DRUG RESISTANCE OF MICROORGANISMS

SCHNITZER and GRUNBERG

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ROBERT J. SCHNITZER
EMANUEL GRUNBERG
Department of Chemotherapy
Hoffmann-LaRoche, Inc.
Nutley, New Jersey

1957
ACADEMIC PRESS INC • PUBLISHERS • NEW YORK

Library of Congress Catalog Card Number: 57–8381

PRINTED IN THE UNITED STATES OF AMERICA

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FOREWORD

To the genius of Paul Ehrlich the world owes chemotherapy. Early this century he set out to find drugs which should cure infections—a unique effort requiring coordination of great resources, mental and material, in the fields of biology and chemistry. Previously less than half a dozen such "specifics" were known—quinine, mercury, ipecacuanha—and they were folk-medicines (except methylene blue, which Ehrlich had tried and which gave some promise of success in treatment of malaria).

The introduction of trypan-red was the first milestone; by means of a single dose an otherwise fatal experimental infection in mice with a trypanosome was sterilized. Now, a large number of compounds are available, potent against various infections in man and domestic animals caused by protozoa, spirochetes, bacteria, and other microorganisms.

It might appear that the complete control of infective diseases by drugs was within grasp, were it not for the menace of drug-resistance. Already certain common bacterial infections, e.g. with staphylococci, which were at first sensitive to a chemotherapeutic agent such as penicillin, have become largely intractable. The basis of this change lies in the parasites themselves and, as a rule, is to be traced to their contact with the compound in question (or sometimes with certain other chemical classes). Although drug-resistance has obtruded itself recently, it is not a novelty. It was discovered in Ehrlich's laboratories within a few years after research on chemotherapy started and the practical significance of the phenomenon was at once recognized. It also furnished Ehrlich with much of the foundation for his famous theory of the mode of action of such drugs, which is still in the foreground.

When once drug-resistant parasites have appeared they may retain this character for an indefinitely long period, and the particular chemotherapeutic agent becomes useless. One is faced with the dilemma of a recurring need to evolve fresh remedies for old diseases or of preventing the emergence of drug-resistance, if not of restoring sensitiveness. So far, no certain solution of those most complex problems is known in spite of a vast accumulation of data and paradoxes.

This book presents a critical survey of the methods, facts, and theories regarding drug-resistance and allied phenomena (e.g., therapeutic interference). It is a fascinating field for the biologist and immensely urgent for the practical therapist. The authors, themselves important contributors to the subject, have performed an arduous and invaluable service in

bringing together more completely than ever before a body of knowledge indispensable for those in any way concerned with progress in chemotherapy.

C. H. BROWNING

University of Glasgow, Scotland
January 29, 1957

PREFACE

This book was prompted by the lack of a comprehensive treatise on drug resistance of microorganisms. It is the first attempt to gather the widely scattered literature on experimental studies in this large and still growing field which covers such systematically different organisms as protozoa, spirochetes, bacteria, and fungi.*

It seemed timely to assemble in this volume the observations on drug-fast organisms as well as the many theoretical aspects of this phenomenon because the problem of drug resistance has gained increasing interest in many branches of biological science. The rapid development of chemotherapy of infectious diseases has been accompanied by more and more frequent occurrences of actual cases of drug resistance in the course of specific antimicrobial therapy. Although drug fastness is still predominantly a medical problem of major practical importance, it has become to an even greater degree a valuable tool for the study of general biological principles which can be analyzed by the investigation of experimentally accessible and easily reproducible functional changes of microbial cells. Moreover, the specific character of drug resistance seemed to allow a correlation with the chemical agents involved in resistance thus offering an approach to the still unsolved problem of the mechanism of antimicrobial activity.

The necessity to incorporate all these different facets of drug resistance into a readable text seemed to require a division into descriptive parts in which the factual observations, e.g., in protozoa or bacteria, would be separated, whereas in the discussion of general principles the different classes of organisms would be treated in a manner permitting a closer comparison.

The result was the thought-provoking conclusion that the concepts derived from single observations or groups of interrelated findings cannot yet be molded into *one* theory which will satisfy all possibilities of explanation. One might even gain the impression that basically different forms of resistance have to be postulated in different organisms. Though we have tried to correlate these seemingly divergent fields and to reconcile rather contradictory opinions, we feel that these attempts hardly approach an answer to the basic question. All we can hope, therefore,

* Indications are that the principles derived from the knowledge of drug-fast larger microorganisms can also be applied to viruses and even malignant cells, but these scanty though promising results have not yet been included in the discussion.

is that our efforts in offering this comprehensive treatise, inadequate as they might be, may serve as a link which will help to connect future work with the experiences of the past and present.

This book has, therefore, been written for all those who directly or indirectly are concerned with problems related to drug-fastness of microorganisms: for the student who is faced with obtaining a general, comprehensive review of the problems concerned with drug resistance, for the investigator desiring a detailed reference which compiles the immense literature, and for the clinician whose interest in the practical problems is aided by a better understanding of the experimental background which comprises this volume.

In the writing of the book and the preparation of the manuscript we enjoyed the able help and generous collaboration of Mrs. Alberta Schumacher who participated in all phases of the work. Miss Margaret Buck and Mrs. Dorothy R. Kelly gave us the benefit of their experience and contributed experimental data used in the text. Miss Edith Titsworth acted as the amanuensis of the junior author. Mrs. Winifred Blencowe and Miss Margaret Thomas assisted in the checking of the references and the preparation of the subject index. Mr. Rocco Russomanno is responsible for a good number of the drawings of graphs and diagrams; Mrs. Anne O'Donnell typed the greatest part of the manuscript. To all these, our co-workers, we should like to express our gratitude.

Our thanks are also due to Dr. Gertrude Berend for her painstaking efforts in correcting chemical expressions, to the staff of the Scientific Library of Hoffmann-LaRoche, and to Dr. J. A. Aeschlimann, vice-president in charge of research, for his help and encouragement.

Mrs. Peggy Schnitzer and Mrs. Eleanor Grunberg not only supported us by their patience and understanding but also typed the numerous notes, fragments, and versions which were eventually condensed into this book.

R. J. S.
E. G.

Nutley, New Jersey
March 1957

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PART I

DRUG RESISTANCE OF PROTOZOA AND SPIROCHETES

1. INTRODUCTION

Drug resistance is as old as chemotherapy. In 1907 when Ehrlich described the discovery of the trypanocidal activity of *p*-rosaniline, he also included Franke and Roehl's observation (1907) that *Trypanosoma brucei* could be rendered resistant by extended exposure to the dyestuff. Observations of "adaptation" of microorganisms to chemical substances (Kossiakoff, 1887; Effront, 1891; Davenport and Neal, 1895) had been reported earlier, but their importance became apparent after Ehrlich had recognized drug resistance as a characteristic paradoxical response of microorganisms towards anti-microbial agents. Since that time the phenomenon of drug resistance has accompanied the development of chemotherapy like a faithful shadow and the history of chemotherapy is also a history of drug resistance. This is the reason why drug fastness of protozoa and particularly of trypanosomes has decisively influenced methods and theories of this phenomenon. Starting with the earliest observations on triphenylmethane dyestuffs, followed by Browning's (1908) classical investigations on arsenicals, to the most recently developed anti-trypanosomal drugs, the study of resistance has formed an integral part in the definition and interpretation of anti-trypanosomal activity. In case of other protozoa, in particular the plasmodia, drug resistance was investigated as soon as experimental methods became available which allowed the evaluation of anti-malarial activity. Interest was heightened when it was observed that the most recent group of anti-malarials such as proguanil (Curd *et al.*, 1945) and pyrimethamine (Falco *et al.*, 1951) possessed the property of producing drug-fast strains of plasmodia much more consistently than the earlier anti-malarial drugs.

Drug fastness of bacteria followed a similar pattern. Shortly after the specific anti-pneumococcal effect of ethylhydrocupreine (optochin) had been demonstrated (Morgenroth and Levy, 1911), Morgenroth and Kaufmann (1912) described drug resistance of pneumococci towards this compound.

The progress of the chemotherapy of bacterial infections during the past decades is also characterized by an impressive—though not unex-

pected—increase in the number of observations on fastness. Since the first demonstration of resistance of bacteria towards sulfonamides by Maclean *et al.* (1939) or to the antibiotics penicillin (Abraham *et al.*, 1941) and streptomycin (Murray *et al.*, 1946) it is difficult to find any anti-bacterial drug either synthetic or of natural origin that has not been successfully used to render sensitive organisms resistant.

The drug fastness of fungi and viruses is still in an early stage of investigation, but there are indications that also these organisms might be rendered resistant towards antifungal or antiviral agents.

The multitude of biological objects is equaled by the number of chemical substances—not all of them drugs in the medicinal sense—towards which these different microorganisms have been rendered resistant. This wealth of observations and the accumulation of single, often unique, and frequently unconnected findings has created a complexity of aspects which seems to obscure the fundamentally simple phenomenon. A definition which contains the few elementary facts common to all forms of drug fastness might be formulated in the following way: drug resistance or drug fastness of microorganisms manifests itself after exposure of the organisms to an anti-microbial agent either *in vitro* or *in vivo*. It can be defined as the temporary or permanent loss of the initial sensitivity of the microorganism to the effect of the active substance. This perhaps oversimplified definition presumes that drug resistance is basically only one of the many variations that microorganisms can undergo under altered environmental conditions. It does not take into account that resistance has become a complex and multi-faceted subject owing to the multitude of different organisms and the steadily increasing number of substances which are involved in the study of resistance. It is, however, not only the quantity of available information, but the distinct individuality of practically every known drug-fast organism which accounts for the complexity of the problem. The pattern of the resistance is determined in every case by the biological properties of the parent strain on one hand, the chemical properties and the type of activity of the resistance-producing agent on the other. These differences are also responsible for the use of the many different methods devised for obtaining drug-fast organisms. The term "exposure" as used in our definition represents in the experimental reality a great number of various and frequently basically different techniques. Every organism and every chemical requires at least certain technical modifications and sometimes entirely new methods.

Another reason or better group of reasons which is responsible for the complexity of drug resistance is the multiplicity of problems involved in the study of resistance. Besides the medical aspects where the principal

concern is the actual occurrence of fastness in patients and the methods used to avoid it, there are two trends in the investigation of theoretical aspects of drug resistance. One is the study of the mechanism of the development of drug fastness which comprises the basic problems of this field and connects it closely with other branches of science, particularly microbial genetics, biochemistry of microbial metabolism, and physicochemical aspects of permeability, to name only a few subjects. The other trend is the use of drug-fast organisms as tools for the interpretation of chemotherapeutic activity and is based on two of the most valuable characteristics of drug resistance: (1) the predictable change of sensitivity of organisms towards chemical substances under reproducible experimental conditions, (2) the specificity of drug resistance which as a rule is limited to the fastness-producing agent and chemically or biologically related substances. The specificity of fastness as well as examples of lack of this specificity seem to allow a certain insight into the rules which govern the relation of chemical constitution and chemotherapeutic activity. Most of the theories which try to elucidate the still unsolved problem of chemical cure by direct effect on the pathogenic cell refer at one or the other phase of their disputation to findings with drug-fast organisms. These theories comprise the reactions on the part of the parasites as well as the chemical aspects on the basis of the constitution and physical properties of the active molecule.

Regardless of the point from which one might attempt to approach drug resistance as an entity, one will always be confronted by the multiplicity of objects, of methods, and of the underlying problems. Drug resistance must, therefore, be approached from all the different aspects offered by the wealth of available observations if one hopes, not perhaps to find a concept which satisfies all possible interpretations, but a means of classification which will allow the marshaling of an amorphous mass of material according to a few guiding facts: the development of drug resistance, the characteristics of drug fast organisms, and the mechanism of drug resistance.

Although the chemical concepts of chemotherapy play an important part in the analysis of specificity, i.e. the phenomena of cross-resistance, the variety of anti-microbial agents of widely different constitution made it inadvisable to attempt the presentation of the problem of drug resistance on this basis. It appeared equally difficult to approach this task from the microbiological point of view. The reason for this dilemma seemed to be the absence of a common denominator (except the fact of resistance) as a subordinating principle, not alone for the theoretical concepts, but also for the multiplicity of observations. The arrangement of the material has, therefore, been determined rather by differences than

by similarities. The most obvious differences are given by the studies in protozoa on the one hand and in bacteria and fungi on the other. Beyond the mere fact of historical development, briefly outlined in the preceding paragraphs, there are more basic divergences between the phenomena of resistance in these groups of organisms.

In a comprehensive review on drug resistance by Schnitzer (1932) published 25 years after Ehrlich's first communication of this subject, about 66 resistant strains of protozoa were listed as being known at that time. Only 6 of them had been made resistant *in vitro*. Of about 64 drug-fast strains of bacteria, only 4 had been produced in animal experiments. Notwithstanding the great increase of observations in this field, the proportion of *in vitro* and *in vivo* experiments has not changed essentially in the past two decades. As far as the experimental demonstration of drug resistance is concerned, one may well state that the fastness of protozoa is as a rule obtained *in vivo*, whereas bacteria are rendered resistant *in vitro*. This differentiation according to organisms and methods is not wholly superficial or based on the historical development alone, though it is true that in the early stages of chemotherapy of both protozoan and bacterial infections the establishment of activity *in vivo* preceded the observation of activity in the test tube. More important is the fact that drug-resistant organisms obtained *in vitro* do not possess necessarily properties identical with those emerging from infected and treated animals. The technical principles involved in the procedures *in vitro* and *in vivo* might be similar, but similarity of procedures does not always infer similarity or identity of biological processes. For that reason the development of resistance *in vitro* and *in vivo* will be treated separately. This necessitates also a separate description of drug fastness of protozoa and bacteria. Tempting as it would be to interpret the phenomena observed in these systematically different organisms on the basis of a common concept, it is not always possible to correlate such findings without misinterpretation. The characteristics of drug-resistant microorganisms are not consistent even in one species.

Certain changes accompanying resistance such as altered morphology, virulence, and other properties are frequently found in bacteria, but are comparatively rare in protozoa. Moreover, biological functions of bacteria which can be studied in cultures occupy an important place in the definition of resistance as a metabolic change. Similar experiences hardly exist in protozoa, where the attempts to define the mechanism of drug activity are in the foreground of interest. Drug fastness of trypanosomes is characterized by the reduction of binding or absorption of the agents by the resistant forms, an observation which as a rule cannot be reproduced in bacteria. The interpretation of the genetic mechanism of drug