Origins of Resistance to Toxic Agents

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PREFACE

The Symposium which is presented here in its entirety was motivated by a desire to conduct a free and unobstructed discussion on a single question with many facets: the origins of drug resistance and related problems.

Practical aspects of this question, no doubt, engage our interest most. The discovery of sulfonamides, penicillin, and other antibiotics appeared to have ushered in a miraculous or golden age of antimicrobial chemotherapy in the history of medicine. As pointed out again by Admiral Furth in his welcoming speech, the belief had been entertained optimistically that infectious diseases were about to become a thing of the past. However, there prevails now a certain degree of disappointment and concern among practicing physicians in regard to the increase in the percentage of resistant microbial strains, or relapse of infections following antibiotic therapy. This spells a serious warning against a false sense of security regarding the control of infection in patients. The situation is also of considerable concern for the reason that, following antibiotic treatment, toxic reactions to too large a dose of the drug, such as systemic or metabolic derangements, states of hypersensitivity, or dermatologic manifestations, become established. To avoid such complications, the use of sub-effective doses of antibiotic would, on the other hand, favor the production or the survival of antibiotic-resistant microbial populations. A further development in the treatment of infectious agents which is causing bewilderment and concern among clinicians is the upsurge of the overgrowth of pathogens which normally were suppressed by the organisms inhibited by the antibiotic.

The concept formulated in this symposium, that the development of resistance to various toxic agents is associated with alterations and or loss in enzyme proteins, can likewise apply to the explanation of the mutational processes, occurrence of cancerous growths by the action of carcinogens, and organic agents via various routes, addiction to drugs and alcoholism, and resistance to herbicides and insecticides, which, as known, have created serious economic and health problems. On this basis, the phenomena of resistance to various toxic agents can therefore be brought into a common focus. From the standpoint of practical solutions of these problems, an accurate appraisal of their theoretical

basis represents a most important issue. A decision as to the origins of acquired resistance in cells may point the way for designing experimental procedures for the prevention of its emergence. The theory that resistant populations of cells arise from a few spontaneously produced resistant mutants has been in existence for a considerable period. But no light has as yet been shed on the question of how spontaneous mutants within a growing cell population arise, and there is as yet no adequate rationale for guidance in the use of drugs in synergistic combinations.

It appeared to us that the theory that attributes the emergence of resistance to the direct action of toxic agents on living cells offered more definitive possibilities for planning preventive measures. The chemical action of an agent would be expected to produce changes in the cells which survive this action. The characterization of these changes, and of the enzymatic differences between the resistant and sensitive cells, as we have discussed in this symposium, has provided a biochemical basis for the prevention of the emergence of a resistant population in several species of bacteria. Expanded, these findings could provide a rationale for synergistic combinations as well. These findings may likewise indicate the possibility that similar successes can be achieved in living systems other than microbes. It is expected therefore that acquaintance with processes of similar character in other superficially nonrelated fields would bring about a highly desirable cross-fertilization for creative thinking. This symposium represents therefore an array of material from various disciplines capable of benefiting physicians, biochemists, pharmacologists, entomologists, plant physiologists, students of cancer, and those who are interested in the theory of the evolution of living matter.

The above considerations naturally formed the basis of this symposium, which aimed to bring together various specialists not merely for the presentation of collected experimental data, but principally for discovering "the one in many," the common element in an apparent diversity of ideas, and for following the implied consequences of the assertion or denial of current ideas around a central theme. In order, therefore, to enable the various participants to partake in the orientation and formulation of each other's thoughts we studied the subject matter beforehand in its entirety and formulated "theorems." These were divided into major component parts of a whole, each part with as many subdivisions as there were questions in regard to unsolved and incomplete problems. The outline of these questions is given in Appendix I under a separate heading, "Posing of the Basic Questions." In reality we wanted to make this a "symposium of search" in a classical sense. The outline

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was distributed to the principal speakers, moderators, and to those who requested detailed information about the event many months before it actually took place. To what extent we were successful in materializing our hopes and aims is left to the reader's judicious analysis of the experimental data and the unity and divergence of points of view presented in this volume. We are of the belief that the organization of any symposium proceeding in this manner could be an immense source of inspiration and of great educational value.

The science of chemotherapy or, more specifically, the finding of microbicides, herbicides, insecticides, etc. has principally an empirical origin. It is true that the empirical findings have in certain instances lent themselves to rationalization and improvements such as Ehrlichian researches and those dealing with the synthesis of a variety of sulfonamides. Nevertheless, despite the overwhelming amount of scientific and empirical experimentation, we have not as yet been able to gather a body of knowledge capable of answering the "whys and hows" of the action of antimicrobial agents, and the basic questions posed by the phenomenon of resistance to toxic agents. Nor does there appear as yet any new beam of light to indicate that the multiplication of our efforts along the familiar beaten paths is suggestive of being more fertile. In view of these considerations and the conditions and prevailing elements in the atmosphere of research institutions this symposium seems to be a timely adventure off the beaten track. It is also in view of these considerations that Dr. Roger D. Reid was prompted to voice the following plea (See "Freedom and Finance in Research," *American Scientist* **41**, No. 2, 286–292, 1953) in an outspoken manner:

"What has happened to the explorer of unknown scientific territories, that pathfinder who did not hesitate to be first to seek, first to speak, and first with new ideas? Have our scientists become less capable of original thought, or are they afraid to promote original ideas, *because they cannot be assured of the intellectual support of their colleagues or of financial support from those who hold the purse*? * I would plead for applications for research projects which lie outside the beaten path in entirely new fields, even on projects that are speculative and uncertain."

In unity with Dr. Reid's plea, Dr. Henry M. Wriston, President of Brown University, on June 4, 1954, at the fourth bicentennial conference of Columbia University on policies for higher education, pleaded the case of the heterodox since it may become the orthodox of tomorrow.

* Italicized by M.G.S.

He pleaded that the "universities must encourage and shield the pioneer. Historically the pioneer, whether a settler or researcher, has had boldness of spirit combined with unorthodox methods and beliefs. He has often been intemperate and headstrong, whether attacking the untamed wilderness, or improving, as an entrepreneur, industry and commerce, or breaking new paths into the unknown in the world of knowledge."

History has already shown us what may be the consequences of having sciences under conditions implied by the above challenging pleas. The Golden Age of Pericles, in which Socrates lived and created, witnessed one of the periodical "bankruptcies of science." *Socrates discovered, to his distress, that though each authority was quite sure that the views of the others were wrong, none of them could give any proof that his own were right.* He believed in science, the science of dialectic, the clear, independent, and ultimate thinking out of the meaning of one's own thoughts. Know thine own mind and meaning was Socrates' interpretation of the old Greek maxim, "Know thyself." In the Socratic method, "his aim is not to win a debater's victory over an opponent, but to clear the atmosphere of false or irrelevant definitions, to arrive at the essential character of an idea." Any challenging problem requires such an idealized treatment, and its practical solution is the ultimate step in arriving at the essential character of the problem itself. This can be realized fully only then when students of science employ liberal approaches and enjoy freedom from the restraints of scientific provincialism.

M. G. SEVAG

March, 1955

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CONTENTS

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Session I

RESISTANCE TO MICROBICIDES

INTRODUCTORY REMARKS

W. D. McElroy, *Moderator*

The introduction of the idea of variation and natural selection by Charles Darwin in the middle of the nineteenth century provided a rational explanation of organic evolution from a primitive state to the present more complex one. In addition, it brought unity to biology, explaining the relationships among living organisms and the recurrent morphological and physiological patterns one finds throughout the living world. This persistence of well defined patterns was used as a basis for the separation of organisms into various species and larger groups. We recognize today, however, that this stability is a superficial one and that tremendous changes are occurring in a population at all times. For evolution, there must be some variants from the general pattern that are themselves relatively stable; that is, once such variants occur, they must be handed on to successive generations without reverting too readily to the ancestral type. Such persistent variant patterns are essentially what we distinguish as mutations and these, according to modern theory, provide the basis for natural selection, i.e., a mutant form if better adapted to the environment has a better chance of survival and is therefore "selected." Minor stable variability may occur without selective advantage. This is merely a play upon the mechanism of heredity. It has become increasingly clear in recent times that problems which were formally considered at the macrolevel can now be experimentally approached at the micro or chemical level. The intervention of the genes at the biochemical level is strikingly illustrated by the tremendous developments in the general area of biochemical genetics which has been so successfully exploited by Beadle, Tatum, and associates.

Experimental work during the past 25 years has shown us, however, that there are many latent or dormant patterns in an organism which are only realized under certain environmental conditions. The formation of an enzyme in high concentration in an organism in response to a specific

substrate is one of the best examples, i.e., adaptive enzyme formation. It seems quite clear from numerous investigations that a particular genotype is essential before this adaptation can take place. In other words, although the genic constitution is present for a certain biochemical pattern, the latter is not entirely obvious except under specific environmental conditions. Likewise it is possible to eliminate what is considered a normal biochemical pattern in organisms, without appreciably affecting growth. For example, if one grows Neurospora in a medium which contains just sufficient zinc for maximum growth, in contrast to the normal medium which has excess zinc, one finds that certain enzymes are greatly decreased or are missing completely while other enzymes may have increased 20 to 30 times their normal concentration. All of these changes are rapidly reversible, however, when the organism is returned to the normal medium.

These examples of alteration in an organism have been used in attempting to explain the adaptation of organisms to drugs and other adverse environmental conditions. They may be classified into two general categories, as follows:

(1). *Genetic adaptation,* wherein individual mutants arise which can propagate themselves more readily in the new environment, thus giving rise to new genotypes which are relatively stable. These new strains eventually substitute for the old population.

(2). *A physiological adaptation,* wherein individuals adapt to the new environment but leave the hereditary machinery unaltered, such as in adaptive enzyme formation. On return to the new environment the adaptive changes usually disappear rapidly.

In other words, with the introduction of antibacterial agents, herbicides, and insecticides, the whole problem of evolution with a play on variation, however small, and natural selection, was greatly exaggerated, with the result that large populations of organisms soon arose which were resistant to these agents. One should emphasize that the above two adaptive mechanisms are the extremes and certain intermediate variations are possible. For example: (A) A drug may itself induce a genetic change, thus leading to a change in resistance. (B) Certain physiological changes appear to be genetic because of the slow return to the normal state. The whole problem of Dauermodification is reemphasized. (C) Physiological adaptation may take many different forms: (1) The organism may form an adaptive enzyme which destroys the drugs itself. (2) On the other hand, detoxification may occur by using existing enzymatic machinery. Under these circumstances an adap-

tive increase in the capacity of the system may be necessary when excess drug is applied. (3) In some cases the toxicity of a drug may be overcome by various nutritional means, either externally or internally. By externally, I mean supplying in the diet certain nutrients which will counteract the toxicity. An example would be found in the cases where the toxicity is due to a block in a specific biosynthesis such as sulfanilamide inhibition of bacterial growth and its reversal by paraaminobenzoic acid. Internally the organism may overcome this toxicity by an adaptation which leads to an increase in the synthesis of the nutrient, possibly by a new pathway. Adaptation of Neurospora to sulfanilamide is such an example. An example in reverse would be the process whereby a normally nontoxic agent prevents decomposition of a toxic agent. As I understand it the body can detoxify certain amounts of alcohol by metabolic patterns that exist normally. However, by feeding antabuse the complete breakdown of alcohol is prevented and highly toxic aldehydes accumulate. Under these circumstances very small concentrations of alcohol are toxic. This type of observation certainly teaches us many lessons on physiological adaptation and variation. As a matter of fact this may offer a reasonable, alternate, but rational approach to the broad area of chemotherapy. I am sure we will hear much more about these various possibilities at this symposium.

The employment of drugs as antibacterial agents, as insecticides, and as herbicides has been so extensive and the action so dramatic that it has hardly been possible to keep track of the basic biological problems which have been either uncovered or reemphasized. The practical importance has been appalling both in peacetime and war. This is particularly true for the herbicides and insecticides which have offered unusual opportunities for the development of new land areas and the reclamation of old for agricultural development. The human and animal health problems and practices of every nation on the earth have felt the impact of this new approach—chemotherapy. It was the magic approach and solution to many problems until field and laboratory reports started emphasizing those horrible words which are the theme of the present symposium—drug resistance. Fortunately, however, some of the basic biological problems underlying this phenomenon of drug resistance were already under investigation in the laboratories and certain answers were already available. Resistance to drugs still is and will remain, however, a real problem both practical and theoretical for some time to come, but some of the important practical aspects are being rapidly approached and solved.

SPONTANEOUS AND INDUCED MUTATIONS TO DRUG RESISTANCE IN ESCHERICHIA COLI

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I. Introduction

This Symposium is concerned with a fundamental biological problem, which can be stated either in a specific or in a general form.

In its specific form the problem stems from a simple and often repeated observation, which briefly is as follows: A large population of bacteria are treated, either in an infected animal or in a test tube, with some antibacterial drug. Most of the cells are inhibited or killed, but sometimes a few of them go on dividing and grow into a new population which is resistant to the action of the drug. The question we ask is: were these few cells resistant before the drug was applied, or did they become resistant in its presence? It is not an easy matter to discriminate between these two alternatives. One of the most direct approaches would be to examine each cell in the original culture, that is to grow each one in the absence of the drug and test its descendents for resistance. But this would involve growing and testing millions, or even billions, of separate cultures, and the amount of work required would be quite prohibitive. It has therefore been necessary to devise other tests of a less direct nature, and with many of these it is not quite so clear just how the results should be interpreted.

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