A STUDY OF
Antimetabolites

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Preface

The purpose of this book is to present an idea, or more correctly, a group of closely related ideas. These have arisen during the past decade as a result of the finding of a number of experimental facts, all of which indicate that the biological functioning of diverse kinds of essential metabolic substances can be antagonized by the presence of other compounds closely related in chemical structure to them. From these facts have arisen an opinion, which is held in several quarters, that useful pharmacological agents may be realized by careful study of this phenomenon, and that a deeper insight into natural processes of the living world may be uncovered with them. Such ideas have been a powerful stimulant to experimentation in this field, and a considerable body of knowledge is being rapidly accumulated. It was felt that a useful purpose might be served if this factual information were assembled in as brief a form as is consistent with clarity, and also if attention were called to underlying principles which seem to lie close to the experimental findings.

In writing a monograph of this sort the author is in an unenviable position. The field is new, being scarcely ten years old, and consequently information is not as complete as one would desire. Hypotheses to explain the few facts have flourished, and many of them are championed vigorously by their originators.

The author of this book has attempted to summarize most of the facts in this field with which he is familiar, and to place these prominently in front. Generalizations intended to help in the assimilation of these facts have been proposed, and opinions about the applications of the knowledge now at our disposal to practical and theoretical problems have been discussed. It is hoped that most of these opinions will meet the searching criticism of new factual information collected in the future, but it is realized that modifications will be needed in many of them. However, they do not represent casual judgments. Rather they have been weighed carefully over a period of years and are presented as the nearest approximation to the truth which present knowledge has permitted. Many of them were set forth in rudimentary form several years ago in short papers in *Physiological Reviews*, **27**, 308 and in *Science*, **100**, 579.

PREFACE

Although the present work has been completed (except for a few footnotes) for the past two years, the question of publication of it was much debated in the mind of the author. It had been written with the intention of presenting an intelligible account of experimental results which have been accumulating over the past decade from the efforts of many individuals, and which have seemed unintelligible to some, unconnected to many others, and meaningful to a few. The book deals with the questions and answers from natural occurrences and from human needs which have unfolded to the author from his own experiments and from the reading about those of others.

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Contents

CONTENTS

INTRODUCTION

Discovery and development of ideas about antimetabolites

To write history respectably that is to abbreviate dispatches and make extracts from speeches, to intersperse in due proportions epithets of praise and abhorrence . . . all this is very easy, but to be a really great historian is perhaps the rarest of intellectual distinctions. Many scientific works are in their kind absolutely perfect. There are poems which we should be inclined to designate as faultless or as disfigured only by blemishes which pass unnoticed in the general blaze of excellence. . . . But we are acquainted with no history which approaches to our notion of what a history ought to be, with no history which does not widely depart either on the right hand or on the left from the exact line. The cause may easily be assigned. This province of literature is a debatable land which lies on the confines of two distinct territories. It is under the jurisdiction of two hostile powers, and like other districts similarly situated, it is ill defined, ill cultivated and ill regulated. Instead of being equally shared between its two rulers, the reason and the imagination, it falls alternately under the sole and absolute domain of each; it is sometimes fiction, it is sometimes theory.

The perfect historian is he in whose work the character and spirit of an age is exhibited in miniature. He relates no fact, he attributes no expression to his characters which is not authenticated by sufficient testimony, but by judicious selection, rejection and arrangement he gives to truth those attractions which have been usurped by fiction. In his narrative a new subordination is observed. Some transactions are prominent, others retired, but the

scale on which he represents them is increased or diminished, not according to the dignity of the persons concerned in them, but according to the degree in which they elucidate the condition of society and the nature of man.

—Lord Macaulay, *Essay on History,* London, 1828

The present concept of the antimetabolites has grown slowly with the acquisition of new knowledge of branches of biochemistry. Its roots reach back clearly to the time of Ehrlich, whose Harben Lectures of 1907 (273) present some ideas akin to those which more exact and detailed information has now allowed formulation in more precise terms. The basic concepts have arisen from studies of bacterial nutrition, of animal nutrition, of enzyme action and composition, and of pharmacological antagonisms of drugs. These, and related fields to a lesser degree, have shaped the concept as it now is seen. Along the path of its growth, the work of particular investigators has determined the direction of the search and has provided the framework upon which subsequent experimental observations have been fitted together permanently or until a better structure could be erected to contain them. Some of these outstanding turns in thought will be traced in this introduction in an attempt to see more distinctly how the present position has evolved. In doing so, the judgment of the chronicler may be in error as to details, but the major features should be revealed.

With the investigations of the antimetabolites as with other branches of science, the sequence of events in the emergence of each new idea has usually been first the chance discovery of isolated and unexpected facts. This is then followed by the recognition of the underlying principle. Such recognition frequently results from the bringing together in the mind of one individual of a group of heterogeneous and isolated cases. Each member of this group is seen to be an example of an underlying new phenomenon. The individuals who make such correlations and thus finish the discovery of the new phenomenon are able to do so usually because they carry over a new idea which has been forming in one branch of science and apply it to these heterogeneous chance findings in an allied field. The two stages of discovery are both essential. So long as unpremeditated and unexplained findings are recorded and left to be forgotten they do not constitute discovery. Only when they are activated by an inspiration of the new phenomenon do they become usable and effective knowledge. Finally, as a result of the discovery, a number of explorations are made and from these the validity of the original principle is established. In addition, from these explorations,

the general features of the phenomenon are recognized and classified. Occasionally, these explorations lead to new discoveries which then follow a course similar to that just outlined.

As just indicated, the carrying over of an embryonic idea from one branch of science to another may be followed by a flowering of the transplanted thought in the new field. After it has gained stature and support on the foreign soil, it is frequently brought back again to enrich the land of its origin. These processes can be discerned in an historical account of the antimetabolites.

The inhibition of enzyme action by substances related in structure to the substrate or to the product

Early in the studies of the nature of enzyme action and in the years from 1910 to 1914, Michaelis and his collaborators (230, 274) investigated the inhibition of carbohydrate-hydrolyzing enzymes and observed that some of the inhibitors were similar in chemical structure to the products formed in the reaction. Thus invertase was retarded in its action by fructose, and α-glucosidase was inhibited by glucose. Both these enzymes form the respective inhibitors during the hydrolysis of their normal substrates. This is readily seen by recalling that yeast invertase acts upon sucrose and catalyzes its hydrolysis according to the following equation:

$$\text{Sucrose} + H_2O + \text{invertase} \leftrightarrows \text{glucose} + \text{fructose} + \text{invertase}$$

α-Glucosidase may be represented as acting on a substrate such as α-methyl glucoside as follows:

$$\alpha\text{-Methyl glucoside} + H_2O + \text{enzyme} \leftrightarrows \text{glucose} + \text{methanol} + \text{enzyme}$$

Some residual linking of the product to the enzyme, with the resultant hindering of the action of the latter, was pictured as an explanation of why fructose and glucose were able to cause the inhibition. Such facts and ideas figured prominently in the formulation of the concept about the nature of enzyme action during the early twentieth century.

These studies were limited to inhibition caused by a product of the reaction, and, although these products might be said to be analogs of the substrate, since they do bear structural resemblance to it, the ideas about the matter were clarified when Quastel and Wooldridge in 1927 (114, 275) were able to show marked, competitive inhibition of succinic dehydrogenase by malonic acid, a structural analog of the *substrate* of

the enzyme. They pictured the mechanism of this inhibition much as it is done today, with the possible exception that now some details might be added about the nature of the union of inhibitor with the enzyme. In many of the instances in which the product acted as inhibitor, the structural relationship of the inhibitor to the substrate was clear. The idea that this structural resemblance was the reason for the inhibition gained acceptance in the early part of the twentieth century. By the 1930's, the currently accepted hypothesis to explain competition between substrate and inhibitors related to it in structure was well established among enzymologists. It had not, however, spread to related sections of biochemistry or of biology. The way in which this transition was made is intimately linked with the antimetabolite idea.

The discovery of haptens in immunology

In the years 1920 to 1940, it was discovered that relatively simple substances related in structure to antigens would inhibit the formation of a precipitate when those antigens were mixed with their antibodies. This phenomenon was studied in detail and has been well described by Landsteiner (392) who was largely responsible for its elucidation. The inhibitory substances were called haptens. They were shown to form unions with the specific antibodies, and from these combinations they frequently could not be dislodged by dialysis. One of the major features of a hapten was the occurrence in it of a chemical structure which was also a part of the antigen with which it interfered. In retrospect, it can be recognized how closely the haptens fit into the concept of the antimetabolites, but the realization of this came only after the investigations with antivitamins and antagonistic analogs of hormones, purines, and amino acids had been in progress for several years. Even today the idea is not generally accepted.

Unpremeditated and unexplained findings on the antagonism between certain vitamins, hormones, and amino acids and compounds related to them in chemical structure

In the biochemical and nutritional literature from 1920 to 1940, several instances can be found in which, as a result of chance observations, structural analogs of a vitamin, or a hormone, or an amino acid were discovered to antagonize the biological action of the related metabolite. Such findings seemed remarkable to those who first observed them. In fact, they seemed so unorthodox that critics frequently branded them as errors. A few of these unpremeditated and unexplained observations are noteworthy for the present discussion.

One of the first of such findings, made in 1938 (92), was that β-acetylpyridine and pyridine-3-sulfonic acid were toxic to dogs suffering from nicotinic acid deficiency but were harmless to animals receiving a normal diet. The observations were made during an investigation of various derivatives of nicotinic acid as substitutes for this vitamin, and the possession of toxic rather than beneficial (i.e., vitamin-like) properties in these two analogs was unexpected. After the recognition of the phenomenon of antimetabolites, both of these substances were studied again in a variety of living things and shown to be analogs antagonistic to nicotinic acid.

In 1938, Dyer (84) was interested in the ethyl analog of methionine, viz., ethionine, as a substitute for this amino acid in the diet of rats. She was surprised to learn that when it was given in place of methionine, it caused toxic effects rather than beneficial ones. The astonishment engendered by such an observation is clearly evident in her publication. This toxicity was partially neutralized when methionine was present along with the analog. Ethionine has since proved to be an effective antimetabolite of methionine in a variety of biological systems.

In a series of investigations culminating in 1940, Kuhn (61) and his collaborators ascribed the activity of hormones which elicited male and female behavior in certain algae, not to single compounds, but rather to the ratio between two closely related substances. Either male or female behavior could be called forth by alteration in the proportion of *cis*- and *trans*-dimethylcrocetin to which the cells were exposed. At that time the idea that specific biological responses were due to the action of single substances was so universally held, that the proposal ascribing one of these effects to a ratio between two analogs seemed hard to believe. This proposal about the control of specific biological effects still has much opposition but is beginning to gain favor.

Another one of the unpremeditated findings of antagonism between structurally similar compounds was the competition between acetylcholine and allylisopropylcholine (276) and other quaternary ammonium bases (277, 278); and between acetylcholine and atropine (279) which appeared between 1932 and 1937. Observations of these antagonisms were made by pharmacologists who were considerably baffled in attempts to understand them. In fact, the perplexing shift from one type of pharmacological effect to its exact opposite which may result from relatively slight changes in the structure of a drug poses a problem which is not completely explained today, even though the ideas arising from recent studies with antimetabolites may constitute a rational approach to the problem.

Clark's appraisal of the antagonism between acetylcholine and structurally similar drugs

In 1937 Clark published his explanation of the competitive pharmacological antagonism which exists between acetylcholine and tetraalkyl ammonium salts, and between this same hormone and atropine (279). Such antagonisms were then demonstrated by application of the substances to hearts of frogs and observation of the response in contraction. Clark recognized the structural relationship of the drugs to the hormone and saw in this relationship the possible understanding of the mode of action of the drugs. In 1950 his explanation could be improved only by the addition of the analogies of other antimetabolite demonstrations.

He was able from a consideration of these competitive antagonisms between structurally related drugs such as atropine and acetylcholine to conclude that each member of a pair of antagonistic agents probably reacted in a reversible fashion with one special site in the cell. Being a pharmacologist rather than a biochemist, he did not emphasize that one of the antagonists was a metabolite but rather tended to view the hormone as a drug.[1] He recognized, however, that it was of ubiquitous occurrence in animal species and concluded, therefore, that all animals probably had receptor sites capable of combining with it. His deductions about the mode of action of certain drugs represent one phase of the discovery of antimetabolites in relation to pharmacology.[2] If he had emphasized the metabolite—rather than the drug—properties of acetylcholine, he probably would have been able to make the postulations which recently have energized explorations in chemotherapy.

Clark's conclusions did not find ready understanding or acceptance among pharmacologists, possibly because they were delayed in circulation by the World War, but, within a few years, the field of antimetabolites expanded so rapidly that his contribution was soon merged in a large number of similar views. Biochemical explanations of the mode of action of drugs (the antimetabolite postulate is a biochemical explanation) are usually viewed with suspicion and accepted with con-

[1] Acetylcholine was discovered pharmacologically early in the twentieth century as just another synthetic compound with powerful actions on animal tissues. Not until twenty years later was its natural occurrence and role as a hormone elucidated. It is, therefore, not surprising that its original character as a drug continued to obscure its metabolite function in the minds of investigators.

[2] Clark recognized clearly that all drug actions were probably not based on competition between structurally similar substances. Quite wisely he pointed out that drug antagonisms were exceedingly common and that his views were applicable only to those cases of competition between pairs of analogous compounds.

siderable reserve in other biological fields. There are far too many good reasons, annotated with experience of the past, why this is probably so.

The discovery of *p*-aminobenzoic acid as an antagonist of the sulfonamide drugs, and postulates about chemotherapy of bacterial diseases which arose from it

In 1940 Woods (34) reported that the bacteriostatic action of sulfanilamide and related sulfonamide drugs was reversed completely and competitively by *p*-aminobenzoic acid. The mode of action of the sulfonamide drugs was therefore pictured as the production in the bacterial cell of a deficiency of the essential metabolite, *p*-aminobenzoic acid. Without this metabolite, the organisms were unable to multiply. *p*-Aminobenzoic acid had not previously been recognized as a biologically important compound, but Woods' prediction that it was such was soon verified amply, both by the finding of several species which could not grow without it (138, 280) and by the recognition of it as an integral part of the vitamin folic acid (281). Woods had been trained at Cambridge where the competitive inhibition of enzymes by structural analogs of their substrates had been discovered, and he had drawn upon this knowledge in making his advance. He was led to his important finding by attempts to isolate a material in yeast extract which was known from the work of Stamp (282) to interfere with the bacteriostatic action of sulfanilamide. Investigation of this substance revealed to him that it was an ether-soluble compound which contained both amino and carboxyl groups, and from this information he was able to predict that it was *p*-aminobenzoic acid. The sulfonamide drugs were thus viewed as structural analogs of this metabolite (see Figure 1, Chapter 1) and were thus said to owe their bacteriostatic properties to this fact.

Since *p*-aminobenzoic acid had now appeared as a metabolite, and the sulfonamide drugs as structurally similar antagonists of it, Fildes, in 1940 (283), proposed that useful chemotherapeutic agents against infectious diseases might be produced by altering the structure of some other vitamin or metabolite so as to achieve an antagonistic analog. This idea was immediately tried, especially since at that time the success of the sulfonamide drugs and the coming success of penicillin had raised lush hopes of triumph over bacterial diseases. In fact, the demonstration of Woods exerted such a great influence because it offered a logical basis for the understanding of the action of some highly useful and practical chemotherapeutic agents, i.e., the sulfonamide drugs. By carrying over from enzymology to the fields of bacterial nutrition and

of chemotherapy the knowledge about antagonism between structural analogs, a new direction was given to these subjects.

Largely stimulated by the Fildes' suggestion, but also partly out of curiosity to see if antagonism between vitamins and their structural relatives was a general phenomenon, many investigators soon produced a variety of analogs of bacterial growth factors and showed that they did indeed inhibit the multiplication of a variety of microbial species, and that such effects could be overcome by the related metabolites. Among the earliest studies was that of Fildes in 1941 (129) who showed that indoleacrylic acid antagonized the growth-stimulating properties of tryptophane for the typhoid bacillus. Also, in 1940, McIlwain (87), an associate of Woods and of Fildes, found that pyridine-3-sulfonic acid antagonized the action of nicotinic acid in several bacteria. In 1941, Snell (171) showed that the sulfonic acid analog of pantothenic acid, pantoyltaurine, competed with that vitamin in several bacterial species. This was confirmed in 1942 by McIlwain (173) and Kuhn et al. (105). Robbins in 1941 (284) and Woolley and White in 1943 (5) found pyrithiamine so to antagonize the action of thiamine. Indeed, studies followed in such profusion that between 1942 and 1947 almost the entire list of compounds to be described in Chapter 1 was accumulated.

The Fildes' hypothesis, however, that useful antibacterial drugs could be formed by suitable alteration of the structure of a given growth factor was not immediately substantiated. Although such analogs would retard multiplication of pathogenic bacteria *in vitro,* they did not seem to succeed *in vivo.* One reason may have been that this postulate lacked an appreciation of the importance of selectivity of action, and a means of predicting what kinds of structural changes should be made to produce useful agents. Furthermore, the idea did not provide a means of discriminating in the choice of metabolite to be selected for alteration but suggested that any essential growth factor might be expected eventually to yield useful agents. It had not been realized that many of the antivitamins would call forth in animals, as well as in bacteria, the signs of deficiency of the vitamins. Even when this had been shown, some investigators still clung to the hope that the deficiency would prove more injurious to the parasite than to the host, or that it would destroy the former in time to allow salvation of the latter by administration of the vitamin. Time and many failures were required to impress the need for understanding of the basis of selectivity of action. Even today the appreciation of this is not common and frequent studies are based on the assumption that, if an analog of some bacterial growth factor can be designed with very high