CRC REVIVALS

Bacterial Interference

Edited by

Raza Aly, Henry R. Shinefield

CRC Press
Taylor & Francis Group

Bacterial Interference

Editors

Raza Aly, Ph.D.
Dermatology and Microbiology
University of California
San Francisco, California

Henry R. Shinefield, M.D.
Chief Pediatrics
The Permanente Medical Group
San Francisco, California

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

First published 1982 by CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Reissued 2018 by CRC Press

Library of Congress Cataloging in Publication Data

Aly, Raza.
Bacterial interference.

Bibliography.
Includes index.
1. Antibiosis. 2. Bacterial diseases—Prevention. I. Shinefield, Henry. II. Title.
DNLM: 1. Bacteria—lmmunology. 2. Antibiosis.
QW 52 B1307]
QR99.A64 616' .014 81-1 8078: ISBN 0-8493-6285-7 AACR2

A Library of Congress record exists under LC control number: 81018078

Publisher's Note
The publisher has gone to great lengths to ensure the quality of this reprint but points out that some imperfections in the original copies may be apparent.

Disclaimer
The publisher has made every effort to trace copyright holders and welcomes correspondence from those they have been unable to contact.

ISBN 13: 978-1-315-89095-1 (hbk)
ISBN 13: 978-1-351-07005-8 (ebk)

Visit the Taylor & Francis Web site at http://www.taylorandfrancis.com and the
CRC Press Web site at http://www.crcpress.com

PREFACE

Microbial interaction between two organisms resulting in protection of the host from a virulent organism has been the subject of sporadic investigation and continuous speculation for almost one hundred years. However, only recently has it been demonstrated convincingly that antagonistic interaction may enhance the host's capacity to resist infection. This phenomenon has been referred as "bacterial interference". Interest in bacterial interference has increased during the last two decades as clearly evidenced by the literature accumulated in this area of research. With the emergence of the antibiotic era, such approaches as bacterial interference used in the prevention of bacterial infections became less popular. However, interest in bacterial interference has been recently rekindled due to the limited usefulness of antibiotics as a prophylactic agent and the increased incidence of antibiotic resistance of some bacterial strains. To facilitate the collection and coordination of data in this important aspect of biology, contributions were invited from a member of well-known investigators. The collection of papers in the field of bacterial interference will provide a useful reference and guide to those who are interested in this important approach of microbial ecology. It is obvious that much remains to be done in order to understand the mechanism(s) involved in this phenomenon. Such understanding will undoubtedly lead to the further use of this concept in clinical situation. It is hoped that these papers will provide the basis for future investigations which will in turn result in a practical biologic approach to the control and prevention of some serious infectious diseases.

THE EDITORS

Raza Aly, Ph.D., is Associate Professor of Dermatology and Microbiology at the University of California-San Francisco, San Francisco, CA.

Dr. Aly obtained his training from the University of Michigan at Ann Arbor receiving the M.S. and M.P.H. degrees in 1963 and 1965, and from the University of Oklahoma, Norman, receiving his Ph.D. in 1969. He served as an Assistant Research Microbiologist at the University of California-San Francisco from 1969-1972, and as an Assistant Professor from 1972 to 1977.

He is the author of over 60 papers, one book, and also is the coeditor of another book. Also Dr. Aly has been listed in Who's Who's in the West, 1978.

He is a member of the American Academy of Microbiology, the American Society for Microbiology, the American Society of Investigative Dermatology, the International Society for Human and Animal Mycology, the Academy of Dermatology, and the American Federation of Clinical Research. Dr. Aly served as Secretary of the Northern California Branch of American Society for Microbiology and is now the Vice-President of the same Society.

He has been the recipient of many research grants and projects from the National Institute of Health, NASA, and the Food and Drug Administration.

Dr. Aly's research interests are to study the mechanism(s) of bacterial interference, to investigate the factors involved in bacterial adherence to mucosal surfaces, and to explore the anti-microbial activity of skin surface lipids. In addition, he has special interest in the development of training and teaching in dermatomycology and medical microbiology.

Henry R. Shinefield, M.D., is Chief of the Department of Pediatrics at The Permanente Medical Group in San Francisco, California.

He received his B.A. degree in 1944 from Columbia University and his M.D. degree in 1948 from Columbia College of Physicians and Surgeons. Beginning at The Mount Sinai Hospital in New York City, Dr. Shinefield has continued his careed in Pediatrics in hospitals on both the east and west coasts. He served as an Assistant Professor of Pediatrics at the New York Hospital (Cornell) from 1960 to 1964 and then in 1965 as Associate Professor of Pediatrics. Currently, in addition to being Chief of Pediatrics at the San Francisco Kaiser-Permanente Medical Center, he is a Clinical Professor of Pediatrics and a Clinical Professor of Dermatology with the University of California School of Medicine.

Dr. Shinefield is a member of several national societies including Society of Pediatric Research, Infectious Diseases Society of America, and American Pediatric Society. Dr. Shinefield is a senior member of the Institute of Medicine of the National Academy of Science. In addition he has served as a consultant to the Federal Drug Administration, the National Academy of Sciences, and the National Institutes of Health.

Presently Dr. Shinefield is a member of the American Board of Pediatrics and is also serving on the editorial boards of two journals. Dr. Shinefield has published over 75 articles. Bacterial interference and *Staphylococcus aureus* infection in children are the subjects of many of his articles.

CONTRIBUTORS

Raza Aly, Ph.D.
Dermatology and Microbiology
University of California
San Francisco, California

Debra Jan Bibel, Ph.D.
Director
Elie Metchnikoff Memorial Library
Oakland, California

Rama Ganguly, Ph.D.
Departments of Medicine and Microbiology
West Virginia University
Morgantown, West Virginia

Curtis G. Gemmell, Ph.D.
Bacteriology Department
Medical School
University of Glasgow
Royal Infirmary
Glasgow, Scotland

David J. Hentges
Chairman, Department of Microbiology
School of Medicine
Texas Tech University
Health Sciences Center
Lubbock, Texas

Richard B. Kohler, M.D.
Assistant Professor of Medicine
Indiana University Medical Center
Indianapolis, Indiana

Grace Leidy
Department of Pediatrics
Columbia University College of Physicians and Surgeons
Columbia University
New York, New York

James Leyden, M.D.
Dermatology Clinic
University of Pennsylvania
School of Medicine
Philadelphia, Pennsylvania

Howard Maibach, M.D.
University of California Hospital
San Francisco, California

William C. Noble, D.S.C.
Department of Bacteriology
Institute of Dermatology
University of London
London, England

Christine C. Sanders, Ph.D.
Department of Medical Microbiology
Creighton University
School of Medicine
Omaha, Nebraska

W. Eugene Sanders, Jr., M.D.
Professor and Chairman
Department of Medical Microbiology
Creighton University
School of Medicine
Omaha, Nebraska

Sydney Selwyn, M.D.
Head, Department of Medical Microbiology
Westminster Medical School
University of London
London, England

Henry R. Shinefield, M.D.
Chief Department of Pediatrics
The Permanente Medical Group
University of California
San Francisco, California

Katherine Sprunt, M.D.
Department of Pediatrics
Columbia University College of Physicians and Surgeons
Columbia University
New York, New York

Robert H. Waldman, M.D.
Department of Medicine
West Virginia University School of Medicine
Morgantown, West Virginia

L. Joseph Wheat, M.D.
Indiana University Medical Center
Indianapolis, Indiana

Arthur White, M.D.
Assistant Professor of Medicine
Indiana University Medical Center
Indianapolis, Indiana

Kristina Wickman
Head, Mycobacterial Section
The National Bacteriological Laboratory
Stockholm, Sweden

TABLE OF CONTENTS

Chapter 1

BACTERIAL INTERFERENCE, BACTERIOTHERAPY, AND BACTERIOPROPHYLAXIS

Debra Jan Bibel

TABLE OF CONTENTS

I. THE PROBLEM OF DEFINITION

Defining bacterial interference is like trying to pick up a wet watermelon seed with one's fingers. Squeeze a little and watch the goal skitter away.

For the liberal lexicographer, the discovery of bacterial interference, like most first observations in microbiology, may be attributed to Pasteur. Much earlier, in 1852, Mosse[1] had become the first to apply a living microorganism as therapy for an infectious disease with his use of yeast for furunculosis. However, he could not offer proof of any antagonistic action simply because the bacterial origin of the skin disease would not be known for yet another 30 years with the independent work of, again, Pasteur[2] and especially Ogston.[3,4]

In 1877 Pasteur and associate Joubert[5] provided such evidence in their study of anthrax septicemia. They noted that when the anthrax bacillus and "common bacilli" were simultaneously inoculated in urine, the anthrax bacilli, instead of thriving as usual in this medium, hardly grew and soon died. This report of antagonism between two microorganisms is among the earliest contributions to the development of contemporary antibiotic therapy.[6,7] Their subsequent experiment of introducing a mixed culture of these two bacteria subcutaneously into the bodies of guinea pigs and rabbits, typically susceptible to anthrax, yielded the astonishing result of the animals' survival. The significance was not lost to these scientific pioneers. "These facts perhaps justify the highest hopes for therapeutics," wrote Pasteur.

This book adopts an expanded use of the term interference. However, from the strict point of view, Pasteur's work did not even come close to conforming to the exceedingly narrow, primary definition issued in 1963 by Shinefield et al.: "the inability of a second strain of coagulase positive staphylococcus to colonize a particular site of a newborn infant following artificial colonization of this specific site with staphylococcal strain 502A."[8] To be fair, this high degree of conservatism seemed warranted, for at the time the therapeutic interaction was unique to scientific knowledge.

In an editorial which prefaced the above and subsequent series of reports, Dubos,[9] nonetheless, equated bacterial interference with the previously categorized "infection-immunity", a phenomenon recognized with latent infections of tuberculosis and syphilis. Dubos, however, was inaccurate, for what the British immunologists of the 1920s and 1930s defined as infection-immunity was resistance to superinfection by the same strain.[10] For instance, a new chancre will not develop at the site of inoculation in those rabbits which are already infected with the same strain of *Treponema pallidum*. Chancres will form when another strain is used or when the animal has been previously cured of its initial infection. Clearly, some interfering mechanism is involved, and perhaps Dubos was justified in enlarging the umbrella of interference to cover superinfection-immunity, but in doing so, he set a precedent which has led to the dilution of the phenomenon's uniqueness such that interference is often mistakenly considered synonymous with antagonism.

Bacterial interference is a functional term of circumstances in vivo and should not describe antagonism in the test tube or on the culture plate unless tissue cells are directly involved, such as in the examination of adherence. Furthermore, the definition is not restricted to mechanism, encompassing as diverse means as antibiotics, bacteriocins, nutritional competition, and modification or masking of tissue receptors. One could thus speak of the alteration of pH and concurrent inhibition of one bacterium by another in a mixed broth culture not as interference but as a mechanism of interference.

In principle, bacterial interference is analogous to viral interference — an infection of a cell by one virus preventing superinfection by another of its kind or a similar variety (homologous interference) or by a completely different virus (heterologous in-

terference).[11] Therefore, by this broader fashion one could say that Pasteur had produced an example of heterologous bacterial interference, there being no biological reason to exclude internal body areas from the general definition. Shinefield and associates[12] have now extended interference to different protective and challenge species. Similarly, interference should also pertain to the ecological situation in which both interacting bacteria colonize the body surface with one blocking the infective process of the other. This further widens the initial definition from purely ecologic to pathologic domains.

In summary, bacterial interference is an oblique term that begs for qualifiers, such as isologous in the case of strain-specific superinfection-immunity. A subset of antagonism, it has come to refer to any interaction in vivo of two bacteria, whereby one bars the progress of colonization or infection of the other. The order of natural or artificial acquisition of the interfering bacterium is only of practical importance as in the distinction of therapy and prophylaxis.

II. A HISTORY OF CLINICAL EXPERIMENTS

A. The Pioneers

Before the advent of chemotherapy and antibiotics, many physicians had explored the possibility of developing interference into a therapy. Despite their crude and inadequately controlled experiments and their negligible appreciation of ecological complexities, they provided several tantalizing claims of success. In 1885 the Italian Arnaldo Cantani[13] attempted to treat pulmonary tuberculosis by spraying into the lungs of his patient thick aerosols of an obscure, harmless microorganism called *Bacterium termo.* This long lost microbe was first described by Ehrenberg[14] during microbiology's dark age in 1832! The intent was to displace the tubercle bacillus from lung tissue with the benign substitute. Cantani's report, declaring a loss of tubercle bacilli from the sputum and an improvement in the patient's condition, is the pioneering document of bacteriotherapy.

Rudolf Emmerich[15] echoed Pasteur by demonstrating in his Munich laboratory in 1887 that streptococci, previously isolated from a case of erysipelas and later added to an inoculum of anthrax bacilli, could protect rabbits from death by the bacilli. Charles Bouchard[16] observed the same effect in 1889 when he used *Bacillus pyocyaneous (Pseudomonas aeruginosa).* Ten years afterwards, Emmerich and Löw[17] introduced pyocyanase, not an enzyme but the culture extract of *B. pyocyaneous.* This metabolic mixture, having been demonstrated lethal to the bacteria of anthrax, diphtheria, typhoid, and plague in the test tube, took the medical profession by storm and paved the way for antibiotics.[6] Pyocyanase proved too toxic for systemic use and soon was relegated to topical and antiseptic functions, particularly for diphtheria patients and carriers.

While others pursued extracts, filtrates, and lysates of microbial cultures for therapeutic purposes, Shiϕtz[18] in Denmark continued along the ecologic road. In 1909 a patient came to his attention whose staphylococcal throat infection had been incorrectly diagnosed as diphtheria. Despite his assignment to the diphtheria ward, the patient did not develop the infamous disease. Schiϕtz surmised the protective ability of staphylococci and put the hypothesis to a test. He sprayed the isolated and cultured staphylococci into the throat of diphtheria carriers, and upon analyzing the cultures from subsequent samples, reported success in ridding the subjects of the hazardous bacilli.

In 1915 at the lively and famous Inoculation Department of St. Mary's Hospital in London, Leonard Colebrook[19] had observed pneumococci inhibit the growth of meningococci upon an agar medium. Such inhibitory interactions of microorganisms were

well known to Almroth Wright's research group long before Alexander Fleming came upon his *Penicillium* contaminant. Armed with the knowledge of this antagonism in vitro, Colebrook attempted the dubious replacement of throat-borne meningococci with pneumococci. He found six volunteers, including his associate Harold Tanner, who were carriers of *Neisseria.* After first treating several subjects with silver iodide to sterilize the target site — a clever innovation — he sprayed a broth culture of a benign yet inhibitory strain of pneumococcus onto the nasopharynx of each carrier. Only one volunteer seemed to lose his meningococci, but the effect was fleeting. In two days agar cultures again displayed the bacterium. "With more knowledge of the precise conditions which enable the inhibitory organisms to establish themselves," wrote Colebrook, "the method might prove of some value."[19]

In contrast to the potentially hazardous pneumococci, the lactobacilli, which figured strongly in early bacteriotherapeutic regimens, are especially benign, common, and significant members of the normal floras of the intestine and vagina. With the slowly developing realization of the indigenous flora's contribution to natural resistance, lactobacilli were utilized to combat acute infections. One such example is David Newman's[20] 1915 treatment of cystitis by injection of the bacteria into the bladder. This clinician of the Glasgow Royal Infirmary had earlier used lactobacilli with dressings for surface wounds, since lactic acid was known to have antiseptic properties. He was attracted by the idea of a self-perpetuating protective agent. Lactobacillus therapy, however, is more widely associated with sour milk, longevity, and a remarkable Russian scientist.

Elie Metchnikoff stands out as the most creative and vociferous advocate of altering one's normal flora for benefit. His approach was prophylactic. Metchnikoff began his career in zoology and comparative embryology, journeyed through pathology and bacteriology, and championed the phagocyte or cellular theory of immunity, a body of research that earned him the Nobel Prize. His last years at the Pasteur Institute were dedicated to investigating the tangled web of aging, diet, and the normal flora.

As far back as 1894, his studies in vitro on cholera produced evidence of both supportive (commensalistic) and antagonistic interactions of the common intestinal bacteria and *Vibrio cholerae.*[21] He was able to infect suckling rabbits with the cholera vibrio only when he introduced as well an inoculum of cooperative bacteria. Without any other supporting data, Metchnikoff believed that individual susceptibility to cholera could be partially correlated with the composition of one's normal intestinal flora — an explanation for his resistance and that of several associates to the swallowing of vibrio cultures in the manner of Max von Pettenkoffer.

Beginning in 1903 with a lecture before the Manchester Literary and Philosophical Society, he theorized that the putrefactive varieties of bacteria in the intestine produce toxins whose slow and cumulative effect is arteriosclerosis and other degenerative diseases.[22,23] Metchnikoff thus regarded these microorganisms as responsible for the symptoms of old age. For him, old age was an infectious chronic disease mediated by the excessive activity of macrophages in disposing of weakened cells and tissues. The public misconstrued the theory and his prophylactic diet, seeking the prolongation of life itself instead of, as he suggested, the hopeful elimination of life-shortening cardiovascular disease.

Because he knew that diet influences the intestinal flora — fermentative lactic acid bacilli dominate breast-fed infants but not babies given cow's milk — Metchnikoff sought means to replace the putrefactive flora with beneficial fermentative microorganisms. Further influenced by reports of healthy, long-lived Bulgarians whose diet consisted largely of yogurt, Metchnikoff advocated the continued consumption of lactobacilli, either in the form of curdled milk or in pure cultures. The organisms were *Lactobacillus bulgaricus* and *L. caucasicus (L. desidiosus).* Metchnikoff's popular

writings launched the health fad of the decade and initiated the first commercial production of yogurt. He soon realized that these lactobacilli were not always able to compete with and dominate the intestinal flora, but claimed that they, nevertheless, diminished the quantity of intestinal toxins.

Researchers in the 1920s found that the lactobacilli examined by Metchnikoff cannot survive the transit through the stomach and small intestine; however, they discovered that *L. acidophilus* does possess this property. While discounting the longevity concept, they observed, as did Metchnikoff, that the diet was beneficial to minor gastrointestinal disorders.[24] Constipation, diarrhea, and colitis seemed to be aided by the drinking of a quart of milk a day containing 10^8 colony-forming units of the lactobacillus. High levels of meat were not conducive to intestinal maintenance of the microorganism, but a diet of bread, lactose, and milk was supportive. Rettger et al.[25] claimed a microbial survival time of over a year under certain cyclic and vigorous regimens; otherwise, the lactobacilli were eliminated after just 3 to 5 days. The Yale clinicians observed an improvement of symptoms in most of their patients and hypothesized that the long-term consumption of massive numbers of lactobacilli permitted the selection of variants which were best suited for survival in the individual intestinal habitat.

For many years the implantation of lactic acid bacilli following intestinal surgery was commonplace, and even today some physicians, especially those following the popular holistic health doctrine, recommend yogurt, acidophilus milk, or other lactobacillus-containing dairy products as a source of a safe, interim replacement flora following systemic antibiotic therapy. The consumption of lactobacilli for the prevention or cure of assorted intestinal disorders remains routine in France, Russia, and East Europe, and commercial products have a large market in Japan, Taiwan, and Brazil.[26]

Acidophilus milk also has a dietary advantage in providing β-galactosidase, an intestinal enzyme that is lost after weaning, except for adults of northern and western European extraction. Without the enzyme, lactose intolerance results — the production of discomforting acid and gas in the large intestine by the metabolism of the bacterial flora. The lactobacilli of cultured milk products split lactose for absorption by the small intestine, depriving the other enteric flora of the sugar.

Besides competing nutritionally, lactobacilli interact with other flora by lowering the oxidation-reduction potential of the immediate environment, producing growth-inhibiting lactic and acetic acids, and secreting antibiotics. One pertinent example offered by Sandine et al.[27] is the antagonistic activity in baby pigs of lactobacilli against often lethal enteropathogenic *Escherichia coli.* Bacteriotherapy has a greater following among agriculturists than among physicians.

The vagina, where lactobacilli are dominant, is the other popular site of bacteriotherapy. A scattering of papers relating favorable results can be found from each of the past 6 decades — each successive generation of physicians making the same discovery — and the use of yogurt for vaginitis has become a folk remedy. In 1974, for instance, Ostrzenski[28] in Poland had examined whether *L. acidophilus* could augment nystatin treatment for candidal infections, and he concluded that the joint use was superior to treatment by the antibiotic alone in preventing recurrent disease. Earlier, in 1960, Butler and Beakley[29] in a similar study attempted to control vaginitis by combining antibiotic or chemical therapy with the application of lyophilized cultures of lactobacilli. They, too, had found that the addition of normal flora to a disease site hastens the loss of symptoms, increases the cure rate, and helps prevent recurrences. For their experimental investigation they had used a strain of vaginal lactobacilli that preliminary trials had determined was best able among several to persist in the habitat. The lactobacilli seemed to aid recovery from candidal and trichomonal vaginitis, and bacteriotherapy alone cured 95% of patients suffering from nonspecific vaginitis. Yogurt itself is a suitable preparation, for in 1975 Gunston and Fairbrother[30] reported

its efficacy for nonspecific vaginitis but not for trichomonal infections. In these various experiments, the intent has been simply to restore acidity to the vagina and to shorten the recovery period by introducing an already plentiful lactobacillus population. Interference was not considered, although it likely had some influence, particularly with nonspecific vaginitis.

No one has yet reported an attempt of employing lactobacilli as a therapy for endocervical gonorrhea with or without standard antibiotic regimens. However, Saigh et al.[31] have provided some suggestive circumstantial evidence that links these members of the normal flora with a degree of natural resistance to the virulent disease. On agar media, some 40% of cervical isolates of lactobacilli inhibited the growth of gonococci. The antagonistic varieties were more often isolated from healthy women than from women with gonorrhea. Furthermore, the menstrual cycle was influential, since Saigh and associates found more women with inhibitory lactobacilli during the 2-week period after menses than the interval before. It was also at this time that epidemiologists had recorded the lowest incidence in carriage of *N. gonorrhoeae.* Normal flora alone, of course, is not responsible for resistance to gonorrhea; their contributory effect, however, should be considered in further investigations of this venereal disease as in all infectious diseases.

In addition to lactobacilli, *B. coli (E. coli)* was used to treat intestinal ailments. Nissle's[32] report of 1916 presents perhaps the first suggestion of classic homologous interference. Finding a strain of *B. coli* that could inhibit several bacterial species, he began to provide patients the microorganism to replace their supposedly belligerent strains of *B. coli.* Cultures soon went commercial and were sold under the name of "multiflor".

In 1956 Sears et al.[33] examined strain stability within the intestinal habitat of dogs and humans. They noted that a given antigenic type of *E. coli* would be carried for several months to over a year before being replaced by another. Usually one but, depending on the individual, several different strains can colonize the intestine. These researchers soon recognized how selective the intestinal environment is when they attempted to establish by artificial means new resident strains of *E. coli* in dogs. The animals were fed capsules solidly packed with the test microorganism for 3 to 4 weeks. Despite the massive dosage and the reduction of original flora by sulfaguanidine or enemas, the foreign strain would not survive beyond a few days. They had experimentally proved that *E. coli* interfered with the colonization of a different strain of its own species.

In 1946 Florey[6] mentioned but did not cite a contemporary French article that proposed a strain of *B. subtilis* as therapy for intestinal infections. This antecedent is of interest, since *Bacillus* species are well known as antibiotic producers. In 1978 Iglewski and Gerhardt[34] described their isolation of a nonsporulating variant of *B. subtilis* from individuals who had little or no coliforms. As few as 10^4 colony-forming units of bacilli per gram of feces apparently could prevent the colonization of enteric bacteria that typically number 10^9 cfu/g of feces.[35] The potent antibiotic-producer could be a candidate therapeutic agent against intestinal infections. Although this idea was not offered by the authors — it being premature — they did test the bacilli against *V. cholerae* in vitro, finding it highly antagonistic.

A team of French and American researchers have used an animal model to demonstrate experimentally that bacilli can interfere with the colonization of intestinal microorganisms.[36] Although essentially artificial, the use of germ-free mice effectively isolated the interaction of an antibiotic-producing strain of *B. licheniformis* and an isolate of *Clostridium perfringens* from the metabolic web of oral and intestinal flora. Both the *Bacillis* and the *Clostridium* easily established residence along the digestive tract of the mice when implanted independently. However, when the *Clostridium* was fed

to those animals wherein bacilli already resided, it failed to survive. When the order of introduction was reversed, both species thrived and the *Bacillus* no longer produced the antibiotic. Various strains of *Eubacterium, Peptostreptococcus*, and *Staphylococcus* yielded similar results.

B. The Emergence of Modern Bacteriotherapy

In general, these assorted trials exploring the advantage of living microorganisms were tantalizing but rudimentary and largely inconclusive. The purification and use of antagonistic agents derived from microorganisms seemed, in comparison, more reliable and certainly easier to approach and to understand. Except for minor ailments or as an occasional supplemental measure, bacteriotherapy, bacterioprophylaxis, and the similar bacteriophage therapy of Felix d'Herelle[37,38] were put aside with the advent of the Antibiotic Age. However, the dramatic and extensive research of Shinefield and associates at the Cornell Medical Center in New York reawakened the biomedical community to the bright potential of bacteriotherapy by proving that living microorganisms directly interacting with virulent pathogens can prevent lethal disease. Despite the splash, the ripples from these series of reports did not endure.

As Fleming's penicillin was not immediately appreciated because of the numerous other reports of antagonism then current in the literature, Shinefield's work seemed merely another, albeit more intensively studied, example of therapeutic interference. The optimism of antibiotic therapy permeated medicine and science, blocking a proper reception to alternative measures. Furthermore, the therapy, first aimed at newborn infants, seemed too restricted, too complex, and because an undisputed pathogen itself was used, too hazardous.

Almost 2 decades have now passed since Shinefield's deduction and discovery, and penicillin can no longer be trusted to cure gonorrhea or pneumococcal pneumonia. While present antibiotic approaches are collapsing, new tactics, such as adherence inhibitors, are being developed. Research is also returning to vaccines and is casting a hopeful eye toward bacteriotherapy, a concept that has persisted because of its ecologic directness and has been stymied because of its ecologic intricacy.

The milestone contribution of Shinefield et al. began in 1961 during an epidemic of *Staphylococcus aureus* type 80/81 in the hospital nursery.[8] Searching for the source, they found a nurse to be a nasal carrier of this particularly virulent bacterium. The clinicians observed that only those newborn infants under 24 hr of age became colonized by type 80/81; older infants apparently were not susceptible because they had acquired a different phage type of *S. aureus* prior to being handled by the nurse. It was not a matter of simple age-related resistance, since 16-hour-old infants transferred from a different nursery were protected.

Shinefield and colleagues[12] took the bold step of testing the concept of interference. A detailed discussion of their large body of research appears elsewhere in this book. Using a low virulent, penicillin-susceptible *S. aureus*, strain 502A, which was originally isolated from a different nurse, they learned that tenfold fewer staphylococci were needed to colonize the umbilicus than the nasal mucosa. Under the pressure of an epidemic that resisted customary remedial procedures, clinical trials clearly demonstrated that strain 502A could prevent the colonization of virulent type 80/81 and thereby eliminate the offending pathogen from the nursery. Several other hospitals attempted this preventive therapy with equal effectiveness.

The system also was successfully tested outside the hospital environment among adult volunteers using strain 502A and an 80/81 challenge strain which were purposefully inoculated onto the nasal mucosa. Interference was not restricted to the interaction of 502A and 80/81, for prior colonization by another strain of *S. aureus* could block the acceptance of strain 502A. To ensure that the prophylactic staphylococcus

would colonize the mucosa, subjects were first treated with an antibiotic to eliminate the primary antagonist and to empty the niche. This experimental tactic fundamentally duplicated the susceptible, virgin conditions of the infants, who entered the external environment in a germ-free state. The investigative team noted that strain 502A, nonetheless, had to be applied in large numbers over a few days in order to survive on the nasal mucosa. However, even after prior antibiotic treatment, strain 502A could not colonize the mucosa of the oro-pharynx. Since this site is the less preferred habitat of *S. aureus*, the result is not surprising.

Bacteriotherapy with strain 502A is not limited to pediatrics. The bacterium is also beneficial in treating chronic furunculosis.[39] A double-blind controlled study of families plagued by skin infections demonstrated that implantation of strain 502A could interrupt chronic familial patterns of staphylococcal disease. At times 502A carriage was lost and patients relapsed with their original strains. The cycle was repeated with the same results. Thus, strain 502A appears to suppress the growth of different strains of *S. aureus*, but does not necessarily eliminate total carriage, except perhaps over an extended period of time. Nevertheless, strain 502A remains in favorable standing within the therapeutic arsenal of Maibach et al., who adopted the interference approach in 1965. Indeed, they believe that the principle of bacterial interference "may have a future in many areas of infectious disease and in burn therapy".[39]

The use of strain 502A has been questioned simply because it is of the species *S. aureus*, a notorious opportunist and versatile pathogen. The microorganism was chosen empirically, and other strains may be substantially better in interference and in persistence. Indeed, 502A has on rare occasion been associated with disease: blisters around the umbilical area of newborns, conjunctivitis, death in a premature hypoglycemic baby who was given an infusion of concentrated glucose after catherization through the inoculated umbilical site, an abscess on a patient given immunity-inhibiting steroids, and various lesions in a few patients with diabetes or eczema. However, these events are exceptional when contrasted with the several thousands of successfully treated infants and adults. Yet, there is no reason why a less opportunistic member of the normal flora, *S. epidermidis*, for instance, could not be used should it be determined inhibitory for *S. aureus*.

In one situation, at least, heterologous interference appears to have a high potential. Burns are infamous breeding grounds for bacteria and are particularly susceptible to pathogenic microorganisms that abound in hospital environments. In Sweden, Wickman,[40] after noting that a particular *S. aureus* did not colonize the burns of patients who were already carriers of different strains of staphylococci, examined interference in a guinea pig model. Previously, Anthony and Wannamaker[41] confirmed the interfering ability of strain 502A in experimental burns of rabbits. Wickman burned her animals on about 2% of their body surface and subsequently inoculated them with *S. epidermidis* derived from their own skin flora. Afterwards, she sprayed *S. aureus* onto the sites. Most guinea pigs inoculated with the interfering strain did not support the challenge bacterium, and of those that did allow the colonization of *S. aureus*, the researcher found a lower population than in control animals. The interfering *Staphylococcus* was innocuous to the animals, and healing time was unaltered. Wickman furnishes further information in a subsequent section of this book.

Also following is a thorough discussion of the experimental procedures and results of the research team of Sanders et al., who have conducted an extensive examination of interference in the oral-pharyngeal regions. Rather than to attempt bacteriotherapy with a strain chosen more or less by whim, their initial design was to define the naturally resistant flora and the ecology of the habitat. Hence, selection of an inhibitory bacterium — an implication of their work — would be based on clear evidence under well-understood conditions.

Particular interest has been placed on the role of viridans streptococci in infections by *Streptococcus pyogenes*, especially pharyngitis. Laboratory tests had indicated that the viridans group is antagonistic to the growth of *S. pyogenes*. A brief survey of children who were or were not infected with *S. pyogenes* yielded the interesting result that more healthy children harbored inhibitory viridans streptococci than infected youngsters.[42]

This information spurred on further studies to determine whether normal throat flora provide natural resistance to infection by *S. pyogenes*. A prospective survey of children all under the same closed environment was undertaken, and the team observed that those children who did not become colonized with *S. pyogenes* tended to carry more normal flora of greater inhibitory activity than those children who subsequently became colonized. In addition, cultures taken from convalescent children showed more inhibitory isolates of α-hemolytic or viridans streptococci than specimens obtained before or during the colonization.[43]

With the intriguing evidence associating inhibitory normal oral flora with inherent, individual resistance to infectious disease, the scientists next examined the effects of antibiotics.[44] Analysis showed that the number of inhibitory isolates had declined. Since viridans streptococci of low inhibitory activity seemed to be more competitive than more active strains, the investigators expressed the concern that penicillin therapy may enhance the susceptibility of some individuals to subsequent infection by *S. pyogenes*.

A year-long survey of close to 1000 persons produced some corroborative epidemiological data.[45] Detailed analysis of previous work had shown that the bactericidal rather than the bacteriostatic inhibitory flora was responsible for resistance to streptococcal infection. Thus, when tests of culture isolates demonstrated an increased prevalence of bactericidal microbes with advancing age, the investigators were presented with a mechanism that possibly may explain the observed resistance of adults to streptococcal pharyngitis. *S. pyogenes* seems to have a selective influence on the presence of inhibitory microorganisms within the resident oral flora. Apparently, each exposure to *S. pyogenes* expands the proportion of antagonists. However, when antibiotic therapy interrupts the normal course of infection, the pattern of inhibitory microbes returns to a juvenile state. These investigations, which appear to be leading to the selection of a strain of bactericidal viridans streptococci for prophylaxis, strongly indicate the advantage of such measures over conventional, ecologically hazardous antibiotic therapy.

The interfering ability of α-hemolytic streptococci in the throat has also interested Sprunt et al.,[46] who are contributors to this volume. This group has already employed bacteriotherapy to pharyngeal overgrowth by Gram-negative enterics and by staphylococci, which is thought to be a preliminary stage of superinfection. The clinical subjects were, like those of Shinefield, newborn infants, particularly the premature and sickly. The protective *Streptococcus* was isolated from a normal neonate. Results in converting the abnormal resident flora to a healthy composition have been encouraging.

III. TOWARD THE FUTURE

Bacteriotherapeutic or bacterioprophylactic agents ideally should fulfill the following criteria:

1. They obviously must be effective against the intended pathogen.
2. They must be indigenous to and be able to survive within the selected habitat, but be eliminated elsewhere.

3. They must not be pathogenic or at least be poorly opportunistic.
4. They should be susceptible to penicillin or to other nontoxic, low-risk antibiotics.
5. They should be easy to grow and prepare by the physician or otherwise maintain viability in the suspending commercial vehicle, such as saline, ointment, or capsule.
6. From the practical laboratory standpoint, they should be reasonably easy to identify among other resident flora.

Significant theoretical advantages are intrinsic to the use of competing, living microorganisms. First, the system is, after all — to use that often abused term — natural, since microbial antagonistic interactions constantly occur in the various ecosystems of the body. Second, it essentially boosts the host's defense, sometimes acting synergistically with secretory antibody.[47] Third, active inhibitory agents such as antibiotics and bacteriocins can be introduced to the exact location of need, saving other regions of the body from any detrimental effect. Fourth, the production of such agents can be effective, yet be too low for systemic toxicity or immunologic sensitization and too high for selection of resistant strains. Last, the effect can be of long duration, protecting the host perhaps for years under optimal environmental conditions. Since we have now entered the age of genetic engineering and recombinant DNA, perhaps a microorganism can be designed to meet these stipulations, if a naturally occurring one cannot be obtained. When based on sound ecologic principles, bacteriotherapy and bacterioprophylaxis seem appropriate and efficient means of resisting infectious microorganisms at the portals of entry to the body.

As in all therapeutic regimens, there are benefits and risks. Among the problems that bacteriotherapy might offer is the selection of resistant pathogens, especially if the mechanism of interference is antibiosis. The spread of such a resistant microbe poses hazards to the community. Experiments have brought forth conflicting data on the rise of antibiotic-resistant flora following bacteriotherapy, and may reflect the different ecosystems under study.[36,48] Fortunately, once the selective pressure is removed, resistant strains are lost from the site of application. Antagonism based on bacteriocins, nutrition, or alteration of local environment is more stable and has no effect outside the host. Mutants more able to survive in the presence of the therapeutic agents may be temporarily selected out, but since the interaction is so subtle and narrow in spectrum, the risk of spreading to other individuals is negligible.

The bacteriotherapeutic agent itself may initiate disease under special circumstances. Immunodeficiency or immunosuppression, burns, drug or stress-induced modifications of the habitat, and climatic variation may afford opportunities for infection. However, if the agent is chosen with care after proper testing in animal models has determined its safety, and the patient is likewise screened as a suitable candidate, then such a misfortune should be exceedingly rare.

The question of whether the purposeful alteration of one's surface environment, even if done therapeutically, is ultimately safe and proper cannot be answered. Long-term bacterioprophylaxis may permit new and different opportunistic infections or detrimentally affect some unknown aspect of natural host defense. All risks and hazards cannot be predicted. Each innovation of medicine and science has brought unforeseen complications to society. Nonetheless, sophisticated, refined bacteriotherapy and bacterioprophylaxis based on the interference phenomena offer means by which the physician can combat infectious disease and exemplify the modern ecologic approach to health.

REFERENCES

1. **Mosse, J. R.,** Of the use of yeast in the treatment of boils, *Lancet*, 2, 113, 1852.
2. **Pasteur, L.,** De l'extension de la théorie des germes à l'étiologie de quelques maladies communes, *C. R. Acad. Sci. (Paris)*, 90, 1033, 1880.
3. **Ogston, A.,** Über Abscesse, *Arch. Klin. Chir.*, 25, 588, 1880.
4. **Lyell, A.,** Alexander Ogston (1844 to 1929) — Staphylococci, *Scot. Med. J.*, 22, 277, 1977.
5. **Pasteur, L. and Joubert, J. F.,** Carbon et septicémie, *C. R. Acad. Sci. (Paris)*, 85, 101, 1877.
6. **Florey, H. W.,** The use of micro-organisms for therapeutic purposes, *Yale J. Biol. Med.*, 19, 101, 1946.
7. **Brunel, J.,** Antibiosis from Pasteur to Fleming, *J. Hist. Med.*, 6, 287, 1951.
8. **Shinefield, H. R., Ribble, J. C., Boris, M., and Eichenwald, H. F.,** Bacterial interference: its effect on nursery-acquired infection with *Staphylococcus aureus*. I. Preliminary observations on artificial colonization of newborns, *Am. J. Dis. Child.*, 105, 646, 1963.
9. **Dubos, R.,** Staphylococci and infection immunity, *Am. J. Dis. Child.*, 105, 643, 1963.
10. **Topley, W. W. C.,** *An Outline of Immunity*, William Wood, Baltimore, 1933, 217.
11. **Singleton, P. and Sainsbury, D.,** *Dictionary of Microbiology*, John Wiley & Sons, Chichester, 1978, 207.
12. **Shinefield, H. R., Ribble, J. C., Boris, M., and Eichenwald, H. F.,** Bacterial interference, in *The Staphylococci*, Cohen, J. O., Ed., Wiley-Interscience, New York, 1972, 503.
13. **Cantani, A.,** Un tentativo di batterioterapia, *G. Int. Sci. Med.*, 7, 493, 1885.
14. **Buchanan, R. E., Holt, J. G., and Lessel, E. F., Eds.,** *Index Bergeyana*, Williams & Wilkins, Baltimore, 1966.
15. **Emmerich, R.,** Die Heilung des Milzbrandes, *Arch. Hyg. (Berlin)*, 6, 442, 1887.
16. **Bouchard, C.,** Influence qu'exerce sur la maladie charbonneuse l'inoculation du bacille pyocyanique, *C. R. Acad. Sci. (Paris)*, 108, 713, 1889.
17. **Emmerich, R. and Löw, O.,** Bakteriolytische Enzyme als Ursache der erworbenen Immunität und die Heilung von Infektionskrankheiten durch dieselben, *Z. Hyg. Infekt.*, 31, 1, 1899.
18. **Schiøtz, A.,** *Ugesk. Laeger*, 71, 50, 1909.
19. **Colebrook, L.,** Bacterial antagonism, with particular reference to meningococcus, *Lancet*, 2, 1136, 1915.
20. **Newman, D.,** The treatment of cystitis by intravesical injections of lactic bacillus cultures, *Lancet*, 2, 330, 1915.
21. **Metchnikoff, E.,** Recherches sur le choléra et ses vibrions. IV. Sur l'immunité et la réceptivite vis-à-vis du choléra intestinal, *Ann. Inst. Pasteur*, 8, 529, 1894.
22. **Metchnikoff, E.,** *The Prolongation of Life. Optimistic Studies*, William Heinemann, London, 1907.
23. **Metchnikoff, E.,** The utility of lactic microbes with explanation of the author's views on longevity, *Century Mag.*, 79, 53, 1909.
24. **Kopeloff, N.,** *Lactobacillus acidophilus*, Williams & Wilkins, Baltimore, 1926.
25. **Rettger, L. F., Levy, M. N., Weinstein, L., and Weiss, J. E.,** *Lactobacillus acidophilus and Its Therapeutic Application*, Yale University Press, New Haven, Conn., 1935.
26. **Sandine, W. E.,** Roles of lactobacillus in the intestinal tract, *J. Food Prot.*, 42, 259, 1979.
27. **Sandine, W. E., Muralidhara, K. S., Elliker, P. R., and England, D. C.,** Lactic acid bacteria in food and health: a review with special reference to enteropathogenic *Escherichia coli* as well as certain enteric diseases and their treatment with antibiotics and lactobacilli, *J. Milk Food Technol.*, 35, 691, 1972.
28. **Ostrzenski, A.,** Lyophilized suspension of *Lactobacillus acidophilus* in supportive treatment of mycotic forms of vaginitis in women, *Pol. Tyg. Lek.*, 30, 925, 1974.
29. **Butler, B. C. and Beakley, J. W.,** Bacterial flora in vaginitis, *Am. J. Obstet. Gynecol.*, 79, 432, 1960.
30. **Gunston, K. D. and Fairbrother, P. F.,** Treatment of vaginal discharge with yoghurt, *S. Afr. Med. J.*, 49, 675, 1975.
31. **Saigh, J. H., Sanders, C. C., and Sanders, W. E., Jr.,** Inhibition of *Neisseria gonorrhoeae* by aerobic and facultatively anaerobic components of the endocervical flora: evidence for a protective effect against infection, *Infect. Immun.*, 19, 704, 1978.
32. **Nissle, A.,** Ueber die Grundlagen einer neuen ursächlichen Bekämpfung der pathologischen Darmflora, *Dtsch. Med. Wochenschr.*, 42, 1181, 1916.
33. **Sears, H. J., Janes, H., Saloum, R., Brownlee, I., and Lamoreaux, L. F.,** Persistence of individual strains of *Escherichia coli* in man and dog under varying conditions, *J. Bacteriol.*, 71, 370, 1956.
34. **Iglewski, W. J. and Gerhardt, N. B.,** Identification of an antibiotic-producing bacterium from the human intestinal tract and characterization of its antimicrobial product, *Antimicrob. Agents Chemother.*, 13, 81, 1978.