saclay.

Christian's science was both rigorous and original. At the time of his death, he was making a major contribution to our understanding of the structure of cholinergic recognition sites. He was an invited speaker at the 36th Oholo Conference in Eilat which, sadly, was the last meeting he attended.

Christian was liked and respected by his collaborators and by those who encountered him at scientific meetings. He was a warm and loyal friend and colleague, with a great sense of humor, often sharp but never unkind. His death, in the midst of a brilliant scientific carrier, is a great loss to all of us, and we will miss him very much.

PREFACE

Acetylcholinesterase plays a key role in cholinergic transmission. By rapid hydrolysis of the neurotransmitter acetylcholine the enzyme terminates the chemical impluse, thereby allowing rapid repetitive responses. Inhibitors of cholinesterases have important applications in agriculture - pest control, and in medicine - treatment of various disorders such as myasthenia gravis, glaucoma and management of Alzheimer's disease. These are some of the reasons for the special attraction of scientists from multiple disciplines such as neurobiology, pharmacology, chemistry, biochemistry, molecular biology and biotechnology to cholinesterase research.

We have been fortunate to organize the 36th OHOLO conference on *Multidisciplinary Approaches to Cholinesterase Functions* at a time when this field of research is bursting with new concepts and ideas, brought about by the recent structural resolution of the catalytic subunit of acetylcholinesterase and by application of methodologies related to molecular genetics and protein engineering. This volume summarizes most of the important advances resulting from these developments as reflected in the OHOLO meeting.

The first article in this volume provides an overview of the large body of investigations accumulated over a century and a perspective on past and future studies on cholinesterases. This is followed by articles dedicated to biochemical and molecular genetic studies that reveal the origin and extent of the intriguing multiplicity of the molecular forms of cholinesterases. Papers describing the regulated expression of acetylcholinesterase in hematopoietic, muscle and nervous systems, as well as in engineered mammalian and bacterial high level expression systems, shed light on various postranscriptional and postranslational modifications of cholinesterases. The section dealing with polymorphism and structure includes papers describing the three dimensional structures of the *Torpedo* acetylchoninesterase and different enzymes sharing common folding patterns, despite the absence of sequence identity. The report on the crystal structure of two complexes of *Torpedo* acetylcholinesterase with ligands of clinical importance is yet another step in unravelling the unique catalytic functions of acetylcholinesterase. These reports together with articles describing site directed labeling and analysis of site directed mutants of cholinesterases, have identified amino acids constituting the catalytic triad and residues related to the peripheral and catalytic anionic subsites. Further insight into functional domains of cholinesterases and the role of particular amino acids, is provided by kinetic studies with various inhibitors and the analysis of natural mutants of human acetylcholinesterase and butyrylcholinesterase, as well as of *Drosophila* acetylcholinesterase insecticide resistant strains. Some articles are involved in the classical challenges of the physiological and developmental functions of cholinesterases, as well as in the possibility that these molecules fulfill functions other than inactivation of acetylcholine at the synapses.

A few papers address the clinical implications of acetylcholinesterase as a pretreatment drug for organophosphate toxicity and the potential use of different acetylcholinesterase inhibitors in the treatment of Alzheimer's disease. Finally, the contribution from a number of researchers from different labs provides a set of recommendations for a unifying nomenclature for the cholinesterase genes, their transcription and translation products, the numeration of amino acids and secondary structural motifs of acetylcholinesterases and related proteins.

We are obliged to all the contributors to this volume and to our colleagues: Palmer Taylor, Jean Massoulie, Hermona Soreq, Gabriel Amitai, Yaacov Ashani and Israel Silman, who were instrumental in formulating the scientific scope of the meeting. We hope that the volume contains a wealth of information that will generate new directions in cholinesterase research.

Avigdor Shafferman
Baruch Velan

June 1992
Israel Institute For Biological Research
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CONTENTS

IMPACT OF RECOMBINANT DNA TECHNOLOGY AND PROTEIN STRUCTURE DETERMINATION ON PAST AND FUTURE STUDIES ON ACETYLCHOLINESTERASE

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It is a pleasure and challenge to open the scientific sessions of the 36th Oholo Conference. The pleasure comes from the opportunity to participate in a conference organized at a most opportune time in the development of the field of cholinesterases. Avigdor Shafferman, Baruch Velan and their colleagues who have served as local organizers have planned this conference with unwavering enthusiasm in what has been a politically difficult period for Israel. My challenge stems from attempting to assemble from a large body of investigations a perspective on past and future studies on the cholinesterases. Studies on cholinesterases have been propelled by advances in recombinant DNA technology, protein chemistry and crystallography over the past six years, and our field of endeavor now fully encompasses the span from gene to protein, both in terms of structure and function. The work of Joel Sussman, Israel Silman and their colleagues has provided us with structural resolution of the catalytic subunits of acetylcholinesterase (AChE) extending to 2.8Å resolution (Sussman et al., 1991). This development now enables us to relate aspects of catalytic function and inhibitor binding to structural domains and to particular amino acid residues. With structural resolution of the cholinesterases at an atomic level, virtual reality emerges within the field.

At the other end of the spectrum, the genes encoding mammalian AChE and butyrylcholinesterase have been characterized (Li et al., 1991; Arpagaus et al., 1990) and localized to the chromosomal positions 7q22 and 3q26 respectively in man (Getman et al., 1992; Gaughan et al., 1991; Allderdice et al., 1991) and the AChE gene localizes to distal region of chromosome 5 in mouse, a region syntenic with human 7q (Rachinsky et al., 1992). While the structural resolution of chromosome localization is orders of magnitude less than that yielded by the crystal structure of AChE, recombinant DNA technology and nucleic acid sequencing enable one to determine rapidly essential elements in gene structure. Moreover, the power of genetics can be applied to the cholinesterases as can be seen from the correspondence

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of AChE and the QT-blood group antigen (Zelinski et al., 1991; Bartels and Lockridge, 1992). Virtually all of what we discuss over the next 4 days will either relate to the boundaries of the gene and protein themselves or extend to intermediary processes of gene transcription, mRNA stabilization, mRNA translation, peptide processing and secretion of the newly biosynthesized enzyme.

WHERE WE'VE BEEN

Cholinesterase research predates its very identification as an enzyme and, in fact, extends back to the mid-19th century. Various investigators were intrigued with the pharmacologic actions of the calabar bean in the early 1860's where both animal and self-administration were used to study its pharmacology. Argyll-Robinson's accounts of his responses to self-administration of extracts into one of his eyes using the other eye as a corresponding control are particularly enlightening (Argyll-Robinson, 1863). The first organophosphate inhibitor of cholinesterases was synthesized in the same period by Clermont.

.It was not until 1914 and the appearance Sir Henry Dale's classic manuscript on the nicotine- and muscarinic-like actions of the esters of choline that the concept of physostigmine inhibiting an enzyme responsible for the degradation of a natural neurotransmitter in the cholinergic nervous system emerged (Dale, 1914). Nevertheless, the name cholinesterase was coined in a paper by the organic medicinal chemist, Stedman some 18 years later (Stedman et al., 1932).

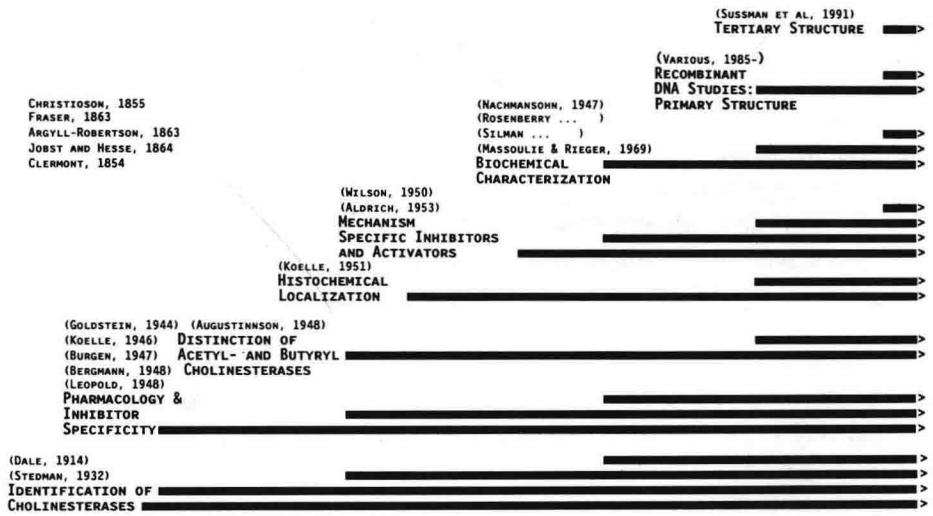

Fig. 1. Developments in the Field of Cholinesterases. An attempt is made to show escalating developments in the field with some of the critical studies and investigators that initiated new avenues of endeavor. New approaches also contributed substantially to ongoing research currents; major synergistic contributions are shown by the staircase additions. The early studies in the mid-19th century were concerned only with the pharmacologic effects of inhibitors without reference to the enzyme and are not included in the staircase.