A Ciba Foundation Symposium

DRUG RESISTANCE IN MICRO-ORGANISMS

Mechanisms of Development

Editors for the Ciba Foundation

G. E. W. WOLSTENHOLME, *O.B.E.*, M.A., M.B., B.Ch.

and

CECILIA M. O'CONNOR, B.Sc.

With 62 Illustrations

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PREFACE

It was Sir Charles Harington, Director of the National Institute for Medical Research, and also Chairman of the Medical Research Council's Committee on Chemotherapy, who put forward to the Director of the Ciba Foundation the proposal of a symposium on drug resistance. It was his belief that the more fundamental problems at the basis of chemotherapy were not attracting as much attention in research as was desirable, and his hope that out of a thorough discussion of the question some suggestions might come which would stimulate fresh investigations, more particularly perhaps on the part of chemists.

The Director of the Foundation thought it would be most profitable, and more in keeping with the facilities of the Foundation, if the subject to be considered were narrowed down to "Mechanisms of Development of Drug Resistance in Micro-Organisms." With the expert advice and ready assistance of Sir Charles, and also of Dr. M. R. Pollock, such a meeting was realized in March 1957, Sir Charles himself acting as its Chairman. The Trustees and the Director of the Foundation remain much indebted to both of them, and to the Members who contributed so freely and informatively in the papers and discussions.

The group was a small one, as usual at the Ciba Foundation, partly because the Foundation's accommodation is severely limited, but mainly because experience has shown that useful discussions can best be conducted when members can get to know each other quickly and well, and can be seated in a convenient and comfortable manner for conversation.

This record of the papers presented and the discussions they aroused is prepared for the many people who could not be invited on this occasion, and the Editors hope it will prove an acceptable substitute for personal participation.

To some readers this book may form an introduction to the work of the Ciba Foundation, and it may be helpful to add a few words about its interests.

Under its eminent Trustees, the Foundation is engaged in a number of activities with the purpose of improving co-operation in medical and chemical research between workers in different countries and different disciplines. At its house in London the Foundation provides accommodation for scientists, organizes conferences, conducts a medical post-graduate exchange scheme between Great Britain and France, arranges a variety of informal discussions, awards two annual lectureships, and is building up a library service in special fields. The Foundation assists international congresses and scientific institutions, and it is hoped that in its hospitality, its meetings, and in such a volume as this, it is also usefully helping the individual scientist.

List of those participating in or attending the Symposium on Drug Resistance in Micro-Organisms 26th–28th March, 1957

P. Alexander	Chester Beatty Research Inst., London
Mary Barber	Dept. of Bacteriology, St. Thomas's Hospital Medical School, London
Ann Bishop	Molteno Inst. of Biology and Parasitology, University of Cambridge
L. L. Cavalli-Sforza	Istituto Sieroterapico Milanese, Milan
B. D. Davis	Dept. of Bacteriology and Immunology, Harvard Medical School, Boston
A. C. R. Dean	Physical Chemistry Laboratory, Oxford
M. Demerec	Dept. of Genetics, Carnegie Inst. of Washington, Cold Spring Harbor, Long Island, New York
H. Eagle	Dept. of Experimental Therapeutics, National Inst. of Allergy and Infectious Diseases, Bethesda, Maryland
P. Fredericq	Dept. of Microbiology and Hygiene, University of Liège
J. D. Fulton	National Inst. for Medical Research, Mill Hill, London
B. Györffy	Inst. of Genetics, Hungarian Academy of Sciences, Budapest
Sir Charles Harington	National Inst. for Medical Research, Mill Hill, London
P. Hartman	Dept. of Bacteriology and Immunology, Harvard Medical School, Boston; and Faculté des Sciences, Brussels
W. Hayes	Dept. of Bacteriology, Postgraduate Medical School, London
Sir Cyril Hinshelwood	Physical Chemistry Laboratory, Oxford
R. D. Hotchkiss	Rockefeller Inst. for Medical Research, New York
W. Howard Hughes	Wright-Fleming Inst. for Microbiology, St. Mary's Hospital Medical School, London
N. D. Ierusalimski	Inst. of Genetics, Academy of Sciences of the U.S.S.R., Moscow
R. Knox	Dept. of Bacteriology, Guy's Hospital Medical School, London
K. V. Kossikov	Inst. of Genetics, Academy of Sciences of the U.S.S.R., Moscow

W. KUNICKI-GOLDFINGER	Dept. of Microbiology, The University, Warsaw
J. LEDERBERG	The Medical School, Dept. of Medical Genetics, University of Wisconsin
ESTHER M. LEDERBERG	The Medical School, Dept. of Medical Genetics, University of Wisconsin
M. R. POLLOCK	National Inst. for Medical Research, Mill Hill, London
G. PONTECORVO	Dept. of Genetics, The University, Glasgow
F. L. ROSE	Research Dept. Chemical Group, Pharmaceuticals Division, Imperial Chemical Industries Ltd., Manchester
P. SLONIMSKI	Laboratoire de Génétique Physiologique du C.N.R.S., Université de Paris
B. A. D. STOCKER	Lister Inst. of Preventive Medicine, London
J. WALKER	National Inst. for Medical Research, Mill Hill, London
M. WESTERGAARD	Dept. of Genetics, The University, Copenhagen
J. YUDKIN	Dept. of Nutrition, Queen Elizabeth College, University of London

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CONTENTS

OPENING REMARKS

Sir Charles Harington

In spite of the great advances that have been made in recent years in the chemotherapeutic treatment of infectious diseases—advances that have brought under some measure of control the majority of protozoal and bacterial infections and some helminthic infections—the subject of chemotherapy remains distressingly empirical. The relationship between chemical structure and biological action in this field is still so ill-defined that we have only one significant guiding principle, based on biological theory, to help us in the search for new synthetic drugs for specific chemotherapeutic purposes. As for antibiotics the attempt to discover new substances of therapeutic value is admittedly based on no scientific principle at all, but is an operation such as oil prospecting would be with no adequate background of geological information.

Even when a new synthetic drug is discovered that proves to be of value in the treatment of a particular infection, it is usually impossible to explain the nature of the action of the drug; indeed the type of activity found is not infrequently quite different from that which is being sought, as is shown for instance by the discovery of a valuable antimalarial, pyrimethamine, in the course of a search for folic acid antagonists. Sometimes the drug proves not to have a direct action on the infecting organism at all, although it can suppress or cure the disease which the micro-organism causes; thus the anti-malarial drug proguanil has no direct lethal effect on plasmodia, but it is metabolized in the body of the host to a substance that has such an effect. Again hetrazan, which is the most effective remedy so far known for filariasis, does not kill the microfilariae directly but so alters them that they become susceptible to attack by the natural defence mechanisms of the host. The most striking example of such indirect chemotherapeutic action is afforded by the high-molecular surface-active

compounds that are curative of experimental tuberculosis and leprosy; in this instance there is clear evidence that the drugs, which are quite innocuous to the infecting organisms, confer on the monocytes of the host the power of inhibiting the growth of these organisms, thus exercising their effect by reinforcing the natural defence mechanisms of the host. The discovery of these drugs again was a totally unexpected outcome of the line of research that was being pursued.

All this means that the life of a chemist working in chemotherapy is apt to consist of long periods of unexciting work, punctuated if he is fortunate by occasional successes; even these successes however, whilst practically satisfying, may well be intellectually disappointing, since they will very likely bear little or no relation to the thought that he has put into his research.

By emphasizing as I have done the uncertainties and lack of fundamental knowledge that bedevil chemotherapy I must appear to have painted a very gloomy picture of the subject. If this is so, it is certainly not because I wish to say anything in disparagement of its importance. On the contrary, my object is to analyse the difficulties that we face, and which are particularly discouraging to chemists, in the attempt to see how they may be overcome.

It might be argued that in spite of all that I have said the situation is not unsatisfactory. New and effective chemotherapeutic agents continue to be discovered and the range of diseases brought under control increases. But so long as we cannot explain the reason for our successes we must remain scientifically dissatisfied, and there is one biological phenomenon, namely drug resistance, which makes the situation much less favourable than it appears even from a strictly utilitarian point of view. We can hardly be easy about a state of affairs in which it is reported that in many hospitals over 50 per cent of the strains of staphylococci causing infections have become resistant to penicillin, even though we now have other antibiotics with which they can be controlled; nor is the encouraging emptying of our tuberculosis sanatoria cause

for complacency, when we reflect that this would not be occurring had not the discovery of streptomycin been opportunely followed by those of the antituberculous effects of *p*-aminosalicylic acid and isoniazid. We cannot be sure that the searchers for new drugs and antibiotics will always win the race.

However hard and successfully we may work in the search for new drugs we shall therefore continue to labour under discouragement so long as we are faced with the bugbear of drug resistance. The problem is one of microbial biochemistry, physiology and genetics, and can only be solved by work in these fields. Until we understand the problem we shall have no hope of overcoming it, and until we overcome it we shall have no real sense of security in our chemotherapy. The subject of this symposium therefore is not only of the greatest scientific interest and importance; it has also a background of practical medical urgency, and I think we should do well to keep this thought in our minds.

There are, I am sure, plenty of chemists who would be eager to devote their abilities to research in chemotherapy if they could see it as a less empirical subject than it still is. This is obvious indeed from the mass of work that has resulted from the Woods-Fildes hypothesis of metabolic interference, a theory which, born by biochemistry out of microbiology, has been the most encouraging lead that the chemists have yet received from the biologist; it has systematized thought in the search for new drugs, and if its practical yield, apart from the folic acid antagonists, has so far been small, this is in my view because full fructification of the idea cannot be expected until microbiology is further advanced.

Now a further lead is needed, which can only come from the biochemists and the microbiologists. The greatest encouragement to chemical research would be the achievement of clearer insight into the development of drug resistance together with even a glimmering of an indication that this phenomenon may ultimately be subject to control. If our discussions bring nearer the day when a confident lead in this direction can be given our time will not have been wasted.

ASPECTS OF THE PROBLEM OF DRUG RESISTANCE IN BACTERIA

A. C. R. Dean and Sir Cyril Hinshelwood
Physical Chemistry Laboratory, Oxford

General Observations

In presenting this brief review of our present ideas on the subject of drug resistance it may be well to begin by mentioning views which have at one time or another been attributed to us, but which we have never held and of which no expression could be quoted from any of our publications.

We have never doubted that the essential characters of a cell are inherent in the structure of certain fundamental units including (though not necessarily exclusively) the deoxyribonucleic acid (DNA). We do not suppose these basic structures to be easily susceptible to change, and indeed in our experience easily provoked changes are normally destructive. The maintenance of species characters is of course a matter of the copying of the genetic patterns, and if and when these have been changed the heredity will be changed. We have never denied that structural mutations leading to increased drug resistance or improved utilization of nutrient sources can and do occur, or the obvious consequence that the mutants so arising would be rapidly selected in the appropriate environment.

On the other hand, we have contested the assumption that random mutation and selection is the sole mechanism (or perhaps even the major mechanism) for adaptation to new media or for the development of drug resistance. We have proposed more direct mechanisms, and quoted what appears to us to be good experimental evidence that in various specific examples these mechanisms operate.

Before outlining the proposed mechanisms and summarizing this evidence another point should be made clear. The primary concern of the work has been to explore the problem of the way in which cell reactions are co-ordinated, and the manner in which adjustments in the cell economy can take place, not to assert the relative importance of this or that evolutionary mechanism. A sound judgement on this latter question will probably be reached only when the number of examples studied is considerably greater than it is at present.

In quoting the kinds of evidence on which our current views are based we shall group together examples of adaptation to drugs and certain examples of adaptation to new substrates. The mechanisms will be often, though not always, the same. The addition of a drug to the medium often impedes certain essential enzyme reactions and so imposes a new reaction pattern on the cell. This is not unlike what happens when an unfamiliar substrate has to be used. On the other hand, drug resistance could arise, as in some cases phage resistance seems to, by a mutation leading to a deficiency whereby receptors for the drug in the cell are extirpated. In such a case the analogy with enzymic adaptation would be absent.

In its essentials the mechanism of adaptive change which we believe to operate in certain examples is the following. Although the major characters are determined by the basic gene structures, their quantitative expression is a function not merely of what structures are present but of the proportions in which they occur in the cell. When the medium is changed so that some parts of the reaction sequence are impeded relatively to others, corresponding changes in the relative proportions of the major cell constituents must occur. If division is governed even approximately by the attainment of a threshold amount of some key substance (and DNA seems to be roughly an invariant in this respect), then it is easily shown that the cell composition adjusts itself automatically to give an optimum growth rate.