THE SEARCH FOR NEW ANTIBIOTICS

G. F. GAUSE

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Problems and Perspectives

by G. F. Gause

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Another great Aristotelian theme—the search for relations between things apparently disconnected, and for similitude in things to common view unlike.—D'ARCY THOMPSON, *On Growth and Form*, Cambridge, 1917, p. 6

FOREWORD

SINCE WORLD WAR II an extraordinary amount of basic research has been directed toward the discovery and behavior of widely distributed microorganisms which produce substances that are of primary importance as antibiotics. These investigations have resulted in the finding of many new types of antibiotics, and the end is nowhere in sight; rather, the field is more and more extended as the studies move to widely remote centers.

All this antibiotic activity has developed from the discovery by Sir Alexander Fleming, a few years ago, that an obscure mold, *Penicillium*, produces a potent substance, penicillin, which is naturally destructive to many disease-producing microorganisms. Penicillin still ranks as one of the antibiotics most widely used in the control of parasitic diseases, but many other antibiotics discovered since penicillin—such as streptomycin, neomycin, terramycin, and aureomycin, to name only a few—have attained high rank in medicine.

The eminent author of this volume, Professor G. F. Gause, has been Scientific Director of the Institute of Antibiotics, Academy of Medical Sciences, Moscow, U.S.S.R., since 1953. After graduating from the University of Moscow in 1931, he continued with advanced studies and received the doctorate in 1936. Five years later he was appointed professor and head of the Department of Antibiotics in the University.

This volume brings together many of the studies of Professor Gause and his co-workers, directed primarily toward the discovery and isolation of antibiotics from a variety of habitats.

Much of this material is of recent origin and therefore not so widely known to investigators around the world as it should be. It is hoped that the publication and wide distribution of this authoritative volume will have an important bearing on the further development and utilization of antibiotics in medicine and many other fields of biology and chemistry.

This second volume in the Trends in Science program follows *The World of the Electron Microscope*, by R. W. G. Wycoff, and is the result of a cooperative effort by the Yale Chapter of Sigma Xi, the Yale University Press, and Yale University.

GEORGE A. BAITSELL

PREFACE

THIS BOOK is based on a Trends in Science lecture sponsored by the Yale University chapter of Sigma Xi and delivered in December 1959. It is a pleasure for the author to express his sense of the great honor done him by Yale University in inviting him to participate in this important series.

The subject I have chosen is the search for new antibiotics and other useful microbial products, which is now going on with increasing intensity in numerous laboratories all over the world. On the one hand, it is strongly felt that the capabilities of microorganisms in the fine art of organic synthesis are truly excellent. On the other hand, the screening for the potentially useful microbial products is usually considered to be an entirely empirical matter. Referring to the search for new antibiotics of therapeutic value, Sir Charles Harington remarked recently that it "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." *

It has been widely felt that there are plenty of microbiologists and biochemists who would be eager to devote their abilities to the search for new antibiotics if they could see it as less empirical than it still is. It seems appropriate, therefore, to point out that some principles representing the scientific basis of the search have already been discovered, and an attempt is made in these lectures to systematize them to some extent.

One principle, somewhat analogous in importance to geo-

* *Drug Resistance in Micro-organisms*, London, Churchill, 1957.

logical information for oil prospecting, is the general microbiological approach to the geography and ecology of microorganisms producing antibiotics. The second principle is early classification of microorganisms in the screening program which, if used properly, can considerably improve the search for new chemical substances. The third principle is the development of new test organisms for the rapid detection of specific selective inhibition of some biochemical mechanisms, as illustrated by the search for potential anticancer products.

These three principles, based on concepts of general microbiology, represent the subject of the three chapters of the present book. The material discussed in these chapters is mainly original, and has been obtained by the author and his colleagues. It is hoped that its publication will bring the results of this work to a wider audience of researchers and students, and in this way stimulate further development of scientific principles in the search for new antibiotics and other microbial products.

I am greatly indebted to Dr. J. W. Lightbown, of the Department of Biological Standards, National Institute for Medical Research, London, England, for the photographs reproduced in the Frontispiece, which were taken at the time of my work in his laboratory. I am also grateful to Professor H. Munro Fox for permission to use material previously published by me in *Biological Reviews* (Cambridge).

G. F. Gause

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Chapter 1 | THE DISTRIBUTION OF MICRO-ORGANISMS PRODUCING ANTIBIOTICS

ANTIBIOTICS are produced mainly by microorganisms living in the soil. The first steps in every search for new antibiotics are therefore to isolate microorganisms from soils and to test their capacity to inhibit growth of various types of cells. As soon as an investigator engages in this kind of work he becomes keenly interested in soil microbiology. He realizes that each gram of soil in the forest or field contains some thousands of spores of fungi, some hundreds of thousands of actinomycetes, and some millions of bacteria. Could one not find among them in a single gram of soil representatives of all the species and varieties of microorganisms populating the earth? Is it not possible that in the course of many years of work one could isolate from a gram of soil all the species and varieties of microorganisms known at present, including those producing all antibiotics heretofore discovered?

Work along these lines very soon leads to disappointment. One can plate upon the surface of various nutritive media hundreds of small samples from the same piece of soil; usually only a few microbial forms are observed, and these are endlessly repeated. But if another sample of soil is taken from some other ecological conditions one sees that it is populated by different kinds of microbes. Having discovered this fact the investigator begins to take samples of soil from different ecological conditions in order to approach the microbial flora of soil in a given locality.

This line of investigation has led many microbiologists to the conclusion that in the search for microorganisms producing new antibiotics it is much more useful to analyze the microbial flora of a great variety of different soil samples than to engage in an intensive investigation of only a few soil samples. Usually it suffices to plate each soil sample upon five or six agar nutritive media containing different sources of carbon and nitrogen and adjusted to different hydrogen ion concentrations, and to repeat these platings three or four times, in order to get comprehensive information about the microbial flora of a given soil sample.

When some experience is gained in the isolation of antibiotic-producing microorganisms from different soil samples taken from different ecological conditions—let us say, in the environs of Moscow—one becomes naturally interested in the following problem: Would the variety of antibiotic-producing microorganisms isolated from soil be substantially increased in the microbial flora of black soil of the Ukraine or of gray soils of Central Asia? * In the light of evidence at present available it is possible to give an affirmative answer to this question.

In modern screening programs for new antibiotics soil samples are usually taken from all over the world: from the estuary of the Ganges in India, from tropical Africa and America, and from many other localities (e.g. Routien and Finlay, 1952). But in spite of these programs, geographic and ecological regularities in the distribution of antibiotic-producing organisms are very insufficiently known at the present time. It is sometimes argued that inasmuch as, for example, *Streptomyces venezuelae,* which produces the antibiotic chloromycetin, has been isolated from the soils of Venezuela and also from soil samples taken in the environs of Chicago and

* Ed. note: The term *Central Asia* refers throughout to the area in the U.S.S.R. between the Caspian Sea and the Pamirs.

Tokyo, scarcely any geographic regularities in the distribution of antibiotic-producing organisms are to be expected. Nevertheless, it is now known that such regularities in distribution do actually exist, and they have recently been studied in some detail by a number of investigators working at the Institute of Antibiotics of the Academy of Medical Sciences in Moscow. It is the object of this chapter to summarize the results of these studies.

THE DISTRIBUTION OF ANTIBIOTIC-PRODUCING MOLDS

To begin with, let us consider the distribution of antagonistic molds in the rhizosphere of a number of trees as well as outside of it, in soils in the environs of Moscow as well as in south Crimea (Nikitsky Botanical Garden; Avraamova, Gavrilina, and Sveshnikova, 1953). One can see from this example the effect of the rhizosphere, if any, as well as the effect of geographic diversities, on the distribution of antagonistic molds.

Soil samples in the zone of the rhizosphere were taken at a depth of 10–15 cm., directly with the growing root tips. Samples outside the rhizosphere were taken from the same depth and place, but at some distance from roots. The molds from the soil samples were isolated in the usual way on the beer-wort agar sucrose medium at a pH of 5.0. The antibiotic activity of all the strains isolated was tested against two species of the usual soil bacteria (*Bacillus subtilis* and *Bacillus mycoides*), as well as against three species of fungi (*Botrytis allii*, *Fusarium* sp., *Helminthosporium cynadontis*). The mold isolates belonged to the genera *Penicillium*, *Aspergillus*, *Alternaria*, *Mucor*, *Trichoderma*, *Rhizopus*, *Fusarium*, and *Trichotecium*.

The cultures of molds possessing antagonistic action against the test microorganisms just mentioned have been divided into three groups: (1) *antibacterial* antagonists, inhibiting the growth of at least one test bacterium; (2) *wide range* antagonists, inhibiting both bacteria and fungi, (3) *antifungal* antagonists, inhibiting the growth of at least one test fungus. The results of this study are given in Table 1, and they show that in frequency of antagonism, as well as in range of antibiotic action, there are no significant differences between mold populations inhabiting the rhizosphere and those living outside it.

In distinction to this result, the differences between mold populations from northern and southern soils are very sharp and entirely significant. First, the frequency of antagonists in the southern soils is about two times higher than in the northern soils (81.4–87.8% versus 39.4–45.5%). Second, in the southern soils antagonists with a wide range of the inhibitory action predominate, whereas in the northern soils antagonists with a narrow spectrum of inhibition are more frequent. It will be shown later that these two regularities are rather general and hold true not only for molds.

It is interesting to record that in antibiotic power, as measured by the zone of inhibition of growth of test microorganisms, antagonistic molds from the southern soils exceed those from the northern ones (Table 2).

A detailed investigation of distribution of antagonistic forms in molds belonging to the genus *Penicillium*, in soil samples of widely different geographic origin, was published by Kochetkova (1957). She studied populations of *Penicillium* in soil samples from a tundra region (environs of Murmansk), podzol soils in the environs of Moscow, black chernozem soils in the environs of Saratov, and subtropical red soils in the environs of Batumi. In the latter case soils collected in mountains at different altitudes were also studied.

All soil samples studied by Kochetkova were taken from

Table 1

The frequency of antagonistic molds (in %) in the rhizosphere as well as outside the rhizosphere of various trees in the environs of Moscow and in south Crimea (from Avraamova, Gavrilina, and Sveshnikova, 1953)

Tree	Rhizosphere				Outside rhizosphere			
	Anti-bacterial	Wide range	Anti-fungal	Total	Anti-bacterial	Wide range	Anti-fungal	Total
Crimea								
Quercus pubescens	6.0	48.0	19.0	73.0	17.6	47.2	11.7	76.5
Quercus lusitanica	12.5	58.3	12.5	83.3	8.3	50.1	20.8	79.2
Quercus ilex	25.0	41.7	8.3	75.0	3.7	51.9	29.6	85.2
Quercus suber	16.7	16.7	33.3	66.7	23.1	46.2	15.4	84.7
Castanea sativa	20.0	66.6	6.7	93.3	14.3	50.1	28.6	93.0
Caya giabra	35.7	28.6	7.1	71.4	31.3	31.3	18.7	81.3
Arbutus andrachne	12.5	66.7	12.5	91.7	32.0	52.0	16.0	100.0
The same	28.6	57.1	4.8	90.5	17.6	70.7	5.9	94.2
Arbutus unedo	50.0	41.7	—	91.7	16.6	83.4	—	100.0
Average	19.7	49.7	12.0	81.4	17.6	52.6	17.6	87.8
Moscow								
Quercus robur	24.0	9.3	—	33.3	36.0	25.0	1.5	62.5
Betula verrucosa	24.5	17.4	13.0	54.9	37.5	10.9	1.6	50.0
Pinus sylvestris	41.7	8.2	—	49.5	23.2	29.7	4.3	57.2
Picea excelsa	30.7	15.3	3.3	49.3	19.3	7.9	7.9	35.1
Average	26.9	10.3	2.2	39.4	28.0	13.3	4.2	45.5

cultivated fields, except those from high mountains. Samples were taken at a depth of 5–10 cm. For the isolation of fungi, soil suspensions were inoculated on the nutritive beer-wort agar in petri dishes. The medium was acidified by citric acid

TABLE 2

Average sizes of the zones of inhibition of growth of two different test microbes by antagonistic molds isolated from soils from the environs of Moscow and from south Crimea (from Avraamova, Gavrilina, and Sveshnikova, 1953)

Test microorganism	*Origin of mold*	*No. of strains*	*Zone of inhibition mm.*
	Moscow		
Bacillus mycoides	Rhizosphere	69	8.8 ± 0.5
" "	Outside rhizosphere	94	7.8 ± 0.3
	Crimea		
" "	Rhizosphere	122	10.4 ± 0.3
" "	Outside rhizosphere	97	11.1 ± 0.4
	Moscow		
Botrytis allii	Rhizosphere	32	6.0 ± 0.4
" "	Outside rhizosphere	30	6.2 ± 0.3
	Crimea		
" "	Rhizosphere	82	8.7 ± 0.4
" "	Outside rhizosphere	68	8.6 ± 0.5

to a pH of 4.5. After incubation at 25°C for five days the colonies of penicillia were isolated and studied.

Kochetkova studied antagonistic action against *Staphylococcus aureus* and *Bacterium coli* in 1,722 strains of penicillia. The penicillium cultures were grown as streaks on glucose-tryptone-agar at a pH of 7.0 for 48 hours, and afterwards cross-streaked with the two types of test bacteria. In most cases of antagonism the staphylococci were alone inhibited; the inhibition of growth of *B. coli* was less common. When it occurred, both *B. coli* and *S. aureus* were inhibited together, and never *B. coli* alone. Kochetkova did not observe any significant differences in the frequency of antagonists in different soil samples within the same geographic area. However, the differences between populations inhabiting remote geographic areas were rather sharp.

It is clear from the data presented in Table 3 that the frequency of antagonists in penicillia increases from north to south, attaining 14.2% of all the strains isolated in tundra soils (Murmansk), 22.8% in the environs of Moscow, 43.7% in the environs of Saratov, and 55.8% in the subtropical soils of Batumi. In the Transcaucasian region (Batumi) the per-

TABLE 3

The distribution of antagonistic penicillia in various soils (from Kochetkova, 1957)

Origin of soil	No. of soil samples	No. of strains isolated	Percentage of antagonists inhibiting:	
			Staphylococci	*Colon bacteria*
Murmansk environs	14	104	14.2	2.8
Moscow environs	18	83	22.8	3.6
Saratov environs	10	96	43.7	12.5
Batumi environs	10	95	55.8	17.8
The same, 1,000 meters above sea level	10	59	50.8	22.0
The same, 2,000 meters	10	42	40.4	7.1
Rio de Janeiro environs	7	75	73.3	32.0
The same, 1,000 meters above sea level	6	74	71.6	39.2
The same, 2,200 meters	4	53	49.0	4.9

centage of antagonists again decreases at high altitudes, from 55.8% at sea level to 40.4% in the mountains at 2,000 meters.

Kochetkova studied also the distribution of penicillia in the tropical soils of South America, from the environs of Rio de Janeiro, as well as from a mountainous region in the same area. The soil samples were taken simultaneously in July. She observed that in tropical soils at sea level the percentage of antagonists among penicillia attains 73.3, at 1,000 meters above sea level 71.6, and 49.0 at 2,200 meters.

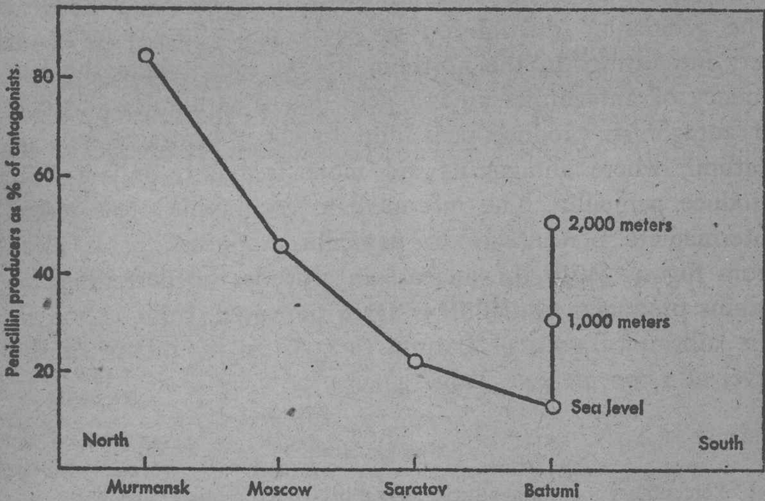

Fig. 1. Geographic distribution of penicillin-producing molds in various soils, according to Kochetkova (1957)

It is therefore possible to conclude that in molds of the genus *Penicillium*, isolated from various soils, antagonistic forms are more frequent in the south than in the north. Besides, the antagonists in the south possess a wider range of inhibitory action. The strains of penicillia that inhibit both staphylococci and colon bacteria make up, in the tropical soils of Brazil, about