Preface and Introduction

Information on inhibitors of metabolic processes or enzyme systems is widely distributed in the scientific literature today. Such inhibitors are being used in all fields of enzyme investigations or in studies of cell metabolism generally, where they are being applied to an increasing extent in a variety of experimental as well as practical aspects ranging from medicine to agriculture. While many investigators use inhibitors in the course of their work, not all are aware of the relevant but scattered information concerning them nor of the full implications of the results obtained through their use. There is not, at the present time, a truly comprehensive treatise devoted to the many aspects of the properties of metabolic inhibitors as we know them today, although certain specific areas have received attention from time to time (as in the *Annual Review of Biochemistry* or *Annual Review of Pharmacology*) and much valuable material may be found in the various monographs, textbooks, and treatises devoted to the properties of enzymes.

It is proposed, in these two volumes of "Metabolic Inhibitors", to provide a comprehensive and authoritative presentation of the properties of metabolic inhibitors which are judged to be of value to the research worker in the various fields of biological chemistry as well as to the advanced student. Moreover, the volumes are intended to be broad in their coverage and to include information that may be of service not only to biochemists and medical research workers but also to others working in the microbiological, botanical, and other agricultural fields. It is hoped that these volumes will present the first comprehensive treatise on the uses of the wide variety of substances which interfere with (and usually retard) metabolic and enzymic processes. The emphasis on the whole is on the inhibitor rather than on the metabolic or enzymic system which is affected.

It is the Editors' hope that these volumes will become a stimulus to further research, that they may help to delineate those areas of knowledge which require further clarification, and that they may emphasize the gaps which exist in our present state of knowledge. "Metabolic Inhibitors" will include data which should enable the investigator to identify the point of attack by an inhibitor of a given metabolic process, whether it be a specific enzyme or a connecting link in a multienzyme system. The importance of

inhibitor techniques in throwing useful light on many biological processes will be emphasized and their application to subjects of great practical value, such as are found in pharmacology, in medicine, or in agriculture, pointed out.

The subject of inhibition kinetics is not covered in "Metabolic Inhibitors" as it is felt that this very specialized area of the field has been exceedingly well documented in a number of recent publications(*1–8*)which are readily accessible. Duplication would serve no really useful purpose.

Some Historical Notes

In the realm of pharmacology or physiology concerned with the mechanism of drug action increasing attention is being paid to the behavior of enzymes. This subject has grown enormously within the last two decades. A glance at the early literature on the effects of poisons and inhibitors of biological systems reveals much empirical knowledge concerning the effects of a wide variety of inorganic substances (e.g., fluoride, cyanide, arsenite, hydrogen sulfide, salts of mercury, lead, iron, barium) and of organic materials (e.g., antiseptics and narcotics such as toluene, chloroform, chloral, dyestuffs) on enzyme preparations of various degrees of purity and on cell life in general. The respiratory inhibitors carbon monoxide and cyanide provided much valuable early knowledge in the development of this field.

A very considerable stimulus to the study of the mode of action of biological inhibitors came with the recognition of the principle of competitive inhibition. This principle is widely accepted today as an aid to the understanding of the mechanisms of action of many drugs and as a means for the investigation of problems of chemotherapy. A number of reviews have been written on the subject (*9–13*).

The modern conception of the principle of competitive inhibition by structural analogues arose as a result of investigations (*14*) carried out with the dehydrogenase systems of bacteria. It was concluded (*14–16*) that the active center of an enzyme is so constituted that it may combine with a variety of substances all possessing a particular type of chemical structure but that, of all the molecules having this structure, only a few are actually substrates, i.e., they are capable of being activated to undergo subsequent chemical change. It followed from this conclusion that many molecules may compete with each other and with the substrate for attachment to an enzyme. Thus the presence of the structural analogue of the substrate of the particular enzyme would have the effect of diminishing the activity of that enzyme toward the substrate. An important example of this phenomenon was the competitive inhibitory effect of malonate on succinic dehydrogenase

(*14*). It was evident that structural analogues, which would combine with an enzyme but which would not be activated, could act as competitive antagonists to the substrate if they were present in the enzyme system at sufficiently high concentrations. The magnitude of the inhibition thus obtained would be dependent on the relative affinities of analogues and of substrates to the enzyme.

That a structural analogue may also act as an antimetabolite in the modern sense of the word was demonstrated quite early (*17*). It was shown that the proliferation of *Escherichia coli* cells in a medium containing fumarate as the sole source of carbon was greatly inhibited if malonate was added to the medium. Although malonate acted as an inhibitor of bacterial growth in this system it was not a cell poison, for it had no inhibitory effects under conditions where the oxidation of fumarate was not required to provide the bacteria with carbon for biosynthetic purposes.

The inhibitory effect of malonate on succinic dehydrogenase has proven to be a most valuable tool in studies of the respiratory systems of living cells. The phenomenon was fundamental to the studies of Szent-Györgyi (*18*) on fumaric acid catalysis in muscle respiration and to the work of Krebs (*19*) and later workers on the tricarboxylic acid cycle.

The practical value of the principle of competitive inhibition was not generally appreciated until 1940 when it was reported (*20*) that the bacteriostatic action of sulfanilamide and related sulfonamide drugs, which had come into prominence following the initial discovery of the antibacterial activity of Prontosil, was reversed competitively by *p*-aminobenzoate. The competitive relationship, taken together with the similarity in chemical structure between sulfanilamide and the antagonist as well as the high activity of the latter, led to a working hypothesis concerning the mode of action of sulfonamides. It was suggested that *p*-aminobenzoate is an essential growth factor for bacteria and that sulfonamides, by virtue of their structural similarity, competitively inhibit the enzyme system involved in the utilization of this substrate. This hypothesis received its most important confirmation by the discovery that *p*-aminobenzoate is a precursor in the biosynthesis of folic acid and other cell constituents. Recognition of the importance of the principle of competitive inhibition as an explanation for the mechanism of action of the sulfonamides stimulated a search for analogues of other known bacterial factors. As a result a large number of structural analogues which inhibit the growth of microorganisms in specific ways have been discovered. Some examples are the folic acid and purine antagonists that have been used in cancer chemotherapy, and nicotinic acid, amino acid, and amine analogues. The list of such analogues which are becoming available is constantly expanding and it can be said with

considerable confidence that, both from the practical as well as from the theoretical point of view, their use has led to a better understanding of the highly complex machinery of the living cell.

Competition between structurally allied substances for receptor sites is a well-recognized principle in the development of chemotherapy today, and among the outstanding examples which might be mentioned are the anticholinesterases and the antihistamines. It is, however, recognized also that a structural analogue may have features in common with those of substrates for different enzymes, or of substances attached to different receptor sites and may, therefore, produce multiple effects in the cell. Such behavior is exemplified by Benzedrine (or phenylisopropylamine). Thus, Benzedrine competitively inhibits amine oxidation in brain (*21*), and it was suggested early that this suppression of amine oxidase by a structural analogue of its substrate was concerned with the known effects of Benzedrine on the nervous system. Later work, however, showed that the affinity of this drug was even greater for another enzyme, viz., choline oxidase (*22*), than for amine oxidase. Perhaps the most recent examples of *in vivo* multiple effects of the most serious nature have been the results observed following thalidomide administration to pregnant mothers in many parts of the world. Pharmacological effects of a drug may thus be dependent upon its affinity for more than one enzyme or receptor site.

It has been recognized also that a structural analogue for a given system may be first converted by the cell into a new substance which is the true inhibitor of an observed metabolic process. Thus, the nicotinamide analogue acetylpyridine leads to the formation of a "false" nicotinamide adenine dinucleotide, azaguanine is first incorporated into a nucleotide, and fluoroacetate leads to the formation of fluorocitrate which is the aconitase inhibitor, to mention only a few. Details of many of these phenomena that have a direct bearing on modern concepts of metabolic inhibition will be found in the various chapters of "Metabolic Inhibitors."

The Future

There seems to be little doubt, when the general field of metabolic inhibition is contemplated, that the role of metabolic inhibitors in the direct control of disease is undergoing serious assessment at the present time. The manner in which inhibitors act, and a firm knowledge of the underlying principles concerning their activities, will provide the means in the future for the development of new drugs with greater potency and specificity. The recognition of the importance of feedback mechanisms, whereby products of enzymic reactions control the rates of their own synthesis, or of cases where a metabolite of one enzymic reaction sequence is also a com-

petitive inhibitor of another, separate but related, sequence is an important step forward in our understanding of cellular reaction processes. An appreciation of the properties of metabolic inhibitors drawn from a great variety of enzyme studies is vital for work which is expected to lead to further understanding of abnormal cell growth, for the development of antitumor agents, for obtaining greater knowledge of genetic relationships to disease conditions, and for the exploration of growth processes as distinct from static metabolic states.

A relatively new field in the realms of biochemistry and physiology is emerging from the recent work on transport carriers across cell membranes. Here again, a knowledge of the effects of structural analogues and other types of metabolic inhibitors on the specific processes governing the activity of such carriers is of paramount importance in the development of experimental approaches to problems of this type. This field promises to be as crucial in future work on the control of cell behavior as knowledge of specific inhibitory phenomena of isolated enzyme systems has been in bringing us to the present stage of understanding.

Editorial Notes and Acknowledgments

We are well aware that, even though we have tried to produce a truly comprehensive treatise, gaps still exist and attempts will be made at a later date to close these. We have attempted to avoid excessive overlap between chapters and duplication of material but have not demanded rigorous shortening of articles since it is our firm belief that authors should be allowed to express their own opinions. In the interest of encouraging original thought and further research we have not sought to alter individual conclusions even though they may differ from those of other authors. The disparity in the lengths of the contributions has been inevitable since we have favored an individualistic approach to each assignment.

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