GAS CHROMATOGRAPHIC APPLICATIONS in MICROBIOLOGY and MEDICINE

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SERIES PREFACE

This series of handbooks is an effort to supply the practical advice that is needed in most laboratories active in the various fields of applied microbiology and to do it without an overdose of theoretical considerations. This, however, does not imply that the books will be of only limited value to a theoretically oriented laboratory. Consider the extensive use of microorganisms as research tools now common among biophysicists, molecular biologists, immunologists, bioengineers and many others—and you will appreciate the need for a quick guide to the accepted techniques for handling bacteria and viruses.

Pure and applied microbiology go together; they are opposite sides of the same coin. The former is a road over forbiddingly steep hills on which the path is always partly hidden from view. The latter is the goal, for, after all, as Orville Wyss has emphasized, applied microbiology constitutes the backbone of our science, even if "we have responded to the gibes of the humanists who have always objected to the university leaving the cloister and entering the market place. It has never been demonstrated that the cloister is in any way superior to the market place for training a man to think, or that applied science is in any way inferior to pure science as an intellectual effort." There are many signs that the young student generation is more keenly aware of this than most of their professors, but this should not make the students forget Louis Pasteur's famous statement: "Without theory, practice is but routine born of habit. Theory alone can bring forth and develop the spirit of inventions." If the student keeps this in mind he will find that microbiology offers more challenging opportunities to make inventions that will affect man's future health and well-being than most other subjects which he might choose to study.

Carl-Göran Hedén

Preface

Gas chromatography, a field of rapid growth and remarkable progress, has in the last decade reached great sophistication and assumed a position of analytical significance in microbiological and medical fields of research. With the development of more sensitive and dependable gas chromatographic instruments, there has been an increased flow of reports on their application to biomedical problems. A critical examination of this technique in the analysis of microorganisms, their metabolic products, and host response, if any, is therefore timely and necessary. The main purpose of this book is to present the results of recent investigations on the potential applications of gas chromatography in microbiology and medicine. Although certain topics related to medical microbiology are emphasized, the scope of ultrasensitive gas chromatography in the areas of clinical chemistry, toxicology, diagnostic microbiology, host-parasite interactions, metabolic disorders, and cancer research is broadly examined.

The basic concepts of gas chromatography are presented briefly in order to familiarize the reader with the fundamentals of the chromatographic process and with the effects of various parameters on column performance, which are essential for the successful exploitation of the potential of this analytical tool. For all practical purposes gas chromatography is still an art and also requires skill and experience in making efficient columns and manipulating the sample, the flow rate, the column temperature, etc. Some of the practical problems such as collections, storage, and preparation of samples, selection of optimal operating conditions, and application of ancillary techniques are discussed in detail to serve as guidelines. Procedures for qualitative and quantitative determination of compounds of medical importance and complex molecules of microbial origin are presented in detail for the benefit of the inexperienced chromatographer. Many chromatographic illustrations and tables of data are included as aids to the microbiologist involved in diagnostic bacteriology.

The last chapter summarizes the capabilities and limitations of gas chromatography in an attempt to put in proper perspective the past, present, and future application of this versatile technique in various fields of microbiology and medicine.

I do not claim that gas chromatography is the answer to all the analytical problems encountered in microbiology and medicine. However, I hope that the book will be valuable and enlightening not only for microbiologists, but also for chemists pursuing biomedical and agricultural research.

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B. M. M.

Dr. Selman Abraham Waksman (1888–1973)

SELMAN ABRAHAM WAKSMAN

Professor Selman Abraham Waksman was born in the Ukraine on July 22, 1888. He was educated at Odessa, and later, after emigrating in 1910 to the United States, at Rutgers University. In 1916 he became a naturalized citizen of the U.S.A. and the same year obtained the degree of M.Sc. at Rutgers University. Two years later he became Ph.D. in biochemistry, University of California at Berkeley. He was a lecturer in soil microbiology and microbiologist at experimental stations of Rutgers University and became an associate professor in 1925. In 1930 he was appointed full professor and head of the department of microbiology. He served in this post and as director of the Institute of Microbiology until 1958, when he retired. During his college years he began to work on *Actinomyces*. By later working in the Cutter and Takamine laboratories, he developed a feeling for the value of active cooperation between industrial and academic laboratories in the development of medically useful products, such as the antibiotics. He held American and foreign honorary degrees in medicine, science, law, and Hebrew letters and received numerous awards and medals from scientific and other societies.

Dr. Waksman has remained the outstanding figure among the pioneers of antibiotic discovery. He was a soil microbiologist and began studying intermicrobial antagonism long before the therapeutic potentiality of penicillin was recognized. He isolated and purified two antibiotics during the early days of the penicillin work at Oxford, actinomycin in 1940 and streptothricin in 1942. The discovery of streptomycin in 1944 was of immense importance as it not only filled a large number of Gram-negative gaps in the "spectrum" of penicillin, including efficacy in plague, but proved to be fully effective in the treatment of tuberculosis. Another effect of Waksman's work was to encourage soil surveys on a prodigious scale to which the discovery of many other antibiotics has been due. His second major discovery, made in 1948, was neomycin. He and his colleagues were also responsible for candicidin, an antifungal antibiotic used in the topical treatment of candidiasis. Streptomycin and neomycin were both patented, and the large income from royalties on their sales was used mainly to support and endow the Institute of Microbiology at Rutgers University, New Jersey, and to establish a Foundation for Microbiology which supports meetings, publications, and lectureships in the field of microbiology.

He was a prolific writer, with about 400 papers and 25 books to his credit. Some of his books remain valuable works of reference, in particular the monumental work in three volumes entitled *The Actinomycetes,* written with H. A. Lechevalier. Volume 3, which describes the antibiotics produced by these organisms, is an encyclopedia of information. Other classical works of Dr. Waksman include *Enzymes* (1926), *Principles of Soil Microbiology* (1927), *My Life With the Microbes* (1954), and *The Conquest of Tuberculosis* (1964). Naturally, honors were showered upon him. In the Festschrift celebrating his eightieth birthday three large pages are occupied by a list of his honorary degrees, prizes, awards, and medals, the latter including a Nobel Prize in 1952 and the keys of the cities of Rio de Janeiro and Tokyo. His achievements as a microbiologist-cum-chemist, will always rank among the greatest, and he deserves a high place among benefactors of the human race.

The work on this book actually started when Dr. Waksman personally called, in November 1971, to tell the author (BMM) that the Waksman Foundation for Microbiology had approved a grant for this project. Without his generous contribution of $2500, the publication of this book would not have been possible.

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New Haven, Connecticut
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PART A

Introduction

CHAPTER 1

Historical Development of Gas Chromatography

B. M. MITRUKA

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INTRODUCTION

Chromatography consists of a group of analytical methods currently available for the separation of compounds on the basis of certain physical properties of the individual substances. The process is carried out by selectively partitioning the components of a mixture between a solid or liquid phase. This chapter deals with the historical aspect of the development of chromatography as a technique. The evolution of various chromatographic processes, namely, column chromatography, paper chromatography, thin layer chromatography, and gas chromatography, is discussed with a view toward their application in biology and medicine. Because of the vast literature on chromatography, only the selected works leading to the development and application of gas chromatographic technique are described here. For other comprehensive reviews, the reader is referred to Grant (119); Littlewood (188); Szymanski (301); Burchfield and Storrs (48); Dal Nogare and Juvet (84); Kroman and Bender (179); and Wotiz and Clark (331).

HISTORY OF TECHNICAL DEVELOPMENT

The chronological order of the development of chromatographic techniques is summarized as follows:

1903—Tswett (308)

- Realized the potentials of chromatographic techniques for separating and identifying materials.
- First separated plant pigments.
- Percolated dissolved compounds through a glass column packed with an absorbant.
- *Beginning of chromatography.*

1931—Kuhn, Winterstein, and Lederer (180)

- Laid the foundation for developments such as frontal analysis, elution chromatography, and displacement analysis.
- Applied the separation of xanthophile pigments.

1938—Reichstein and Van Euw (264)

- Introduced the liquid or flowing chromatogram.
- Extended the application of chromatography of the separation of colorless substances.

1938—Neuberger (237)

- First implemented column chromatography for separating routine amino acids.
- Found that partition coefficients of acetylated amino acids between water and immiscible organic solvents developed for various amino acids.

1938—Izmailov and Schraiber (152)

- Discussed the uses of a thin layer of unbound alumina spread on a glass plate.

1940—Tiselius (306)

- Developed and revised the chromatographic techniques of displacement, frontal analysis, and elution chromatography.
- Responsible for developing the technique of column chromatography as it is known today.

1941—Martin and Synge (198)

- Introduced column partition chromatography.
- Used an inert support (silica gel) to hold water phase and pass the solvent.
- Developed paper chromatography.
- Discovered liquid-liquid partition chromatography.
- Suggested using gas as the mobile or flowing phase in the column.
- *Beginning of gas-liquid chromatography.*

1944—Consden, Gordon, and Martin (74)

- First described paper partition chromatography.

1946—Claesson (68)

- Developed displacement chromatography and the associated technique of frontal analysis.

1947-1949—Boyd, Tompkins, and Spedding (33)

- Developed ion exchange chromatography.

1949—Meinhard and Hall (207)

- Demonstrated the feasibility of incorporating a binder into the adsorbent to hold it to a glass plate.
- *Beginning of thin layer chromatography.*

1951—Kirchner (172)

- Introduced thin layer chromatography.
- Implemented starch and gypsum binders and increased the size of the glass support.
- Increased the separation capabilities.
- Developed ascending and descending direction.
- Achieved excellent separation and identification of terpenes from essential oils on long glass strips or plates.
- Developed the use of the open column method.

1952—James and Martin (153)

- First demonstrated the separation of volatile fatty acids by gas-liquid chromatography.
- Used titration method for the detection of compounds.
- First used a gas density balance as a sensitivity detector for use in gas-liquid chromatography.

1952—Griffith, James, and Phillips (120)

- Developed a katharometer which depends for its operation on changes in thermal conductivity of effluent gas stream.
- Widened the scope of gas chromatographic application for the detection of volatile substances.

1954—Ray (263)

- Found industrial applications for gas-liquid chromatography.
- Demonstrated separations of such compounds as hydrocarbons, alcohols, ethers, esters, aldehydes, and ketones.

1956—Sober and Peterson (289)

- Introduced ion exchange celluloses.

1959—Stahl (293)

- Used thin film over a finely devised adsorbent spread evenly over a smooth inert glass surface.
- Developed modern thin layer chromatography techniques.

1959—Porath and Flodin (257)

- Developed cross-linked dextran for molecular sieving.

1960—

The work of James and Martin and others firmly established gas chromatography as an investigative tool for separation of materials. However, it was assumed that the technique was suited exclusively for the analysis of volatile substances. Horning and coworkers (140) demonstrated that high molecular weight steroids could be separated by this new technique. The use of the gas chromatographic technique was then extended to a wide variety of high molecular weight, polar, nitrogenous substances such as phenolic amines (101); nucleosides (210); barbiturates (245); and carbohydrates (296). Currently, efforts are being directed toward developing new techniques for the separation of individual components of macromolecules (molecular weight 2000–10, 000). The investigations of Giddings (117), Horvath, et al. (145), Knox (176), Pretorious and Smuts (259), Scott and Rowell (282), Horvath and Lipsky (144), and Huber (146) have contributed to the instrumental development of gas chromatography as it is known today.