IONIC INTERPRETATION OF
DRUG ACTION
IN CHEMOTHERAPEUTIC RESEARCH

by

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1955

CHEMICAL PUBLISHING COMPANY, INC.

212 Fifth Avenue, New York, N. Y.

Printed in the United States of America

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Preface

Drugs have been broadly defined as chemical agents which affect living protoplasm. For many decades chemists, pharmacologists and physicians have wrestled with the problem as to what properties a chemical must possess to affect living protoplasm, especially in the highly specific manner which is expected of most modern medicinal agents.

The growth of the art of organic synthesis, and the development of the ability to produce homologs and analogs of active compounds almost at will, have been associated with the tendency to explain the biological activity of drugs on the basis of a purely chemical relationship between structure and activity. The quantitative gradations in activity which accompany variations in the length of the side chain in certain homologous series, as well as the great alterations in activity which may follow the substitution of one type of grouping for another, lend tempting support to this type of explanation. Nevertheless, it should not be forgotten that in the great majority of cases, demonstrations of structure-activity relationships have been retrospective. Few important drugs have been developed as a result of a conscious intent on the part of the chemist to proceed swiftly and unerringly to a new and active compound, solely on the basis of *a priori* considerations. Rather, it has been a tedious process of synthesizing and testing hundreds and sometimes thousands of structural permutations, in the hope that somewhere in this mountain of chaff one may uncover the golden kernel of novel and superior activity.

Against the concept that biological activity is the result of a specific chemical structure are the numerous observations that drugs

of entirely dissimilar constitution may possess identical actions. Furthermore, the same drug often affects the function of tissues which diverge widely in function, species derivation and histochemical characteristics.

In this clouded situation, Dr. Tolstoouhov's book represents a significant contribution by deemphasizing the purely structural characteristics of medicinal agents and by bringing into focus the role played by their physical aspects. He has correlated and integrated a considerable body of otherwise isolated observations by demonstrating the unifying influence of similar physico-chemical properties in the face of quite dissimilar chemical constitution. He has given explanation of the localization of activity of structurally dissimilar drugs on the basis of the relationship of such properties to the complementary characteristics of the site of action. Biological activities at diverse sites are viewed not merely as undesirable side effects, but rather from the perspective that a group of diverse tissues can be anticipated to represent preferential sites of attraction for a particular chemical agent if they possess physico-chemical characteristics in common.

It may be anticipated that only by such thorough exploration of the physico-chemical attributes of compounds can the importance of these properties for medicinal activity be estimated.

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Acknowledgments

The studies of staining, the first determinations of dissociation constants of some common dyes, and the determination of the isoelectric points of tissues were made in the Laboratories of Kings County Hospital, Brooklyn, N. Y., Director: Dr. William Hala. The determination of the isoelectric points of tissues ampholytes and the effect of various fixing fluids on the position of the isoelectric points of tissues was continued in the Laboratories of Sydenham Hospital, New York, N. Y., Director: Dr. M. Goldzieher. The experiments were continued in the Ostro and Hopkinson Laboratories of New York, Directors: Dr. Ivan I. Ostromislensky and Mr. Russell Hopkinson, and Nepera Chemical Co., Directors: Dr. E. Tisza and Dr. D. M. Green.

I am grateful to the Directors of these Laboratories for giving me all facilities to continue my studies and for supplying me with the expensive equipment. I deeply appreciate the assistance of the members of the staffs of these institutions in contributing numerous compounds, making endless determinations of pH, and procuring data of animal experiments. Among them, I wish to mention: W. Jankee, S. Semenoff, V. Troy, R. Thomson, Dr. Merritt C. Fernald, and Miss E. Doran. I deeply appreciate the most painstaking professional corrections of the manuscript by Professor David Lewis. Many thanks are due for the friendly encouragement and criticisms given to me by Dr. E. Tisza, Dr. H. L. Friedman, Dr. F. Anderson, A. Galat, and L. Braitberg. I am very thankful to Miss Susan Morton for the correction of my English and to the Misses J. Gizzardi and D. Abrams for typing the manuscript.

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Chapter 1

Introduction

In the study of chemotherapy, as in the study of other problems where the reaction involves many variables, it becomes necessary to select the dominating variables, without which the reaction simply cannot take place. As these studies will demonstrate, the physicochemical approach to chemotherapy, neglected up to now, is most promising. This was suggested by the work of S. P. L. Sørensen, J. Loeb, L. Michaelis, Carl L. A. Schmidt, Edwin J. Cohn, and others on the physical chemistry of proteins and amino acids. The studies of E. Knecht, A. Seyewetz and L. Pelet Jolivet, and V. Garuti are the basis of our work on staining.

Tissue and Bacteria Staining

We found that staining itself is the formation of poorly soluble salts or complexes of the dye and the tissues or bacteria and that this type of staining depends almost exclusively on four variables: (1) The dissociation constants of the dyes; (2) the isoelectric points of the ampholytes of the tissues or bacteria; (3) the pH of the staining solutions; and (4) the solubility of the poorly soluble complexes or salts of the dyes with the tissues or bacteria, expressed as stability constant or solubility. Using mixtures of acid and basic dyes or two basic dyes, it was found also that the staining, like the simultaneous precipitation of two or more salts (see in the text the precipitations of silver salts), is the result of the distribution of base among several acids or vice versa.

It was also observed that the structure of dyes was the least important for staining. Any investigated dye, at the proper pH and

concentration, was capable of staining any tissue. This eliminated the earlier theory that the structure of the dyes was of great importance.

It was recognized that, in spite of the colloidal state of dyes and tissue ampholytes, their reactivity depended on the presence of electrical charges in the same manner as that of simple electrolytes. The heterogeneous media, precipitated tissue slides or blood smears, only retarded the staining reaction. (Equilibrium was usually reached in 5 minutes.)

The fact that staining is an ionic reaction makes it a unique method for the determination of the isoelectric points of not only whole tissues but also the smallest inclusions inside of cells, such as granules of leukocytes or polar bodies of bacteria. The knowledge of the isoelectric points of tissues and bacteria permitted us to understand the real reason for the selectivity of staining.

The identity of dissociation constants leads us to the assumption that the biological activity of local anesthetics, antiseptics, and many other types of drugs depends on the same four variables as staining. The experimental evidence was very striking. Here again it was shown that the structure previously emphasized was the least important.

The work on the general toxicity of so-called "local anesthetics" convinced us that their action on the central nervous system is similar to that of some of the alkaloids. Here again it was recognized that biological activity is dependent on the values of the dissociation constants of local anesthetics and alkaloids. The great differences in the chemical structures of alkaloids and local anesthetics do not appear to affect their biological activity.

In drawing these general conclusions, we do not wish to say that the dissociation constant of a compound alone is sufficient to explain its biological activity. There are compounds with identical dissociation constants which show different stability in animal organisms.

It is interesting to note that the application of the same ionic interpretation of the reaction between sulfa drugs and the tissues permitted us to predict that the nervous system would be affected as a result of therapy with sulfa drugs. This effect of sulfa drugs on the nervous system demonstrates that they, like many others, do not act on one particular tissue only. They act also on tissues which have isoelectric points nearest in value to those of the tissues of their main activity. In the case of sulfa drugs, this means activity on

bacteria, which have preferably the lowest isoelectric point, pH_i, i.e., which are Gram positive, and on the nervous system. For the same reason, it may be expected that they will be damaging the white blood cells.

The same physicochemical approach permitted us to predict the low general toxicity of sulfa drugs. This is due to their amphoteric nature. It was known from the early work of Carl L. A. Schmidt [112] that two ampholytes with similar isoelectric points had the least chance to react with each other. Therefore, the application of the same reasoning to the problem of neutralization of poisonous bases, toxins, viruses, etc., will be probably even more successful. Working with a mixture of two dyes: (1) a basic and an acid dye; or (2) two basic dyes, it became evident that selectivity of staining is nothing else but redistribution of the base or bases among several acids or vice versa. This principle is illustrated by the precipitation of poorly soluble salts of Ag and Cl, I, CNS, or CrO_4.

From the findings on staining, local anesthetics, antiseptics, and other types of drugs, we have inferred that the biological activity of various basic substances depends on the formation of poorly soluble complexes or even salts with negatively charged bacteria or the nervous system. We tested some acid substances for their biological action. First we studied the precipitation of some local anesthetics, like nupercaine, or alkaloids, such as strychnine, by various organic and inorganic acids, including a number of acid dyes. The experiments pointed to the existence of a correlation between the acid strength of these compounds and the solubility of their salts. The effect of pH on the formation of these salts was evident. It was found that acids containing, e.g., four sulfonic acid groups ($-SO_3H$) can form only neutral salts which are poorly soluble. For example, 1 mol of acid with 4 mols of strychnine will form a poorly soluble salt. The acid salts of the same acid with 1, 2, or 3 mols of strychnine are very soluble. Inorganic chemistry provides many similar examples, e.g., from mono-, di-, and tribasic calcium phosphates only the tricalcium phosphate ($Ca_3(PO_4)_2$) is poorly soluble.

It was possible to prove both biologically and chemically that in the case of soluble acid salts, the base was combined with the acid in the form of an undissociated ion. See for example the articles by S. S. Kety and T. V. Letonoff [97, 98] on the dissociation constant of acid lead citrate and on clinical application of sodium citrate for lead poisoning.

The formation of undissociated soluble salts of poisonous bases with polyvalent acids is extremely important from a therapeutic standpoint. By forming soluble salts, the base can be neutralized and eliminated from the body. The formation of insoluble salts may be dangerous. The precipitated salt can block the blood vessels and kill the animal.

The experimental evidence on animals supporting this theory of neutralization of poisonous bases was satisfactory. Some preliminary work was done on a similar neutralization of some bacterial toxins. We have good reason to believe that viruses, such as that of poliomyelitis anterior, can also be neutralized in a similar manner. In 1949, Dr. Fred R. Klenner[118] reported a very successful treatment of various virus diseases, such as poliomyelitis anterior, virus pneumonia, chicken pox, encephalitis by large doses (1–2 g) of ascorbic acid every 2 hours intravenously.

We realize that the animal body has other means for the neutralization of poisonous bases, e.g., acetylation, formation of anil, ethereal sulfates, glucuronates, and oxidation. However all these means of destruction of poisons are too slow in comparison with neutralization by formation of undissociated salts of basic poisons with polybasic acids. In addition, this type of neutralizations of poisons can be studied quantitatively.

In investigating soluble, but poorly dissociated salts, first of all their dissociation constants had to be measured. The existance of such salts had been assumed by several workers, one of the first among them being L. Michaelis.[4] The measurements of dissociation constant of the salts of bivalent metals with several inorganic and organic acids, done by C. W. Davis[105] and R. Keith Cannon and Andre Kibrick,[110] were of great help. We deal only with the biological effect of ionizable substances, such as common biological dyes, antiseptics, local anesthetics, and alkaloids, on also ionizable tissue ampholytes. So far we have had no chance to work with such substances as hydrocarbons, ethers, or alcohols from the physicochemical standpoint.

We do not claim that the physicochemical approach to chemotherapy is the only one possible; we just want to emphasize that this approach is the most promising and the most neglected.

In this connection, attention is called to the following instances of the structural approach. Starting with the two good and well-known antimalarials: quinine and Atabrin, during World War II in the

United States alone, up to 12,000 compounds were synthesized and biologically tested. To these should be added another 12,000 compounds prepared in Germany during the research in the course of which Atabrin was discovered. Out of these 24,000 compounds perhaps two or three can compete with the original two compounds.

A similar, although less disappointing, picture is found with a new class of therapeutic agents: the antigoiterous compounds. Dr. R. O. Roblin states that there were prepared and tested up to 800 different compounds biologically. There is a possibility of using at least one of them (6-propyl-2-thiouracil) in the treatment of thyrotoxicosis.

Attempts to synthesize the derivatives of the analgetic, amidone, (synthesized in Germany during World War II) were quite futile, as none of the derivatives, prepared by F. F. Blicke, had any analgetic properties.

All this looks very discouraging as it indicates that the relation between the chemical structure and the nature of the action of a drug is unknown. In lack of this knowledge, it is even possible to destroy the biological activity of compounds by preparing the wrong derivatives. Actually, after testing a great many compounds, we learn practically nothing.

Without understanding the real reason for the biological activity of different compounds, the structural chemist becomes lost in a maize of contradictory results which he obtained in trying to correlate structure and biological activity. Also, he often forgets that the effect of various radicals on the properties of compounds, including their biological activity, cannot be summarized by simple addition or subtraction. This was found especially in experiments with local anesthetics where it was possible to study the biological activity without interference from the many complicating factors involved in the distribution of the drugs inside the entire organism.

The selective action of drugs has been known from time immemorial. The biological action of alcohol, quinine, ipecacuanha, digitalis, adonis vernalis, willow bark (salicylates), opium, etc., was recognized at an early stage of human development.

It is quite natural that since the beginning of synthetic organic chemistry, every new organic compound has been tested biologically. This led to the discovery of the therapeutic effect of phenols, aniline derivatives, ether, chloroform, chloral hydrate, organic arsenicals, dyes, etc.

In the later part of the nineteenth century, histologists observed the peculiar selectivity of textile dyes, when they tested them on tissues. It became known that carmine and hematoxylin, after being mixed with salts of iron or aluminium, stain only the nucleus of cells. On the contrary, certain dyes, such as picric acid and eosin, stain only the cytoplasm.

This supplied the background for the work of P. Ehrlich and his well-known side-chain theory. He found that methylene blue not only stained bacteria selectively, but was able to destroy plasmodia malariae in the living organisms.

More important, both from a practical and a theoretical standpoint, was Ehrlich's work with organic arsenicals. After the biological testing of about 1,000 organic arsenicals, he was able to introduce two of them, i.e., Arsphenamine and Neoarsphenamine as specific remedies for syphilis.

All the arsenicals investigated differed from one another in their side chains. They were derivatives of arsenobenzene with different radicals attached to the benzene ring in different positions. Ehrlich noticed great variations in the biological activity of compounds as a result of even small changes in their structure. As he could not find any satisfactory correlation between the biological activity and the structure, he drew the following conclusions:

(1) The biological activity of a compound is strictly qualitative, meaning that for each type of cell or for each type of parasite, it should be possible to find a compound which acts only on this particular cell and not on the others.

(2) The extreme specificity of each drug depends exclusively on the structure of the compound, more particularly on the presence or absence of certain side chains.

It is important to note that Paul Ehrlich's school believed in a strictly qualitative specificity of drug action. This was, however, contrary to their own experiments. For example, the arsenicals were active on *Spirochaeta pallidum* and on a series of trypanosomas.

The nature of specificity was explained by "tropisms" or "philias." For example, the different types of white blood cells were divided into neutrophiles, basophiles, and eosinophiles because some of them "liked" neutral dyes, others basic dyes, and a third group eosin.

The specificity of drugs was described by the term "parasitotropism," which means that a certain drug "likes" a particular parasite, without explaining the real nature of that phenomenon.