CIBA FOUNDATION SYMPOSIUM

Jointly with

CO-ORDINATING COMMITTEE
FOR SYMPOSIA ON DRUG ACTION

on

ENZYMES AND DRUG ACTION

Editor for the Co-ordinating Committee

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PREFACE

THIS symposium is the eighth in the series organized by the Co-ordinating Committee for Symposia on Drug Action. In choosing so general a subject as Enzymes and Drug Action it was hoped to take account of some of the many growing points in biochemistry which are likely to be of importance for future advances in pharmacology.

The symposium was divided into two parts. The first took the form of a meeting at the Wellcome Building with papers and discussion before an audience of about three hundred. The second took the form of a small meeting at the Ciba Foundation consisting almost entirely of unscripted discussion by a panel of about thirty participants. The proceedings at these two meetings are published in separate sections of the present volume as one of the series of Ciba Foundation Symposia. It is edited jointly by the Co-ordinating Committee for Symposia on Drug Action and by the Ciba Foundation.

The main responsibility for the meeting lay in the hands of the British Pharmacological Society with the co-operation of the Biochemical Society, the Physiological Society and the Royal Society of Medicine. The Organizing Committee consisted of W. A. Bain, F. Bergel (Chairman), A. C. Dornhorst, H. McIlwain, J. L. Mongar (Secretary) and H. O. Schild. The Committee is grateful to the Wellcome Foundation for the facilities provided at the Wellcome Building, to the Wellcome Trust for financing the Symposium, and to the Ciba Foundation not only for generous hospitality and travelling expenses for some of the overseas members, and for providing a unique Conference Room whose intimate atmosphere provided an ideal setting for the second part of the meeting, but also for the excellent editorial facilities which make possible the early appearance of the proceedings of the symposium.

List of those presenting papers or contributing to the discussion on Enzymes and Drug Action held at the Wellcome Building and the Ciba Foundation, 20th–23rd March 1961

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R. J. P. WILLIAMS	Dept. of Inorganic Chemistry, University of Oxford
R. T. WILLIAMS	Dept. of Biochemistry, St. Mary's Hospital Medical School, London
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Session 1: Enzymes as Primary Points of Drug Action

CHAIRMAN: F. Bergel

CHAIRMAN'S INTRODUCTION

F. BERGEL

Chester Beatty Research Institute, London

THIS symposium aims to cover a wide field involving a number of highly controversial aspects, but to begin with Drs. Wilson and Davenport will review investigations in which we have relatively more knowledge.

In introducing the session I would like to consider in a general way what one can do from a biochemical or pharmacological point of view with enzyme systems *in vivo* (and for precise measurements *in vitro*) to bring about changes in their activities, which might be followed in certain cases in men and animals by physiological changes.

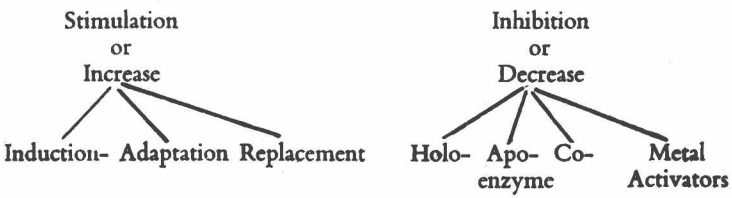

FIG. 1. Possible alterations of enzyme activities.

One can stimulate the enzyme activity either by a process of induction-adaptation, that is by an initiation or increase of its biosynthesis, or by providing the organism with more co-factors,

namely, coenzymes and metals (I am not touching on the replacement of apoenzymes). Alternatively one can inhibit the enzyme or antagonize its activities. In both cases these procedures will lead to observable pharmacological effects in animals or man only if the enzyme or enzymes immediately or decisively control events, whose alteration through drugs may produce an imbalance of some essential physiological functions. Adaptation and induction

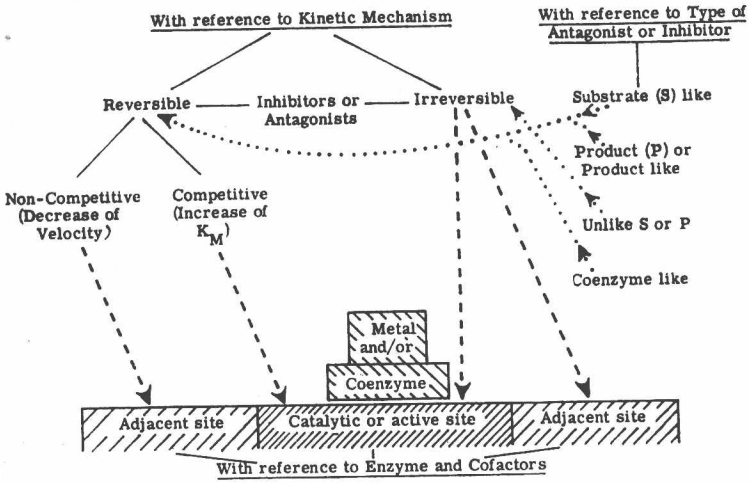

FIG. 2. Mechanism of action of enzyme inhibitors and antagonists: ------, interaction with sites; ·····, interaction with antagonists. [Courtesy of Charles C. Thomas, Publisher, Springfield, Ill., *from* Bergel, F. (1961). Chemistry of Enzymes in Cancer.]

phenomena have been more frequently observed in micro-organisms than in mammals. But in the form of increased intake of substrate (in the widest sense), hormonal application and dietary changes (proteins, vitamins, minerals) one might be able to influence levels of activity, though pharmacological effects are infrequent. Later sessions will return to these problems particularly in connexion with drug metabolism.

With reference to enzymes as primary points of attack, inhibitors or antagonists are the more important drugs.

In Fig. 2 I have assembled a number of general points. First, the classical inhibitors can be substrate-like, product or product-like, coenzyme-like, or they can be unlike the substrate, product or coenzyme. In the latter case the interaction with the enzyme takes place by formation of covalent or ionic bonds, stronger than those of the normal substrate, and is usually "irreversible"; for example, organic phosphates as anti-cholinesterases. This has to be contrasted with substrate-like reversible inhibitors which can act either competitively (neostigmine etc.) or non-competitively (interfering with sites adjacent to the catalytic centre). Mixed types are being developed (compare Baker's non-classical antagonists) which may fit structurally and sterically into the active site, but also carry an additional group which by chemical interaction with the adjacent site or the coenzyme could fix themselves conveniently there and so facilitate antagonism to substrate absorption at the catalytic site (phosphostigmines). Interference with coenzymes and/or metals may also result in paralysis of enzymes. These general points represent of course an oversimplified chart of the phenomena which may be complex and multiple in nature, but they may help as a guide to the detailed reports and discussions to follow. Future developments should aim at still more specific inhibitors and future investigations may discover either enzymes or whole metabolic pathways the blocking of which could produce further desirable pharmacological effects. Such results could also explain the mechanisms of drug actions as yet little understood. The potentialities of enzyme model systems cannot be assessed yet, but it is hoped that enzymo-mimetic substances will contribute to our general and specific knowledge in the field of enzymes and drug action.

INHIBITION OF ACETYLCHOLINESTERASE

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OUR state of knowledge concerning the inhibition of acetylcholinesterase is sufficiently advanced to make it profitable to present the theory in a deductive fashion. We start with an hypothesis concerning the chemical behaviour of the enzyme. This hypothesis was, of course, arrived at in no small measure from the study of inhibitors. If it does no more than sum up and thereby explain our knowledge, it would be valuable but it also leads to new ideas.

The active site of the enzyme is considered to consist of two subsites, an anionic site which binds and orients substituted ammonium ions and an esteratic site containing an essential acidic and basic group, which binds ester (and similar) functions and reacts further in the hydrolytic process (Fig. 1).

The forces of binding at the anionic site are coulombic (Adams and Whitaker, 1950; Wilson and Bergmann, 1950) and hydrophobic (Wilson, 1952*a*). The electrical potential is the sum of the potentials arising from all the charges on the protein molecule and is equivalent to a single negative electronic charge at the distance of closest approach. It may be, perhaps, that the charge is borne by an atom at the surface of the anionic site and that all other charges are so removed that their contribution is small, but we can at present make no decision. This force contributes 2 kcal. (a factor of 30) to the binding.

Nonpolar substituents, alkyl and aryl groups, tend to be driven from the water phase and to combine with nonpolar side chains

in the protein. The forces involved can be referred to loosely and nonspecifically as van der Waals forces but the more descriptive term hydrophobic bond is coming increasingly into use. Each methyl group bar one, contributes 1·2 kcal. (a factor of 7) on the average to the binding of small molecules. It appears that one

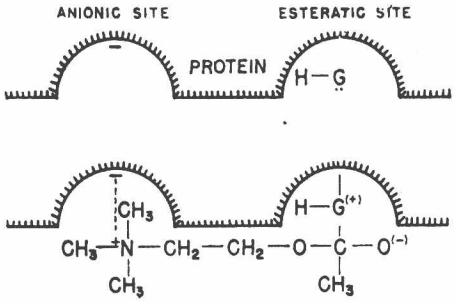
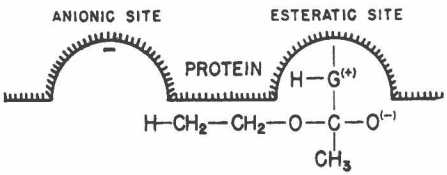


FIG. 1. Representation of the active site of acetylcholinesterase and of the Michaelis-Menten complex with acetylcholine.



Representation of the Michaelis-Menten complex of acetylcholinesterase and ethylacetate.

group projects into the solution phase and so makes no binding contribution.

We can compare the binding of ethyl acetate with the binding of acetylcholine. Compared to acetylcholine, ethyl acetate lacks a positive charge and two contributing methyl groups and should therefore be bound about 1500 times less strongly. The binding constants cannot be measured but the ratio of the Michaelis constants is of this order of magnitude. The specificity is to a very