AN ABSTRACT OF THE THESIS OF

DONALD J. McGRAW for the degree of DOCTOR OF PHILOSOPHY

in BIOLOGICAL SCIENCE presented on May 3, 1974

Title: THE ANTIBIOTIC DISCOVERY ERA (1940-1960):

VANCOMYCIN AS AN EXAMPLE OF THE ERA

Abstract Approved: Redacted for Privacy

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An examination of the technical and historical literature concerning the discovery and development of antibiotics suggests the possible existence of an era of discovery. This era appears to have a well-defined beginning (about 1940) and a well-defined close (about 1960). It is the purpose of this dissertation to examine the various strands of evidence that support the thesis that a definable antibiotic discovery era does indeed exist. An examination of several antibiotics discovered and developed during the period 1940-1960 support the thesis. One antibiotic in particular, vancomycin, discussed in depth is shown to be exemplary of the trends established about 1940 and which ceased to function about 1960. In concluding, a definition for the discovery era is proposed.

The Antibiotic Discovery Era (1940-1960): Vancomycin as an Example of the Era

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed December 1975

Commencement June 1976

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Date thesis is presented May 3, 1974

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ACKNOWLEDGMENTS

Not unlike the team effort which brought vancomycin into existence, this history was dependent upon the assistance of a great many people. Foremost among them was Mr. Eli Lilly to whom this history is dedicated. The Eli Lilly Company, discoverers and developers of vancomycin, have supported and encouraged this research. To them the author is most grateful. Special thanks are accorded to Dr. Earl B. Herr, Jr., President of Lilly Research Laboratories, for his guidance and assistance. Many others at the Lilly Company deserve special mention and thanks for their help. They include: Miss Libby Bland, Mrs. LeeAnn Bertram, Dr. Albert Drumley, Mr. Lee G. Crawford, Mrs. Louise B. Randall, Mrs. Helen Davidson, Mrs. Peg Evans, Mr. Ralph Ernesberger, Miss Gloria Gruber, Dr. Richard S. Griffith, Miss Adele Hoskins, Mr. Donald W. Hollings, Mr. Marvin M. Hoehn, Dr. Harvey M. Higgings, Mr. Robert Higgs, Mr. William C. Herman, Miss Jane Johnson, Dr. Edmund C. Kornfeld, Dr. Kenneth G. Kohlsteadt (retired), Mrs. Louise C. Lage, Mrs. Bernadine Kinder, Mrs. Lynda McBee, Dr. Mack H. McCormick, Dr. Henry K. Nelis, Dr. C. W. Pettinga, Mr. James H. Pearcy, Mr. Richard A. Porter, Mrs. Bernice L. Roberts, Mrs. Hendretta Reagen, Dr. W. Max Stark, Dr. Robert W. Squires, Mr. James L. Shaver, Mrs. Judy A. Stenger, Miss Phyllis

Wente, Dr. Robert H. Williams, and Mr. Frederick G. Winters. Also special thanks go to Dr. W. Brooks Fortune (retired) who helped begin the project and to Mr. Robert T. Doerr for such a great variety of things. Mr. Gene E. McCormick was inspirational *in extenso*, and that has been acutely appreciated. Others whose help is acknowledged include the Library Staff of the Indiana University School of Medicine (Indianapolis) and Mr. Arni Shareski of the Christian and Missionary Alliance (New York City). I wish to thank Dr. Paul Farber, my major professor, and Drs. Robert Becker, Daniel Jones, Richard Morita, and David Willis, from whom I have learned so much. The extensive aid given by Dr. Harry Phinney is especially appreciated. The foundation laid by Drs. Edward Towne Roche, Jerome E. Dimitman, and Ralph W. Ames remains as warmly appreciated as it always has. Thanks are also accorded Mrs. Clover Redfern for her typing of the final manuscript. A proper completion of the work would have been impossible without the devotion and labors of wife Laura and daughters Adrienne and Holly who deserve the greatest thanks of all.

Though the phrase is oft repeated, its importance does not diminish--any mistakes in this history are my own.

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1. THE ANTIBIOTIC DISCOVERY ERA

1.1. Statement of the Problem

When one reads the technical literature concerning antibiotics distinct similarities are seen regarding both the discovery and development of a wide variety of such agents over a period of years. The mode of discovery and the subsequent developmental history of each agent follows a pattern which, in its general aspects, and most of its particular aspects, is almost predictable. When examining the histories of such agents as penicillin, streptomycin, aureomycin and others, it will be found that the genesis of these agents as medically important therapeutic tools occurs within a few years before or after the midpoint of this century. If one looks for the discovery and development of antibiotics prior to about 1940, nothing is to be found. Again if one looks for this same apparently predictable pattern of discovery and development after 1960, the pattern is found quite altered from the pattern familiar in the preceding two decades.

Is this observation indicative of a trend in the progress of chemotherapeutic history which had both a definable beginning and definable end? If so, are the characteristics of the pattern of discovery and development such that one could actually define the period

of 1940-1960 in antibiotic history? Can the characteristics which seem so similar in the growth of a wide variety of antibiotics be used as elements to define a historical period? Finally is the definition peculiar to a certain time-span, and to no other?

The several elements in these questions can be condensed into one simple question--was there a discovery era in the history of antibiotics and can it be defined? Repeated references in the secondary literature, or that literature which examines the histories of individual antibiotics, leads to the conclusion that a period did exist in the history of antibiotic medicine which can be rather clearly circumscribed.

It is the purpose of this dissertation to define the period of antibiotic discovery. This is accomplished by examining factors peculiar to the genesis of the period, to its active years, and to the factors which brought about its close. This is done by considering the histories of several antibiotics to demonstrate the existence of a general pattern of discovery and development functioning from 1940 to 1960. Not a great deal of a historical nature has been written on most antibiotics. Therefore, one agent, vancomycin, discovered and developed during the postulated discovery era, has been examined very closely. This has been done by using original sources of information. When combined with material that is available in the

literature on several other antibiotics, similarities in their histories can be seen.

Since it is stated that a definable era in antibiotic history did exist it is first necessary to examine the argument supporting the genesis of the era. Following that it is equally as important to examine what factors were operative at the close of the period.

1.2. Dating the Discovery Era

At the outset two terms need defining. First, what is an antibiotic? For the purposes of this history the term as first defined by S.A. Waksman (b. 1881) will be utilized. His definition is that which has been most generally accepted since 1941, when it was first used. Waksman defined an antibiotic as any naturally occurring compound that is produced by one microorganism and is antagonistic to others.[1] Included in this definition is one critical phrase which has a bearing on the definition of the second term (that is, discovery era). Waksman stated that the agent must be naturally occurring, hence excluding any synthetic or semi-synthetic agents. Synthetic antibiotics are totally fabricated by man. Semi-synthetic ones require use of molecular moeities elaborated by microorganisms. Through molecular manipulation these moeities are used to produce desired

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[1] D. M. Schullian, "Notes and Events: History of the Word Antibiotic," J. Hist. Med., July (1973), 284-286.

(improved) variants of nature's basic molecules.

The second term, discovery era, depends upon following Waksman's original definition. By discovery era is meant a period when naturally produced antibiotics were being found (discovered) by researchers seeking such agents. The discovery era, then, must end when new naturally occurring antibiotics are no longer being found. As will be demonstrated below, the discovery era is characterized by two distinct time periods. In between beginning and ending dates, all the chemical families of naturally occurring antibiotics were discovered. The era closes when semi-synthetic processes must be relied upon as a source of new agents because no new natural ones are being discovered.

One additional provision is required. The eventual definition of the discovery period is predicated upon the assumption that most antibiotics being discussed are medically useful. If one takes Waksman's definition and searches the literature prior to 1940, several antibiotic discoveries will be noted. This goes back into the past as well as present century. None of those earlier agents were ever proven to be of use in treating human diseases, however. Furthermore, none inaugurated any type of major productive search for more such chemicals. Each case was fortuitous and isolated in time. It was not until the appearance of penicillin that it can be said that an era was inaugurated. After penicillin a great many

discoveries of useful compounds occurred. The inaugural date of the era, then, is set in association with penicillin and no earlier antibiotic. The evidence for choosing 1940 to begin the defined era can now be considered.

In this connection of setting the beginning of the era, Waksman and others have recounted events during late December, 1940 at St. Louis, Missouri. It was there that the Society of American Bacteriologists (now the American Society for Microbiology) was having its annual meeting. About 200 guests were present. One person arose to query the members as to whether anything more had been heard since the 1940 announcement in Oxford, England of the isolation of a compound called penicillin. As Waksman recounted it, no one had anything to say upon the subject.[2] Despite the fact that so many microbiologists were unaware of the status of what soon proved to be so important a discovery. Waksman reminds us of the watershed nature of that time period.

It was in 1938 that Howard Walter Florey (1899-1968) and his colleagues began the penicillin research (see Chapter 2). It was in 1940 that the germinal publication on the first true antibiotic substance possessing soon-to-be-proven utilitarian value occurred.

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[2] S.A. Waksman, "A Quarter-Century of the Antibiotic Era," Antimicrob. Agents and Chemotherapy--1965, ed. by G.L. Hobby. (Washington, D.C.: Amer. Soc. for Microbiology, 1965), p. 10.

Neither Waksman nor any other writer sets the beginning of the era any earlier than the work on penicillin, because other antibiotic agents (for example, Waksman's own gramicidin and tyrocidin of the late 1930's) were not medically useful.[3]

Other authors have chosen the year 1940 as the germinal one for the era. L.H. Conover is exemplary in this matter. A careful reading of the following quotation will indicate just what technological progress had been made by 1940.

> By 1940, basic knowledge and experimental techniques were in hand which permitted (1) facile collection, isolation, and growth of cultures of fungi, molds, bacteria, and actinomycetes; (2) detection, biological assay, purification, isolation, and structure of proof of complex, unstable metabolites having antimicrobial activity; (3) evaluation of the chemotherapeutic efficacy and safety of antibacterial drugs in laboratory animals and man; (4) artificial mutation

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[3]Waksman has not formally set 1940 as the beginning of the era, for in another publication he uses both 1940 and 1939. (That is in S.A. Waksman, "Successes and Failures in the Search for Antibiotics," in Advances in Applied Microbiology, ed. by D. Perlman. (New York: Academic Press, 1969), p. 1.). He terms the period from 1939-1960 as the "Golden Age of Chemotherapy" and "The Antibiotic Era," (Ibid., p. 1-2). In this publication Waksman indicated that in 1940 he was fully aware then that a new era was aborning. Indeed, he said that two personal experiences, to him, "prove that it all began that year [i.e. 1939-1940]" (Ibid., p. 4.). One incident has been recounted above (the 1940 Society meeting). The other was his experience at the Third International Congress for Microbiology in New York in 1939, that just on the even of Chain and Florey's classic paper on penicillin (see Chapter 2). Waksman noted that in Alexander Fleming's presentation at the congress absolutely no mention of penicillin was made, despite the fact that Fleming knew what was occurring at Oxford.

> of antibiotic-producing microorganisms with selection of mutants having improved productivity; and (5) development of industrial-scale submerged, aereated fermentations, and of recovery processes for the antibiotics produced thereby.[4]

In seeking a definition for the discovery era the above statement is very useful. It indicates what could be expected, from a technological standpoint, during the decade of the 1940's and beyond. As the history of the several antibiotics considered within the text of this study unfolds it will be seen that all of the above quoted points were to play major roles. The Conover statement does not consider several other important points, however, and in the evolution of the era's definition such points must be considered. As an example, one must cite the role of the industrial team approach to development of a given antibiotic. This and similar elements in the final definition are discussed in the concluding chapter.

Purely for reasons of surveying the literature, another author has chosen the year 1941 as a beginning point, not solely as a starting year for the antibiotic era, but in reference to drug discovery in general. The DeHaen New Product Survey, first published in 1949, was used over subsequent years by Barry Bloom in his analysis of the

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[4] L.H. Conover, "Discovery of Drugs from Microbial Sources," in Drug Discovery: Science and Development in a Changing Society, ed. by B. Bloom and G.E. Ullyot. (Washington, D.C.: Amer. Chem. Soc., 1971), p. 38.

rate of new drug discovery.[5] His findings will be considered more closely below.

In regard to establishment of the ending date of the discovery era it is possible to turn to a herculean survey of the literature of chemotherapy completed by A. Neelameghan in 1968.[6] The author surveyed the literature concerning antibiotic discovery and rediscovery over the period from 1907 to 1967. Although the author's point was to show that rediscovery of an already known antibiotic could be avoided by the proper literature search, his findings are exceptional in indicating the end point of the discovery era. He began by defining antibiotics to exclude synthetically prepared correlates. This assured, for the present use, that his findings represent those naturally occurring antibiotics considered in this dissertation.

His study was immense in that he had to sort through the over 500 agents named by the year 1960. Most of these were produced by actinomycete microorganisms. This is true of vancomycin, considered in depth later, and most other major antibiotics to be discussed also.

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[5] B. Bloom, "The Rate of Contemporary Drug Discovery," in Drug Discovery: Science and Development in a Changing Society, ed. by B. Bloom and G.E. Ullyot. (Washington, D.C.: Amer. Chem. Soc., 1971), p. 176-184.

[6] A Neelameghan, "Discovery, Duplication and Documentation: A Case Study," Library Sci. With A Slant to Documentation, 5(1968): 264-288.

During the decade 1927-1936, no actinomycete-produced antibiotics were discovered. However, in succeeding decades many were discovered. For example, between 1937 and 1946, 15 were discovered; 1947-1956 saw 375 new compounds, and 1957-1967 saw 767 new names (not all new agents, however). In fact over 96 percent of reports published between 1907 and 1967 occurred in the latter half of that span of years.

Neelameghan noted that

> A piece of seminal research [e.g. penicillin research] or a breakthrough stimulates a considerable amount of pure, applied and developmental research in the field. After a time, the field becomes saturated and research may be directed towards greener pastures.[7]

This very thing happened with the discovery of new antibiotics, particularly those from actinomycetes. The amount of rediscovery of antibiotics during the latter part of the time period studied was very high. For instance, during 1947-1956, 606 publications asserting the discovery of a new antibiotic were seen. Of these, 163 turned out to be duplicates of the compound described. Of the remaining 548, only a handful ever reached medical employment. Duplication and rediscovery reached a peak between 1957 and 1967. That is in direct relationship to the end of the discovery period. Between 1907 and 1967, 1,714 reports were published. Of those, 470 were actual

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[7] Ibid., p. 275.

rediscoveries. Of the 470, 83 percent were published upon between 1947 and 1967 and a significant 54 percent between 1957 and 1967.

Such information indicates that a peak of new discovery of antibiotics occurred sometime prior to 1967 and more closely to the period of the mid-1950's. Rediscovery of some compounds occurred as many as 19 times, though 25 percent of the compounds were rediscovered twice.

Considering actinomycete antibiotics, Neelemeghan noted

> For fifteen years [after 1943] the rate of production of [new] antibiotics with therapeutic value from Streptomyces [the principal antibiotic-producing actinomycete] has been almost one per year. However, in the last decade the rate of discovery of useful antibiotics from Streptomyces has been comparatively low.[8]

The study by Bloom, cited above, provides even more revealing data. Bloom examined many classes of drugs, in addition to the antibiotics. By dividing the period 1941-1970 into five-year units he noted that drug discovery peaked between 1955-1960.

During 1946-1950 many major breakthroughs were made. This was done by the molecular manipulation of many classes of drugs--but not antibiotics--and also by the discovery of entirely new drug classes. During the period 1951-1955 many new antibiotics were added to the inventory and these were "produced directly by

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[8] Ibid., p. 287.

fermentation."[9] (The one exception during the period 1940-1960 in antibiotic discovery wherein an antibiotic was actually synthesized, in part, was that of tetracycline by hydrogenolysis of naturally-occurring chlortetracycline.)

During 1955-1960 valuable new antibiotics were being discovered and the first semi-synthetic ones were being produced toward the end of that period. During 1957-1962, 13 new antibiotics appeared. During 1963-1967, 10 new ones appeared. But, and this is the crucial point, production of so many new antibiotics was due to the introduction of new semisynthetic antibiotics and not to the discovery of totally new, naturally occurring ones.[10]

Ernst Chain (b. 1906) helps to explain this. In 1955 (twelve years after the elucidation of penicillin's structure) an American team succeeded in opening the possibility of molecular modification of a naturally occurring antibiotic. In 1957 the first semisynthetic penicillin was made.[11] Waksman adds that even though screening programs for new antibiotics continued they are today aimed primarily at control of viruses, not bacteria.[12] (Our concern here--

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[9] Bloom, "The Rate," p. 179. Emphasis added.

[10] Ibid., p. 182.

[11] E. B. Chain, "Twenty-Five Years of Penicillin Therapy in Perspective," in Antimicrobial Agents and Chemotherapy-1965, by G. L. Hobby, (New York: Amer. So. for Microbiol., 1965), p. 4.

[12] Waksman, "Quarter-Century," p. 12.

viruses are not microorganisms if one follows Waksman's antibiotic definition closely.) Waksman elsewhere even suggested that since the discovery era seemed to end about 1960, and since 1960-1967 has seen essentially no new discoveries of naturally occurring antibiotics, screening soil and water for them may be a waste of valuable research hours.[13]

The final reason for setting the end of the discovery era at about 1960, however, lies in a statement made by Conover, in which he said,

> During this period [1940-1959] every important class of antibacterial antibiotic known was recognized.[14]

Thus it appears that the dates of 1940-1960 can be put forward as those encompassing the antibiotic discovery era.

1.3. Format of the History and Methods of Research

The format or presentation of this history is as follows. Because of the germinal nature of penicillin in the inauguration of the discovery era, one chapter (Chapter 2) comprises a short historical discussion of that agent. Since it is germinal and precedential it is possible to point out how the pattern of discovery and development of succeeding agents seem to parallel that of penicillin. Therefore,

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[13] Waksman, "Successes and Failures," p. 7.

[14] Conover, "Discovery," p. 39.

Chapter 3 presents a brief historical discussion of three agents which played a vital role in antibiotic medicine during the 1940-1960 era and which fit the era pattern. The rationale for the choice of the three is considered at greater length in the introduction to that chapter.

Since vancomycin is the central example of the discovery era pattern, it is considered at length. However, since its medical application is as a rather highly specialized antistaphylococcal agent the discussion of vancomycin requires a prefacing chapter on the staphylococci. These bacteria played a major role in the practice of medicine during the period 1940-1960, especially. In order to appreciate the place of vancomycin in medicine, the staphylococcal threat in hospital environments and in the general population is considered at length in Chapter 4.

Chapters 5 and 6 comprise the history of vancomycin itself. Chapter 5 examines the discovery and also the development insofar as laboratory and industrial technology are concerned. Chapter 6 is concerned with the use of vancomycin in human medicine.

Finally, in Chapter 7, the definition of the discovery era is proposed.

Three methods of research were used in this study. The first was the use of the published literature, both historical and technical, and is, of course, a well-established technique.

The second method was the use of unpublished materials (reports, notes, laboratory notebooks, minutes of committee meetings) concerning vancomycin made available to the author by the Eli Lilly Company (producers of vancomycin). Within that corporation, at least, such an approach was unique, no non-employee having ever been allowed such access to corporate records.

The third methodological technique, the use of tape-recorded interviews, bears brief discussion. One author, who has had considerable experience with oral history, has said that

> One of the great advantages and complications of contemporary historical research is the opportunity to interview those who participated in the events described. Most historians are necessarily limited to the written record, and their traditional distrust of recollections long after the fact is justified in many ways. But when such sources are available, the historian has no choice but to use them.

and

> the experience of interviewing the major participants in a . . . story was perhaps more helpful than any other phase of the study . . . .[15]

With the aid of interviews the human elements central to decision making could be reconstructed for analysis. Using corporate reports alone did not always allow for this. Within the discussion whenever tape-recorded statements are used in interpreting the

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[15] R. G. Hewlett, "A Pilot Study in Contemporary Scientific History." Isis, 53(1962): 31-38. Quotation on p. 35.

history of the vancomycin project such use is made clear in footnotes or in the text itself. In no case has a conflict been found to exist between the written record and the interview record. For this study both sources are valid.

2. PENICILLIN--THE PRECEDENT

Alexander Fleming (1881-1955) was a physician whose driving interest, since he took his first and only professional position, was the search for an efficacious antibacterial agent. He completed his M.D. degree at St. Mary's Hospital Medical School in London early in the century. He thereafter took a position in that hospital's Inoculation Service and remained there for the rest of his life. The Service (later Department) was essentially a medical bacteriology laboratory involved not only in routine hospital inoculations, but also in basic research. Under the direction of Sir Almroth Wright (1861-1947) the service went as a unit to France during the first world war. Fleming was sickened by the deaths from wound sepsis and gangrene that he saw in the hospital there. He began research in the field hospital on disinfection of severe wounds such as those caused by artillery and explosives. These wounds are complex in that great tissue disruption leaves many inaccessible niches. Here sepsis can take firm hold and resist disinfection. Fleming recognized that no antiseptic approach could ever hope to achieve any beneficial results. Over the next several decades he, like Paul Ehrlich (1845-1915) and others, saw the clear need for an effective systemic antimicrobial agent.[16]

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[16]This discussion of Fleming is to be found in the first two chapters of A. Maurois, The Life of Sir Alexander Fleming (New

Fleming continually searched for an effective antimicrobial agent, but with few positive results. There were two exceptions, however. One was penicillin and the other was the substance lysozyme, which was discovered much before penicillin and was his special interest. Lysozyme (Wright coined the word for Fleming), so Fleming discovered, was a substance common to leucocytes. Along with phagocytosis and antibody formation, Fleming realized that lysozyme was another important natural defense mechanism of the mammalian body. Fleming pursued lysozyme research with great vigor and found that the substance was very widespread in nature. He found it in tears, in egg whites, and in seeds. It seemed an ideal antibacterial agent as it lysed bacteria rapidly. By virtue of its leucocytic origin, it was obviously not systemically toxic. To collect lysozyme (before discovering eggs to be the most convenient source), he rubbed lemons in his and his colleague's eyes to get tears to flow. He had, therefore, a small, but usable source. For many years he pursued research on lysozyme and as he believed that since it was derived from the human system it would be the safest systemic antimicrobial substance. His hopes were never realized in achieving a practical use for the substance, however.[17]

York: Dutton, 1959).

[17]R. Hare, The Birth of Penicillin (London: Allen and Unwin, 1970), Chps. 3 and 4.

That was not so for penicillin. Yet much as penicillin may be a twentieth century discovery, Fleming was not completely original in his observation. He pushed it further than his predecessors, it is true, but others had seen the action of penicillin at least as early as 1876. John Tyndal (1820-1893) described the action of a species of Penicillium mold growing in broth tubes. His hypothesis on the mechanism by which it lysed bacteria was faulty.[18] That was evidently the earliest observation on Penicillium per se. Observations on similar antagonistic activities on the part of other fungi, however, go back to, at least, the seventeenth century.[19]

Fleming evidently only recognized penicillin as an isolation tool for detecting penicillin-insensitive pathogenic bacteria in vitro. Although he did perceive of the antibiotic as useful in topical administration, that realization did not apply to systemic use.[20] Howard Florey, on the other hand, did see the great potential of penicillin.

Florey was a M.D., as was Fleming, but had a much longer and apparently much better education than Fleming. He was

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[18] H. Florey et al., Antibiotics (2 vol.; London: Oxford Univ. Press, 1949) I, p. 3.

[19] A.G. Cranch, "Early Use of Penicillin(?)," J. Amer. Med. Assoc., 123 (1943), 990. See also Florey, Antibiotics, I, p. 3ff.

[20] Hare, Penicillin, p. 108.

well-trained in chemistry, an area virtually a terra incognita for Fleming (and which had much to do with Fleming having never pursued penicillin chemically). Florey was also interested in pursuing the search for an ideal antibacterial agent.

Florey's research led him into the question of the behavior of tissues in health and then into asking questions about what disease is and how can it be treated.[21] In the late twenties he heard of lysozyme and during the same period formulated his life-long philosophy that advances in medical practice would have to come from imaginative experimentation in the medical sciences.[22] Florey wrote upon lysozyme the same year (1929) that Fleming reported his discovery of penicillin.[23] Indeed, that report interested Florey who wanted then to proceed with a further examination of penicillin. But for want of sufficient biochemical training, he did not do so.

After a professional period at the University of Sheffield, and further study of biochemistry, he returned to Oxford to take the Sir William Dunn Chair in Pathology. He was its first holder actually

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[21] L. Bickel, Rise Up to Life: A Biography of Howard Walter Florey Who Gave Penicillin to the World (London: Angus and Robertson, 1972), p. 30.

[22] Ibid., p. 33.

[23] A. Fleming, "On the Antibacterial Action of Cultures of Penicillium, With Special Reference to Their Use in the Isolation of B. influenzae," Lancet, 10(1929), 224-235.

trained in experimental physiology. As such he came to be in charge of a large laboratory. He began building up a research team to staff that new laboratory. Amongst its number was included Ernst Boris Chain. Chain joined what was to soon become known as the Oxford team in 1933. The beginning of the employment of the team approach to discovery and development, so important during the 1940-1960 period, is first seen with penicillin research.

Since Florey was interested in discovering an efficacious antibacterial substance, he and his team researched lysozyme, reptile toxins, and other materials. They found lysozyme the most intriguing. But by 1937 it became abundantly clear to Florey that lysozyme was a dead end. By the summer of 1938 Florey and Chain sought out a new approach in chemotherapy. Their method was to review the whole of the published literature on chemotherapy of bacterial diseases and to choose from it that which seemed to be the most promising field for emphasis. The literature, to the surprise of all, was full of references to Penicillium. A re-examination of Fleming's 1929 paper, and finally a decision (in 1938) to attack penicillin research with full force, led to certainly one of the most important projects in medical history.

At the Dunn laboratories there existed a subculture of the Penicillium strain Fleming took from his Petri plate culture in 1928. It was with that culture that the Oxford team began its work. The

growth of the mold was not rapid not did it produce much penicillin, but a sufficient amount was produced to extract and analyze further the nature of the crude substance. The methods employed were similar to those given by Conover in his statement cited in Chapter 1. Like several others in the early 1930's the Oxford team determined certain basic chemical facts concerning penicillin. One of the earliest pieces of evidence to surprise the team was the power of antibacterial activity they found. Penicillin was still active against one strain of Staphylococcus in dilution of 1:500,000. The material used was a very impure solution containing only 1% of the active substance.[24] Mouse tests indicated only slight host toxicity which would later be shown to be due, not to penicillin itself, but to the impurities in those first impure crystalline extracts. At that point the team members began specializing, some studying improvements in the extraction procedure, others microbiological effects, and still others the chemistry of the molecule. This diversification within the team became typical of the team approach over the next two decades.

It became clear that the team was dealing with the most effective and least toxic chemotherapeutic agent ever discovered. The next several years at Oxford were aimed at improvement in growing the mold to get the highest yield, improving the extraction

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[24] H. W. Florey and E. P. Abraham, "The Work on Penicillin at Oxford," J. Hist. Med., 6(1951), 304.

techniques, and extending the clinical trials. Such activities were repeated many times over with other antibiotics during the following years. Eventually, because England was under severe war pressure and could not adequately take on industrial production, Florey journeyed to America to seek assistance. It was in America that the major industrial portion of the story took place. A significant development of that American involvement was the rapid, indeed phenomenal, growth of the new antibiotic industry in America. Why this should have been so was in part explained by Florey some years later (1951). He said that the small scale of laboratory yield of an antibiotic is too small to carry out meaningful human trials. Since the "American pharmaceutical firms . . . devote very great resources to researches of new antibiotics," it was in the United States that the antibiotic revolution was so much furthered.[25]

Penicillin's impact upon medicine can be considered to be composed of five elements. First it was capable of curing many gram-positive infections. Second, unlike salvarsan or the sulfas, it worked in the minutest of concentrations. Third it penetrated to deep-seated infections which the sulfas could not. Fourth it worked much more rapidly than salvarsan (antiprotozoal only) or sulfa (anti-bacterial). Fifth, and certainly the most significant, was that

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[25]H. Florey, Antibiotics: Being the Fifty-Second Robert Boyle Lecture (Springfield: Thomas, 1951), p. 31ff.

(barring fairly uncommon allergic responses) it was non-toxic when administered systemically. As evidence of that last and most notable feature, a case history was reported where the patient received 100 million units of penicillin daily for two weeks with no side effects. This was equivalent to about 60 grams per day of pure sodium penicillin.[26]

All of those factors made it the ideal antibiotic--or so it seemed. Penicillin lacked one very important attribute. It could not affect the gram-negative bacteria. Although the wide range of cocci (staphylococcus, streptococcus, meningococcus, gonococcus, pneumococcus) were killed by it, such bacteria as the tubercle bacillus, certain pus organisms, and others, were left untouched. That, combined with the intense fervor of the period, set others to search for other useful antibiotics. That search has diminished since the close of the discovery era.

During the discovery era of the 1940's and the 1950's, a great stock-pile of new antibiotics began to accumulate. Waksman, a pioneer of soil microbiology, helped solve the biggest gap in antibiotic therapy. That was the chemotherapeutic attack upon gram-negative infections. He provided medicine with streptomycin (discussed in the following chapter). Though it was far from non-toxic,

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[26] K. Raper, "A Decade of Antibiotics in America," Mycologia, 44 (1951), 15.

it became, along with penicillin, one of the most important agents in early antibiotic history. A variant of it (dihydrostreptomycin) is still of major significance in tuberculosis therapy. Several other early antibiotics (such as aureomycin and terramycin, to be discussed in the following chapter) extended the range of antimicrobial therapy. They were known as broad-spectrum antibiotics for they are effective against not only gram-negative and gram-positive bacteria, but rickettsia, and certain viruses, as well.

Penicillin clearly brought about a revolution in therapeutic medicine. Prior to the advent of antibacterial agents, such as the antibiotics, many bacterial infections were entirely untreatable. But even as this revolution was aborning, a phenomenon termed bacterial resistance was discovered. It was shown by members of the Oxford team that the action of penicillin could be resisted by some bacteria.[27] The agent was then not effective against such bacteria. The importance of that discovery was not appreciated in 1940, however.

The realization of the value of antibiotic substances was becoming rapidly apparent at the opening of the decade of the 1940's. Many researchers sought new agents to extend the range of treatment

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[27] E. P. Abraham and E. Chain, "An Enzyme from Bacteria Able to Destroy Penicillin," Nature, 146(1940), 837.

believed possible by the employment of antibiotics. Thus the precedent set by penicillin was followed, and in a predictable pattern, over the next two decades.

3. REPRESENTATIVE ANTIBIOTICS OF 1940-1960

3.1. Choice of the Representative Antibiotics

It has been stated that a pattern in discovery and development for virtually all antibiotics discovered during the period 1940-1960 is similar. If this is so, it should be possible to pick at random any agent from that period and examine its history and show how it fits the general pattern. If one reads the technical literature for the medically useful antibiotics discovered during 1940-1960, they seem to fit the general pattern. The pattern combines the several elements mentioned by Conover. There are others which he does not consider, but which are integral to the final era definition. One cannot build a definition from one example, nor can one repeat in altered form information concerning dozens of antibiotics from the technical literature.

Three examples are considered in this chapter. Penicillin, about which more has been written by far than any other antibiotic, was given consideration in Chapter 2. Very little of a historical nature has been written about any other antibiotic. This is a crucial point. So in order to consider historically any such agent, one must seek out those that have been discussed in literature beyond the purely technical. The alternative is to have access to the corporate records of that company which discovered and developed the agent.

Researching such records has been possible in the case of vancomycin.

What is the basis for choosing any one agent as an example? In the case of penicillin it was simple. It inaugurated the era and essentially set the developmental pattern. That much history has been written on it was also helpful. Three other agents were chosen because, 1) they were very important medically during the 1940-1960 period, and 2) a very large volume of technical literature and some historical literature exists concerning them. These three are streptomycin, aureomycin, and terramycin. The above four alone constitute a representative sample, for during the peak period of the antibiotic discovery era only about one dozen agents comprised the spectrum of the most useful such medicines.[28]

However, in order to produce a viable definition with wide application, a fifth agent has been studied by examination of all the original source materials available. Vancomycin was selected for several reasons. Primary among these was because of its great importance in the treatment of highly drug-resistant staphylococci, major infectious agents in and out of the hospital environment. As well, the possibility of use of all original corporate research notes,

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[28] A.E. Hussar and H.L. Holley, Antibiotics and Antibiotic Therapy. (New York: Macmillan, 1954), p. viii.

reports, etc., made it possible to appreciate the mechanisms of discovery and development. This cannot be done relying upon technical literature only (as would have to be done with most other agents).

In the following sections several features of the three agents noted above are given. These points are given more consideration in developing the era definition (concluding chapter).

3.2. Streptomycin

The discovery of the penicillin-producing fungus was a fortuitous one made by Fleming in 1928. However, after Florey began the serious study (in 1938) of the Penicillium mold, additional discoveries of other antibiotic-elaborating microorganisms were not so fortuitous. In a concerted effort to find new antibiotics, Selman Waksman and his associates discovered streptomycin. In September of 1943 that team isolated the producing streptomycete.[29]

Within two years of the isolation (discovery), extensive "bacteriological, chemical, pharmacological, and clinical studies" were accomplished.[30] Waksman indicated that that rapid progress was due, in part, to the "spectacular rise of penicillin between 1941

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[29] S.A. Waksman, Streptomycin: Nature and Practical Applications (Baltimore: Williams and Wilkins, 1949), p. 1.

[30] Ibid.

and 1943," for it "suggested the possibility of finding other such useful compounds."[31]

At the onset of the most intensive work on streptomycin eleven chemical and pharmaceutical companies became involved in streptomycin production. That program "constituted the first privately financed, nationally coordinated clinical evaluation in history."[32] That approach, or variations of it, were integral to the developmental patterns of so many of the other discovery era antibiotics. Most commonly, however, only one company (the one whose researchers discovered a given agent) developed and produced it. Streptomycin was discovered at Rutgers University and was controlled at first by the Civilian Production Administration (a World War II governmental organization).

As with penicillin, the first isolate found to produce streptomycin was not a high-yielding strain. Thus strain selection became a major strand in the developmental history of streptomycin. Waksman and his colleagues employed radiation as a selective tool just as had been done with Penicillium only a few years previously. But that was not found to be effective with Streptomyces griseus (the

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[31] Ibid., p. 2.

[32] Ibid.

producing species).[33] Therefore his team pioneered two new methods. One, the use of actinophages (actinomycete viruses) as strain selecting agents, proved effective with streptomycin. Natural selection of strains was the second method (see aureomycin discussion).

In respect to another phase of the developmental history of streptomycin, Waksman suggested that the growth of fermentation chemistry was directly associated with the rise of antibiotic medicine.[34] Indeed, the establishment of industrial-scale fermentation techniques, introduced during penicillin's early history, made it possible for the parmaceutical industry to report over $100 million in sales for penicillin and streptomycin combined during 1947. That figure equaled half the sale of all synthetic drugs produced in the United States during that year.[35] Such figures serve to demonstrate the impact that antibiotics had on the practice of medicine at the onset of the discovery era.

In regard to the overall production of streptomycin, Waksman noted that the basic steps are fermentation, recovery, purification,

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[33] Ibid., p. 29. Waksman noted that only two strains out of 2,300 screened proved higher-yielding. Waksman considered that a poor showing when compared with penicillin research.

[34] Ibid., p. 32.

[35] Ibid.

and finishing.[36] Those four provide a convenient framework by which to compare and contrast the histories of all discovery era antibiotics. Each such agent progressed through those steps. Vancomycin as an example, will demonstrate in detail the nature of the steps. Given only that different agents with their different chemical compositions required certain variations in development, the above four steps remain identical for all agents between 1940 and 1960.

During the early work on penicillin various fermentation techniques were tried. One, submerged fermentation, was settled upon as most ideal. That precedent was followed during streptomycin's history. Integral to the use of fermentation is use of the most ideal cultural medium. So important to high yield is this, that Waksman said

> The most important increases in the level of production have been brought about by microbiologists through use of improved media and selection of high-yielding strains.[37]

In the developmental history of vancomycin this aspect of media composition was found to play an important role, and is discussed in detail in the appropriate chapter. The central role of ideal media composition was another integral unit in the homogeniety of the

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[36] Ibid., p. 33.

[37] Ibid.

discovery era pattern. Waksman's description of the culture and fermentation of Streptomyces griseus reads virtually the same for Streptomyces orientalis (the vancomycin organism) a decade later.[38] Filtration, adsorption, and recovery of crude streptomycin were techniques repeated very little altered over the next decade (and beyond).

An examination of two other significant discovery era antibiotics in briefer detail will but confirm further aspects of the era pattern.

3.3. Aureomycin

Another actinomycete, Streptomyces aureofaciens was shown, in 1948, to produce a new antibiotic, aureomycin. The pattern of the era had been so well set that workers with aureomycin stated

> Our personal experience in this area [antibiotic medicine] led to the idea that an analogy could be possible with the already known types of basic antibiotics and aureomycin. That is the reason we undertook a general study on the production and isolation of aureomycin. We followed the general plan designed by other investigators.[39]

In literature citations at the end of the paragraph quoted above, the authors mention publications on both penicillin and streptomycin.

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[38] Ibid., p. 34.

[39] P. Van Dyck and P. DeSomer, "Production and Extraction Methods of Aureomycin," Antibiotics and Chemotherapy, 2 (1948), 184. Emphasis added.

If just a few aspects of the development of aureomycin are considered, production and extraction methods and others, strong similarities in the era pattern are once again noted. In media composition, for example, the use of corn steep liquor is seen. Some years later the vancomycin team employed this substance. The choice of the corn steep liquor was a direct result of its initial employment by the penicillin team. The aureomycin researchers, both in the United States and Europe, chose to experiment with it, as did the vancomycin team. Both later dispensed with it.

Both vancomycin and aureomycin production efforts employed soybean meal, also. In the case of aureomycin it was said that

> In the basal medium we tried especially to replace soybean by a more preferable organic N [nitrogen] source.[40]

It will be seen later that the same desire to eliminate soybean meal by vancomycin researchers was expressed. Again, elements of the era pattern are seen to reemerge with the development of successive new agents.

In the case of selection techniques, the use of those established during penicillin's and streptomycin's development were tried with aureomycin. Both ultraviolet irradiation (pioneered during penicillin research) and natural selection (one of Waksman's two original

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[40] Ibid., p. 186.

techniques with streptomycin) were employed with aureomycin-producing cultures.[41]

The four basic steps (fermentation, recovery, purification, and finishing--the core of the era pattern) mentioned in connection with streptomycin hold true for all discovery era agents. In the case of aureomycin oxalic acid was found effective in extracting the antibiotic from its fermentation broth. For vancomycin picric acid was used (later replaced by other methods). Such minor variations reflect differences in the chemical composition of differing antibiotics, yet reinforce the proposition that a predictable discovery pattern existed.

With the discussion of another agent (terramycin) further pattern fit can be demonstrated. It should be pointed out that but few elements of the repeatable pattern are being considered in this and the preceding chapter. Vancomycin as exemplary of the era serves to illustrate all components later proposed as elements of the era definition.

3.4. Terramycin

The name terramycin reflects the origin of the producing organism, that is, the earth. The choice of name served in a sense

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[41] Ibid., p. 188 ff.

to illustrate the value of soil as an environment where antibiotic producers could be found. The majority of discovery era agents were produced by soil-borne streptomycetes. The discovery of such useful organisms came only through massive and world-wide soil sampling programs. Such a program is considered in detail with vancomycin. The pioneering work on the technique was done by Waksman, repeated with aureomycin, and greatly amplified by the terramycin team at the Pfizer pharmaceutical company.

Soil sampling played a major role in bringing about the close of the discovery era. It finally became apparent about 1960 that soil continued to yield up the same organisms which produced the same agents. After this soil sampling programs began to diminish. An age of semi-synthetic agents was aborning.

The finishing step, one of the four basic steps mentioned by Waksman, can involve several diverse aspects. One would be the final purification of the product, another the packaging, another the distribution. An interesting aspect regarding these elements occurring in the late developmental period is how the establishment of the era pattern allowed more rapid evolution for each succeeding agent. For instance, penicillin could be viewed as requiring about 13 years

to evolve, aureomycin about two years, and terramycin only a matter of months.[42]

This did not hold for all succeeding agents, however, for vancomycin took nearly six years from discovery to marketing. It was atypical in that regard, but in few others.

3.5. Vancomycin

Vancomycin is typical of most discovery era antibiotics. Its history could be considered as exemplary of the era in the broadest terms and most of the particulars. In this and the preceding chapter a few notable aspects of discovery and development of four agents have been considered. In order to examine each aspect closer, and take in many others required for proposing an era definition, one agent's history must be minutely examined. Since corporate records were available for this agent, it was especially attractive. It was of special medical significance during the 1950's and remains so today. Its history deserves complete documentation in its own right, yet in the greater view it helps make the construction of an era definition possible.

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[42] F. J. Stock, "The Story of Terramycin," Drug and Cosmetic Industry, 68 (1951), 177.

Since vancomycin's medical use is highly specialized, as noted in the introductory chapter, it is necessary to preface a discussion of it with a consideration of staphylococcal diseases. The following chapter considers that subject and lays a foundation for appreciating vancomycin's status as a useful and valuable discovery era example.

4. STAPHYLOCOCCUS AUREUS AND ANTIBIOTIC MEDICINE

4.1. The Use and Misuse of Antibiotics

The rise of antibiotic-resistant Staphylococcus aureus during the 1940's and 1950's (documented below) gave impetus to a search for a truly efficacious anti-staphylococcal agent. Broadly-based screening programs at the major pharmaceutical houses were aimed at general expansion toward new antibiotic capabilities. A fortuitous result of one such program was vancomycin, which is and has been produced exclusively by the Eli Lilly Company. Their aim was to look for antibiotics in general, but they specifically hoped to find a new anti-staphylococcal agent.[43]

The subject of antibiotic resistance in general, and that with the staphylococci in particular, is the central focus of this chapter. There are two reasons for considering this subject matter. In the first place antibiotic resistance in staphylococci was a major topic in bacteriological literature at the century's mid-point. That had a great influence on antibiotic searches. Vancomycin's discovery was fortuitous, but required impetus both to begin and to pursue the search for an ideal anti-staphylococcal agent. Secondly, the success of vancomycin cannot be as well appreciated without an understanding

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[43] C.W. Pettinga, personal communication, October 20, 1973.

of the importance of the problem presented by the resistant staphylococci.

If the appearance of penicillin initiated a new era in the history of medicine, it was not without some immediate difficulties. The curative power of penicillin was impressive. Because of this it was used in great quantities. In fact, "the American public is like a huge sponge that absorbs antibacterial agents like water."[44] This excessive use of the new tools (penicillin and others), had resulted in the resistance seen with certain bacteria. Diseases formerly susceptible to the action of penicillin were no longer so. And disease organisms treated later by streptomycin became resistant so rapidly that after a patient had been undergoing streptomycin therapy for four weeks, chances were 93% that he would harbor totally resistant microbes.[45]

The antibiotic industry grew rapidly after the early production difficulties in the production of penicillin were overcome. The discoveries of new antibiotics came quickly and industrial technology and production facilities grew just as fast, supplying the demands of the

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[44] H. Welch, "Antibiotics 1943-1955: Their Development and Role in Present Day Society," in The Impact of the Antibiotics on Medicine and Society, ed. by I. Gladston (New York: International Univ. Press, 1958), p. 85. Why excessive use caused a rise in resistance will be appreciated after reading the section on bacterial genetics below.

[45] G. L. Hobby, "Microbiology in Relation to Antibiotics," J. History Med., 6 (1951), 380.

new medicine. By the early 1950's there were 13 producers making available at least 17 different antibiotics.[46] The production levels had expanded greatly. In 1943 only 29 pounds of crude penicillin were produced. In 1953, 756,000 pounds of much purer penicillin was made available to be absorbed by the "sponge" of the American public.[47] At the same time there was a rapid increase in streptomycin production from 3,800 pounds in 1946 (its first year on the market), to 375,000 pounds by 1953.[48]

The increase in the availability of an antibiotic, particularly in that period when the oft-heard phrase "miracle drugs" could not be stilled, led to an increase in their employment. It was this extensive utilization of these new tools that threatened their very utility (see below). Some bacterial strains had been found to resist the effects of many antibiotics and in some cases (as with streptomycin) even became dependent upon them to survive. It was the rise of bacterial resistance, and especially staphylococcal resistance that concerns us here, for vancomycin was the antibiotic whose birth was engendered by the need to overcome antibiotic-resistant staphylococci. In order to appreciate the role of Staphylococcus aureus in antibiotic medicine,

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[46] Welch, "Antibiotics," p. 72.

[47] Ibid., p. 25.

[48] Ibid., p. 76.

it is germane to briefly consider the history of man's knowledge of the organism.

4.2. Ogston of Aberdeen

Prior to 1880 concepts of blood poisoning etiology were chaotic.[49] But soon thereafter much light was shed on the subject. That came only after the careful investigations of Sir Alexander Ogston (1844-1929), a Scottish bacteriologist. He studied the origin of acute suppurative processes in man. His studies were not aimed so much at scientific nosology as at practical application in surgery and medicine. During the course of his investigations Ogston made clear the etiology of suppuration, septic wounds, and related infectious processes.

The discovery of the use of aseptic surgery led physicians and surgeons of the period (1870's) to question whether surgical sepsis was not, in fact, of bacterial origin. Ogston, like others, wondered at what may cause sepsis. He "often meditated on the subject and became the more convinced that there was a single cause . . . some special germ."[50] During that period several individuals reported

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[49] S. Elek, Staphylococcus pyogenes: And Its Relation to Disease (Livingston: Edinburgh, 1959), 2.

[50] Cited in Ibid., p. 2. The quotation is a remake of another one cited by Elek, but for which he gives no source.

seeing cocci or micrococci in various pathological processes. But others strongly opposed any suggestion that such organisms could be implicated in the disease mechanism.[51] Elek credits Ogston as having settled the debate clearly in a German language publication in 1880, which subsequently became generally available as an English version in 1881.[52] By infecting laboratory animals with micrococci and demonstrating typical suppurative lesions, Ogston was able to implicate the microorganism. The organisms were grouped, he said, "like the roe of fish, into clusters," and to them Ogston gave the name Staphylococcus.[53]

The turning point in the understanding of the etiology of various septic disorders set off many investigations during the decade following 1880 on the staphylococci. Much attention was accorded the color phenomenon of the organism. It was generally felt, even before Ogston's reference to it, that the golden, yellow, or orange hue of pus should be considered a sign for much concern by the physician.

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[51] Ibid., p. 4. Such others included Louis Pasteur (1822-1895) who grew the organisms in broth in 1880. His thoughts on the role such cocci played were not well known to others. The Pathological Society of London held (in 1879) that the cocci might be the agents of suppuration. Ibid.

[52] A. Ogston, "Report Upon Micro-Organisms in Surgical Diseases," Brit. Med. J., 1 (1881), 370. See Elek, Staphylococcus aureus, p. 4, also.

[53] Ibid. Staphylos is the Greek for a cluster of grapes.

Septic wounds often led to fulminating septicemias and, as Ogston demonstrated, the Staphylococcus was the agent. As Ogston had shown the virulence and pathogenicity of staphylococci in animals, others would within a half dozen years demonstrate it in man.[54]

The first attempts at classification were based upon whether or not the organisms were pathogenic for man and/or animals, or were non-pathogenic commensals. The method was not satisfactory, however, as it was impractical to test every culture for pathogenicity by animal inoculation. Serological typing, used to such great advantage with the streptococci later, was attempted at the turn of the century, but also proved fruitless.[55] Development of a feasible systematics matured over a very long period of time.

The history of nomenclature in the staphylococci is very complex and an examination of it here is not necessary. The monograph (see fn. 49) by Stephen Elek discusses in detail the subject of nomenclature. The work on staphylococci during the 1940's and early 1950's, which is the central period of interest for this chapter, employs several synonyms, but all refer to the same organism.[56]

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[54]Elek, Staphylococcus, p. 6.

[55]Ibid., p. 6 ff.

[56]The name Staphylococcus was applied, as mentioned above, by Ogston, in 1880. Nevertheless it is a nomen nudum and the valid publication of the name falls to Rosenbach (1884). This organism is

4.3. Microbial Resistance

Vancomycin was discovered in 1953 and made commercially available five years later. Beginning in 1954, the first human beings had been administered the drug. It was still possible to say in that year that

> While other species of bacteria have demonstrated resistance to some of the antibiotics, the Staphylococcus has been the most consistent in exhibiting prompt resistance to each of the antibiotics, and infections due to this species pose the most serious clinical problem of antibiotic resistance today.[57]

What led up to this situation began with the first appearance of an antibiotic (i.e., penicillin). But in fact, microbial resistance in general had been of significance very much earlier than that. Coincident with the very beginnings of modern chemotherapy resistance had been encountered. Thus Ehrlich, even before 1910 and the release of salvarsan, discovered that the microbes were able to repulse the attacks of chemotherapeutic agents (see below). The trypanosomes

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the species most commonly indicted in staphylococcal diseases and is the species of central interest to this history. Other synonyms will be seen in the literature. These include Micrococcus pyogenes aureus (Rosenbach) Zopf, 1885; Micrococcus aureus (Rosenbach) Zopf, 1885; Micrococcus pyogenes Lehmann and Neumann, 1896; and others. For further amplification the reader is referred to E. Buchanan, et al., eds., Index Bergeyana (Baltimore: Williams and Wilkins, 1966), p. 1062 ff.

[57] W. Spink, "Staphylococcal Infections and the Problem of Antibiotic-Resistant Staphylococci," Arch. Int. Med., 94 (1954), 167.

with which he was working are very different from the bacteria and it is unlikely their biochemical defense system is analogous to that of the bacteria. (That is, many antibacterial agents disrupt the cell wall, a structure not found in protozoa.) Be that as it may, it is clear that the earliest workers could foresee problems in chemotherapy presented by the resistance phenomenon. Ehrlich found that atoxyl, trypan red, trypan blue, and parafuchsin (all chemotherapeutic agents) could be ignored by strains of resistant trypanosomes. In the case of atoxyl-resistant microbes in mice the dose required to inhibit or kill the parasite exceeded the lethal dose for the host.[58] Such resistance was often long in coming (in vivo), but could develop as quickly as in two weeks after the onset of treatment. He also found that it was a general law that once resistance was acquired it remained heritable.[59] (This is not a general law in bacteria; see below.)

Mutual resistance (we would now call it cross-resistance) was also seen in the case of the dyes--resistance acquired to trypan red would also obtain for trypan blue. That type of resistance much later would make the broad spectrum antibiotics virtually ineffectual against the staphylococci (see below). Ehrlich surmised that use of related

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[58] P. Ehrlich, "Chemotherapeutic Studies on Trypanosomes (Third Harben Lecture)," in Collected Papers, 3, p. 131.

[59] Ibid.

compounds against parasites might lead to mutual resistance and, in fact, saw a great research tool in this. A physician, but yet a consummate scientist, Ehrlich was much intrigued by mutual resistance. He realized he could use it as a technique to differentiate between various antimicrobial agents whose chemical structure might not otherwise be known. Thus, he felt various specific resistances might lead to classify a new agent by chemical family. By using this therapeutic sieve or, as he called it, his "cribrum therapeuticum," such classification would be accomplished.[60] Thus, "if a substance is found to have a destructive effect on . . . three different strains, it necessarily belongs to a fourth chemical group."[61]

Resistance, then, was not the exclusive property of the antibiotic era. Not only did Ehrlich find it during pre-antibiotic times, but it was seen later with the sulfonamides acting against bacteria. In the first publication on this sulfonamide-resistance the authors reported the resistance of various bacteria, including the staphylococci, to sulfa drugs.[62] This resistance was seen not only in vitro, but in vivo as well. And as Ehrlich had noticed with the trypanocide

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[60] Ibid., p. 132.

[61] Ibid.

[62] J. J. Vivino and W. S. Spink, "Sulphonamide-Resistant Strains of Staphylococci: Clinical Significance," Proc. Soc. Exper. Biol. Med., 50 (1942): 336-338.

atoxyl, the onset of resistance could be sudden. In that first sulfonamide case investigated a Staphylococcus strain became resistant (in man) within eight days.[63] The speed of resistance development, as shown later, could be very rapid with some antibiotics. This was true of streptomycin against staphylococci (as discussed below).

The year 1942 must have been a depressing one for the medical community, for not only was sulfonamide-resistance first reported, but so also was penicillin-resistance. Charles Rammelkamp (b. 1911), who had been one of the very first physicians to employ penicillin, was also the bearer of news of its first known defeat. It is not surprising that the title of his paper bears the name of Staphylococcus in it, for from the very beginning of antibiotic-resistance history the staphylococci would be in the foremost role.[64]

In contrast to this, other bacteria (such as many streptococci, for example) have remained highly sensitive to penicillin for three decades. For example, Rammelkamp noted in his report that, unlike Staphylococcus, a strain of hemolytic Streptococcus did not develop

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[63] Ibid., p. 338.

[64] H. Rammelkamp and T. Maxson, "Resistance of Staphylococcus aureus to the Action of Penicillin," Proc. Soc. Exper. Biol. Med., 51 (1942), 386-389.

resistance to penicillin.[65] That encouraging observation proved to point out that the staphylococci were the primary offenders. Even in 1955 when the resistant staphylococci had grown to be a problem of major proportions, streptococci remained penicillin-sensitive.[66] Furthermore, an additional two decades still did not change that picture. It was possible in 1972 to say, "Strep[-tococcus] pyogenes is always very sensitive, and sensitivity testing is not required [i.e., prior to employment of penicillin in streptococcal infection]."[67]

On the dismal side of the situation, however, Rammelkamp had shown a rapid acquisition of resistance by staphylococci (16-fold in 2 days). The mechanism of that resistance seemed unclear because Rammelkamp could not demonstrate penicillinase as Florey's Oxford team had done only a short time before (see Chapter 2).[68] Penicillinase was then assumed the only mechanism of penicillin resistance (though it was later found that others were also possible).

The matter of bacterial resistance to antibiotics became for the clinician a matter of great concern. What was he to do when a

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[65] Ibid., p. 387.

[66] A. Berntsen, "Unaltered Penicillin Susceptibility of Streptococci," J. Amer. Med. Assoc., 157 (1955), 331-333.

[67] A. Kucers. The Use of Antibiotics (London: Heinemann, 1972), p. 4. This book is the only current handbook on the use of presently available antibiotics.

[68] Rammelkamp, "Resistance," p. 388.

resistant staphylococcal sepsis occured in a patient? If he could not turn to penicillin, would some other antibiotic resolve the dilemma? Much success, but many failures, marked anti-staphylococcal antibiosis over more than a decade from Rammelkamp's observation to the early vancomycin period (later 1950's).

How resistance developed and what its mechanism was occupied various investigators beginning in the mid-1940's. Most significant was the work of M. Demerec (1895-1965). A geneticist at the Carnegie Institution (Cold Spring Harbor, New York), Demerec elucidated the mechanism of penicillin resistance. The organism of choice was Staphylococcus. It is a fact that investigations into general bacterial resistance to antibiotics and specifically staphylococcal resistance to antibiotics go hand in hand. Studies on general resistance seem invariably to employ staphylococci. The history of our knowledge of mechanisms of bacterial resistance is based upon that one genus. This is not surprising because Staphylococcus was first to be implicated in resistance phenomena and proved to be the most refractory to chemotherapy. If it had not been one of the most central concerns of infectious medicine prior to the antibiotic era, it certainly became so quickly after the inauguration of that period.

By the mid-1950's, a great many papers had been published on bacterial drug-resistance. In the 1940's, there was a tendency toward controversy on which mechanism might be correct. But by the

mid-1950's, those controversial questions had "lost most of their original interest."[69] The reason being that one (or two) mechanisms were generally conceded to be the most likely ones operative. The central mechanism, mutation and selection, was suggested by Demerec.

In 1945, Demerec set out on a quantitative study to "clarify the genetic aspect of the mechanism through which resistance is formed."[70] He posited two possible mechanisms: (1) resistance is an acquired characteristics, or (2) it is an inherited characteristic arising through mutation which origin was not penicillin dependent. That is, resistant mutants would occur at random and be selected for in the presence of penicillin, the drug killing the sensitive or non-resistant individuals. Demerec, after some very elegant experimentation, decided in favor of the second postulate.[71] The penicillin seemed to affect only the dividing bacterial cells,[72] and the pattern of

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[69] W. Szybalski and V. Bryson, "Origin of Drug Resistance in Microorganisms," in Origins of Resistance to Toxic Agents, ed. by M. G. Sevag, et al. (New York: Academic Press, 1955), p. 22.

[70] M. Demerec, "Production of Staphylococcus Strains Resistant to Various Concentrations of Penicillin," Proc. Nat. Acad. Sci., 31 (1945), 16.

[71] Ibid., 19.

[72] Demerec's discovery, though it had no special historical significance then, helps now to illuminate another problem. Hare, in his Birth of Penicillin (see Fleming discussion in Chapter 1) points out

the appearance of resistance was step-wise and distinctive. In a somewhat later study (1948) Demerec found a second pattern distinctive for streptomycin.[73] Since 1948 antibiotics have been shown to develop resistance generally in accord with either the penicillin pattern or the streptomycin pattern. How these two patterns operate is fascinating, but for the clinician they did not solve the practical problem of the resistant staphylococci. Yet the literature of bacterial resistance is filled with a discussion of these two modes (see fn. 78 below).

The penicillinase problem, however, was a refinement of one of Demerec's two possibilities and would, in the broadest use of the term acquired, fit in as an example of that hypothesis. The production of penicillinase is adaptive and homogeneous throughout the population challenged by penicillin.[74] Not all penicillin-resistant staphylococci which were isolated from infective processes were

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in extenso reasons why the discovery of penicillin was so extremely fortuitous. The fact that penicillin affects dividing cells sets a definite temporal relationship for the appearance on the culture dish of the penicillium spore and its subsequent product penicillin. Had, as Hare points out, the spore arrived on the dish at a somewhat different point in time than the seeding of the plate with Staphylococcus, penicillin would have been missed.

[73] M. Demerec, "Origin of Resistance to Antibiotics," J. Bacteriol., 56 (1958): 63-74.

[74] M. Barber, "Antibiotic-Resistant Staphylococcal Variants," in Adaptation in Micro-Organisms (Cambridge: The University Press, 1953), p. 235.

found to produce penicillinase, although in general that was found to be "the main source of their resistance to penicillin."[75] Also the cells were not necessarily permanently penicillin-resistant, as Ehrlich's trypansomes were to atoxyl (see above). By the mid-1950's "mutation, associated with a process of selection," explained the emergence of penicillin-resistant staphylococci.[76] Those were not all resistant due to the ability to produce penicillinase, though. At least three other types of penicillin resistance had come to be noticed by 1954. Cells which do not produce penicillinase, but were penicillin-resistant: (1) did not combine with penicillin (reason(s) unknown); or (2) did not degrade the penicillin intracellularly; or (3) had components of the cell which would be penicillin-vulnerable and which had a low reactivity with penicillin.[77]

Those were the mechanisms for explaining bacterial resistance at about the time of the discovery of vancomycin. Resistance against streptomycin, chloramphenicol and other early antibiotics seemed primarily due to random mutation as no adaptive enzymes (such as penicillinase) were demonstrable with those agents.[78] The

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[75] Ibid., p. 238.

[76] Ibid., p. 238.

[77] H. Eagle, "The Multiple Mechanisms of Penicillin Resistance," J. Bacteriol., 68 (1954), 615.

[78] Barber, "Resistant Staphylococci," 243 ff.

conclusions of workers in the field of antibiotic resistance were uniform. A multiplicity of highly similar publications became available.[79] Each stressed the importance of Demerec's work. Each concentrated on resistance in staphylococci in particular. None offered a basis by which a specific anti-staphylococcal agent could be purposely designed. Such an agent would have to come from an empirical search, but that would come as a result of the realization of the threat the resistant staphylococci offered. Mechanisms of resistance were being illuminated, but what of the clinical status in anti-staphylococcal therapy?

4.4. Penicillin and Staphylococcus aureus

The use of penicillin against staphylococci presented in most instances a none too hopeful picture. There were repeated successes, failures in treatment became more and more common. The reason for this was not so much because the staphylococci could grow in high

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[79] In addition to those publications on bacterial resistance mentioned in the preceding few footnotes, several others should be consulted. These include V. Bryson and M. Demerec, "Bacterial Resistance," Amer. J. Med., 18 (1955): 723-737; A.E. Hussar and L. Holley, Antibiotics and Antibiotic Therapy (New York: Macmillan, 1954), pp. 19-27, 34-39, 59-60; M. Welsch, "La Résistance Bacterienne aux Antibiotiques," Schweizerische Medizinische Wochenshrift, 85 (1955): 274-279; "Resistance of Micro-Organisms to Antibiotics," the editors, Research Today, 13 (1957): 22-41; and W.W. Spink, "Staphylococcal Infections and the Problem of Antibiotic-Resistant Staphylococci," Arch. Int. Med., 94 (1954): 167-196.

concentrations of penicillin, but because they inactivated the antibiotic outright.[80] That was usually due to the action of penicillinase.

The enzyme penicillinase was first observed by the Oxford team in 1940 (Chapter 2) and was recognized, by 1953, to be the main source of penicillin resistance in staphylococci. Staphylococcus aureus was not the only bacterium capable of penicillinase production, indeed its production was shown to be wide-spread among the eubacteria. The Oxford team had originally demonstrated it, not in staphylococci, but in the mammalian gut bacterium Escherichia coli. Shortly thereafter (1944), penicillinase production had been demonstrated in such diverse bacteria as Bacillus cereus, Bacillus anthracis (anthrax bacillus), Aerobacter aerogenes, Shigella dysenteriae (etiologic agent of bacterial dysentery), Pseudomonas species, and a great many others.[81] A significant point was the mistaken belief that penicillinase-positive organisms were generally non-pathogenic (excepting Shigella spp.).[82] Although penicillin-resistant staphylococci had been observed prior to 1944, it was not known even then why they were resistant. In time the production of penicillinase by one group of bacteria would become the center of interest. That group,

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[80] Barber, "Resistant Staphylococci," 243.

[81] A. Bondi and C. C. Dietz, "Production of Penicillinase by Bacteria," Proc. Soc. Exper. Biol. Med., 56 (1944), 133.

[82] Ibid., 134.

unrecognized in 1944, was the staphylococci.

In 1943, case histories of penicillin failure against the resistant staphylococci began appearing in the literature. In its early use the new antibiotic was in competition with the sulfa drugs as well as being in short supply. Its use as a last-resort effort in some cases made an observer wonder if it was being used against a resistant staphylococcus or merely being used too late on a given patient. For example, a 32 year old woman who had undergone sulfonamide therapy for 19 days for a fulminating staphylococcal bacteremia, showed no improvement.[83] A last-resort two-hourly dose of 40,000 units of penicillin for five doses was insufficient as she died the next day with no apparent improvement before death. Mary Florey (1900-1966) and the other physicians on the Oxford team had gotten remarkably rapid recoveries many times though during that period of the early 1940's. In another example a patient had been treated simultaneously with massive doses of oral sulfa drugs and intramuscular penicillin for two months. He eventually died of staphylococcal septicemia having never shown signs of improvement. That the physicians in the later case were dealing with a penicillin-(and sulfonamide)-resistant staphylococcus is only too clear.[84]

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[83] Case Records of the Massachusetts General Hospital. Case 29371., New England J. Med., 229 (1943): 481-485.

[84] Ibid., Case 29162, 519-522.

Within a few years individual case history reports were being displaced in the literature by impersonal lists of statistics attesting to antibiotic failures against the resistant staphylococci. In 1947, 81% of 239 strains of Staphylococcus aureus were penicillin-sensitive, in 1948, 78%; in 1949, 62%; and in 1951, only 47%.[85]

In that year of 1947, Mary Barber (1911-1965), an astute observer of staphylococcal resistance, noted that the incidence of strains of Staphylococcus aureus resistant to penicillin was "increasing rapidly [and had become] somewhat alarming."[86] That understatement underwent a maturation over the next several years. Soon all such articles opened in much the same manner--each showing an increasing tendency toward greater alarm. In 1955, one typical opening statement was: "the enormous increase in resistance of staphylococci has raised . . . important questions for physicians."[87]

The cause of the increase was that the intensified use of penicillin was causing a shift in the gene pool, therefore strains that were more resistant were appearing in greater numbers in the

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[85] E. Reiss, et al., "Penicillin Sensitivity of Staphylococci," New England J. Med., 246 (1952), 64.

[86] M. Barber, "Staphylococcal Infection Due to Penicillin-Resistant Strains," Brit. Med. J., 2 (1947), 863.

[87] V. Knight and H.S. Collins, "A Current View on the Problem of Drug Resistant Staphylococci and Staphylococcal Infections," Bull. N.Y. Acad. Sci., 31 (1955), 549.

population. Demerec had demonstrated the mechanism for this and the growing literature attesting to the increasing rate of resistance was a proof of it. Barber's 1947 findings showed a resistance level of 38% (with apparently 100% of these strains penicillinase producers), and a year later she found the resistance increasing. In 1948 that percentage had risen to 59%, whereas in 1946 she had found only 14% resistance.[88] The work of Mary Barber was by no means isolated for other investigators world-wide were making similar discoveries. In 1948-9 in Sydney, Australia, 53% of hospital-isolated strains if Staphylococcus aureus were resistant; in Minneapolis in 1950, 56%; in Boston in 1951-2, a stunning 73%.[89]

Until the oral form of penicillin became available in the later 1940's the only way one could receive the antibiotics' benefits was in a hospital. Early administration was by intravenous infusion only. Somewhat later intramuscular injections were possible, but a rapid decrease in blood levels of the active penicillin required repeated administrations. Finally, longer lasting intramuscular preparations made possible a workable regimen less offensive to patient and physician alike. Because oral penicillin was later in coming, the observations on the increase of staphylococcal resistance to penicillin

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[88] M. Barber and M. Rozwadowska-Dowzenko, "Infection by Penicillin Resistant Staphylococci," Lancet, 2 (1948), 641.

[89] Knight, "A Current View," 551.

was primarily hospital-associated. The so-called "hospital staph." was recognized early and to this day the major problems of staphylococcal resistance are in hospitals. When vancomycin became available the earliest advertising literature introduced the new antibiotic as of special importance in hospitals. This situation has not changed. (With one exception, noted in Chapter 5, vancomycin cannot be administered orally--thus the need for a hospital environment.)

Mary Barber's work had "aroused much interest and not a little alarm" by 1949.[90] The rising resistance was clearly hospital oriented. In one example of 78 cases of staphylococcal diseases, 40 were out-patients and 38 were in-patients. Of the 40, a 12.5% resistance was found, but of the 38, 68.4% resistance was shown.[91] The question of the origin of those resistant strains was asked. The discovery that carriers were present on the staff of the hospital provoked much discussion in the literature. The longer one stayed in the hospital, of course, the greater the risk of exposure. Hence more "hospital staph." was available from more hospital staff. It behooved the patient to stay but a short time in the hospital lest he acquire an unwanted infection. Of the patients staying in the hospital 0-1 day the percentage of resistant strains isolated from them was 25%, those

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[90] G. B. Forbes, "Infection With Penicillin-Resistant Staphylococci in Hospitals and General Practice," Brit. Med. J., 2 (1949), 569.

[91] Ibid., p. 570.

staying 2-7 days yielded 52% resistant strains, and those over 8 days had 68% of such strains in infective processes.[92]

The staff who carried the resistant strains included everyone from doctors and nurses to maids. Of 50 ward nurses, 46% carried resistant strains in their anterior nares. In a comparison study a number of office workers, totally unrelated to the hospital environment workers, were shown to have among them only 2% of carriers.[93]

Not unexpectedly by 1956, staphylococcal resistance in the community at large was increasing as it had done theretofore in the hospital community. The 12.5% of outpatients with resistant strains demonstrated in 1949 (above) had increased to 38% by 1956.[94] Staphylococcal infections of varying types were not uncommon in the general population. But, as noted above, in the hospital they were much more common. A cycle of reinfection of patient and staff continued to occur and a good many staff members in a large hospital could at any one time be carriers, convalescents, or patients themselves. That was especially true in earlier years and was

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[92]H. J. F. Cairns, "Penicillin-Resistant Staphylococci: Incidence in Relation to Length of Stay in Hosptial," Lancet, 1 (1950), 446.

[93]Forbes, "Infection," p. 571.

[94]M. Finland and W. F. Jones, "Staphylococcal Infections Currently Encountered in a Large Municipal Hospital: Some Problems in Evaluating Antimicrobial Therapy in Such Infections," Ann. N. Y. Acad. Sci., 65 (1956), 193.

demonstrated at Boston City Hospital at one point in the mid-1950's. A survey of nosocomial infections[95] indicated just how severe hospital staphylococci had become. Of the in-house physicians with varying staphylococcal infections, 18 had carbuncles or furuncles, 9 others were convalescing from other staphylococcal diseases, 7 nurses were out with similar ills, 8 ward attendents had known ongoing infections, and other similar infections were suspected.[96]

Clearly from as early as penicillin became available until well into the mid-1950's, the staphylococci had presented a difficult problem. Penicillin resistance was not (and is not) universal among the staphylococci, but its occurrence was so notable that it soon became apparent that alternatives would have to be sought.

4.5. The Search for Alternatives

By the mid-1950's there were a goodly number of different antibiotics available. As of 1954 the antibiotics in common use included: penicillin, streptomycin, aureomycin, terramycin, tetracycline, chloramphenicol, bacitracin, polymyxin, neomycin,

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[95] Reinfection in the hospital by hospital-harbored agents is so common that the situation has warranted its own term (i.e. nosocomial).

[96] Finland, "Staphylococcal Infections," p. 193-194.

tyrothricin, erythromycin, and carbomycin.[97] Not all of those were effective against the staphylococci for varying reasons. Tyrothricin, though antagonistic to gram-positive organisms (staphylococci are gram-positive), could not be used systemically and hence was never of any particular value except in topical application. The antibiotics aureomycin (=chlortetracycline), terramycin (=oxytetracycline), tetracycline, and chloramphenicol formed the closely related group known as broad-spectrum antibiotics. They antagonized both gram-positive and gram-negative bacteria, but their antimicobial activities were all virtually the same. When resistance to one occurred it automatically occurred with the others, hence they possessed mutually susceptable cross-resistance.[98] For that reason only one, aureomycin, was heavily employed against the staphylococci. Streptomycin, since its greatest activity is against gram-negative organisms was never particularly significant as an anti-staphylococcal agent. Streptomycin also lost its effectiveness against the staphylococci very rapidly (see below). Erythromycin was heavily used against the staphylococci and since carbomycin was subject to mutual cross-resistance with erythromycin, it was rarely employed. Bacitracin, though active against staphylococci, appeared to operate like penicillin.

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[97] A.E. Huser, and H.L. Holley, Antibiotics and Antibiotic Therapy (New York: Macmillan, 1954), p. xii.

[98] Ibid., p. 410.

Resistance to it developed much as it had with penicillin, hence it never played a major role. Polymyxin was only a gram-negative antagonist and neomycin was somewhat cross-resistant with streptomycin and it was very toxic.[99]

Other antibiotics became available during the 1950's. A variety of semi-synthetic penicillins are now available, as well as more tetracyclines. Many of the antibiotics available in 1954 are still available two decades later. Some have fallen into disuse (e.g., tyrothricin, carbomycin).[100] Many other new classes of antibiotics exist today (e.g., the cephalosporins).

The literature of the pre-vancomycin period concentrated upon three antibiotics as penicillin-alternatives in staphylococcal treatment. Those were aureomycin, erythromycin, and to a much lesser extent, streptomycin. In 1952 aureomycin (and related tetracyclines) and streptomycin were considered the principal penicillin alternatives.[101] In that same year erythromycin became available and at first looked extremely promising. Streptomycin, though, was the first alternative considered when it became apparent that the staphylococci were becoming penicillin-resistant. Aureomycin and

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[99] Ibid., p. 171.

[100] Kucers, Use, passim, various chapters.

[101] W.D. Linsell, "The Antibiotic Sensitivity of Pathogenic Staphylococci," J. Clin. Path., 5 (1952), 166.

erythromycin then were considered in that order.

The discovery of streptomycin was first announced in the literature in 1944, but it became available for clinical use in 1946. Streptomycin-resistant organisms were reported that same year, and there were even more reports the following year. Several reports concerning staphylococcal-resistance to streptomycin had appeared by 1947. By 1948, some strains of Staphylococcus aureus (as well as four other pathogens) had been shown to be streptomycin-dependent for their growth.[102] This dependence was not as permanent a characteristic as simple resistance, but under conditions of dependence, strange, pleomorphic forms were demonstrated.[103] Although resistance to penicillin did not seem to be permanent, that to streptomycin evidently was. These findings seemed to suggest that the application of streptomycin in staphylococcal diseases was of little value. At any rate, it was early appreciated that streptomycin was much more active on gram-negative organisms than on gram-positive and it was

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[102] T. F. Paine and M. Finland, "Observations on Bacteria Sensitive to, Resistant to, and Dependent Upon Streptomycin," J. Bacteriol., 56 (1948), 209.

[103] In 1948 Klimek, Cavallito, and Bailey reported that they witnessed pleomorphism and conversion to the gram-negative state in penicillin-grown Staphylococcus aureus. They had been ridiculed by various authors. Such things must have been contaminants so it was thought. The observation by Paine and Finland, however, tends to lend credence to that of Klimek, et al. See J. W. Klimek, et al., "Induced Resistance of Staphylococcus aureus to Various Antibiotics," J. Bacteriol., 55 (1958): 139-145.

not surprising that there were "rather wide variations in the sensitivity [of staphylococci] to streptomycin."[104] The pattern of development of resistance (noted earlier) by its nature led to a very rapid increase in streptomycin resistance by many microorganisms. The success of streptomycin was primarily in the treatment of tuberculosis, where it has had a great impact. It still remains today the drug of choice for plague, tularemia, and brucellosis. In most other earlier applications it has been superceded by other more effective agents.[105]

Chronologically the next alternative to penicillin against the resistant staphylococci was the tetracycline group. Aureomycin was discovered in the very year (1948) that streptomycin-dependence was demonstrated. Aureomycin, like streptomycin and vancomycin, and in fact most major antibiotics, was derived from species of the genus Streptomyces of the order Actinomycetales. Many of the antibiotics so derived, not unexpectedly, have similar chemical structures. Thus streptomycin and carbomycin are quite similar. Aureomycin, terramycin, and tetracycline are virtually the same, structurally though they differ from streptomycin and carbomycin. For that

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[104] M. Finland, et al., "In vitro Susceptibility of Pathogenic Staphylococci to Seven Antibiotics," Amer. J. Clin. Path., 29 (1950), 332.

[105] Kucers, Use, p. 122-123.

reason the broad-spectrum group (=tetracyclines and chloramphenicol) antagonize staphylococci similarly.

As noted earlier, the use of a given antibiotic, extended over time, selects for resistant mutants. The tetracyclines in general, and aureomycin as exemplary of them, provides an excellent example of this situation. In early 1949, of 50 strains of Staphylococcus aureus isolated, 34 were penicillin-resistant. Of this number, 14 were also streptomycin-resistant, but all 34 were very sensitive to aureomycin.[106] Later on, as shown within the same 1949 study, an increase of aureomycin-resistance was appearing. For example, in one case use of aureomycin seemed to irradicate staphylococci in the patient's blood. The blood remained sterile for 26 days, after which time staphylococci reappeared. Where 0.78 micrograms of aureomycin per milliliter of serum had seemingly sterilized the blood at the onset of treatment, 3.2 micrograms were required to successfully treat the patient after reappearance of the bacterium.[107] Actually that rapid

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[106] R. Nichols and G. M. Needham, "Aureomycin in the Treatment of Penicillin Resistant Staphylococci Bacteremia," Proc. Staff Meet. Mayo Clin., 24 (1949), 310 ff. It should be noted that the definitions of "sensitive" and "resistant" are not fixed as to so much weight or volume of antibiotic being the dividing line between these classes. The definitions were routinely set by the various investigators in each of their respective studies. For that reason statistics vary, over time in their qualitative interrelationship. Nevertheless this points out a trend, not absolute (mathematically), but very real.

[107] Ibid., 313.

rise of resistance during the treatment of a single patient had occurred with streptomycin too. Such resistance to aureomycin, which had been known only one year, did not bode well for the future.

In the same year (1949), however, successes were registered. Five infants (noted in one study) with serious staphylococcal infections that were refractory to sulfas and penicillin, recovered quickly under aureomycin treatment.[108] A survey of seven antibiotics tested against staphylococci (in vitro) in 1950, showed a "rather high susceptibility" of most strains to aureomycin. The study did not conclude as optimistically, however. There was "some intimation" that some strains had a "relatively high" resistance to aureomycin. And "it seems not unlikely that [with increased use] . . . more strains which are relatively resistant . . . will be found."[109]

Evidently strains varied widely in their response to aureomycin, for in 1952, one investigator still saw "an optimistic picture."[110] But a quantitative examination of the rise of resistance to aureomycin by staphylococci, showed the rise to be statistically significant. That was found to be true with streptococci, Proteus species, and colon

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[108] C. A. Chandler, et al., "Observations on Staphylococcal Infections Treated with Aureomycin," Pediatrics, 4 (1949): 149-156.

[109] Finland, "In vitro Susceptibility," 333.

[110] Linsell, "Antibiotic Sensitivity," 168.

bacilli, in addition to the staphylococci.[111]

In 1953, the level of aureomycin-resistant staphylococci in the general population was unknown, but in the hospital it was on a strong increase.[112] The related members of the tetracycline family experienced increasing resistance as did aureomycin. In one hospital in 1951, 4.8% of strains were resistant to these agents, and one year later it had risen to 78%.[113] It had become abundantly clear, particularly in hospitals, that not only was streptomycin not a viable alternative to penicillin, but also neither was aureomycin.

During that very period (1952) a new anti-staphylococcal agent, erythromycin, became available. The range of antimicrobial activity of this new agent was found to be quite large. It antagonized gram-positive bacteria, and gram-negative, as well. Although the early failures of erythromycin against staphylococci seemed dismal, those failures were related primarily to the hospital staphylococci. In 1972 erythromycin was still antagonistic to most staphylococci, but

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[111] S.S. Schneierson, "Changes in Bacterial Sensitivity to Aureomycin and Chloramphenicol in the Course of the Past Three Years," J. Lab. Clin. Med., 40 (1952), 56.

[112] H.F. Dowling, et al., "Observations on the Epidemiological Spread of Antibiotic-Resistant Staphylococci, With Measurements of the Changes in Sensitivity to Penicillin and Aureomycin," Amer. J. Pub. Health, 43 (1953), 860.

[113] Knight, "Current View," 551.

the resistant forms remain, even today, a hospital problem generally untreatable by the agent.[114]

In its first year, only rarely had a strain of Staphylococcus aureus been found that was resistant to erythromycin.[115] Provided endocarditis had not developed, septicemia was well controlled by erythromycin. In six of eight cases of varying staphylococcal diseases erythromycin proved highly effective. In the remaining two (with endocarditis), though, the bacteria were seen to develop resistance with "extreme rapidity."[116] For that reason the investigators recommended against erythromycin when treating staphylococcal endocarditis.

Possibly the best example of rapid development of resistance toward an antibiotic can be given in respect to erythromycin. In September of 1952, no erythromycin resistant strains were reported in one hospital, but only one month later resistant strains were found (in the anterior nares of staff carriers). By the following January most strains were resistant. Discontinuing use of erythromycin in the hospital caused a rapid drop in the incidence of resistant strains,

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[114] Kucers, Use, p. 207.

[115] W. Herrell, et al., "Erythromycin for Infections Due to Micrococcus pyogenes," J. Amer. Med. Assoc., 152(1953), 1601.

[116] Ibid., 1602.

but even so 20% remained resistant.[117]

By 1947, Mary Barber had been alarmed. Her sentiments were expressed again by other investigators a half dozen years later. If the alternatives to penicillin were not alternatives in fact, where could one go from there? The answer was to combine two antibiotics. That approach was taken and with success at times.

4.6. Antibiotics in Combination

The trends in antistaphylococcal therapy were event-dependent. The introduction of a new antibiotic was an event. After the appearance of each antibiotic a series of studies of clinical applications of the agent would appear. In the case of new antistaphylococcal agents, great hope would be expressed early. Some time thereafter the warning aura of the decline of the new agent became clear. Hopes of something new coming to fore were then expressed. That cycle was repeated several times and by 1953, no new antistaphylococcal agents were in ascendence (except vancomycin). The only apparent alternative was to use the previous antibiotics of choice in combination against the resistant staphylococci.

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[117] H. F. Dowling and M.H. Lepper, "Clinical Significance of Antibiotic-Resistant Bacteria," J. Amer. Med. Assoc., 157 (1955), 328.

The rationale of antibiotic-combination therapy was not ill-founded. Although combination therapy had been employed as early as 1944 (see below), the practice became common only in 1953. That later year seems to have marked the point at which the clinicians felt obliged not to hope further for the ideal antistaphylococcal agent. Instead they looked at the older antibiotics and asked, if manipulated differently, might they yield improved results? Manipulation by combination proved a not entirely ill-based hope.

The use of sulfa drugs had enjoyed great success on many gram-positive cocci, but not on Staphylococcus aureus. By 1944, not only were the antibacterial sulfas extant, but so also was penicillin. Because there was reason to believe that the two agents antagonized bacteria by different modes of action, the use of them in combination seemed justified. The rationale of combination therapy was based upon the realization of the differences in modes of actions of antibiotic A versus antibiotic B. If A destroyed a significant sector of the invasive microbial population it may still leave survivors which were resistant to A. Had B been employed instead in the first place, similar results may have been obtained. A simultaneous use of both, however, would tend to eliminate the survivors to either.

When, in 1944, Joseph Bigger (1891-1951), a British army physician, attempted to use the combination of penicillin and

sulfathiazole against Staphylococcus aureus, he found it highly effective.[118] Note only did the two function well together, but in fact seemed to exceed the expected. Bigger had discovered that the presence of a small amount of sulfathiazole actually enhanced the action of an amount of penicillin which, by itself, was non-inhibitory to the test bacterium. He had discovered a synergistic action.[119] Much later when many more antibacterial agents were available, the importance of synergism had become the central rationale for the employment of combination therapy. It could be said that

> The ultimate justification for combined therapy then should be based on a combined effect that is greater than that achieved by the safe margin dosage of either drug alone.[120]

Until 1953 nothing more was seen in the literatature on combination therapy. Nor does this seem surprising even given the good results shown by Bigger. New antibiotics were appearing rapidly and penicillin's success was growing as well. Not until the continued rise of resistant Staphylococcus aureus untreatable by single

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[118] J.W. Bigger, "Synergic Action of Penicillin and Sulphonamides," Lancet, 247 (1944): 142-145.

[119] Bigger noted that the only previous combination therapy was done in 1943 by J. Ungar who used sulfaphyridine and penicillin. Although Ungar found evidence of synergism, Bigger showed his sulfathiazole to be much more synergistic than Ungar's sulfapyridine. See Bigger, p. 145.

[120] M. Klein and S.E. Schorr, "The Role of Bacterial Resistance in Antibiotic Synergism and Antagonism," J. Bacteriol., 65 (1953), 454. Emphasis is Klein's and Schorr's.

agents, did combination therapy once again give appeal.

The use of penicillin, streptomycin, and erythromycin singly had led to the development of resistance to each. By combining them it was hoped that the development of resistance could be eliminated or delayed. If one could delay this development within a single patient during therapy, he might be cured. Doubly-resistant strains could be generated in the process, nevertheless. These may or may not be a threat to the initial patient, but would likely to so to the population at large later on. It was found that the development of resistance to streptomycin when in the presence of penicillin was "uniformly rapid."[121] Such development was less rapid when erythromycin was substituted for streptomycin, but still occurred. Carbomycin caused cross-resistance much like erythromycin. When carbomycin was combined with the penicillin, poor results were obtained. Those studies were done with levels of penicillin, which used singly, constituted an ineffective dose. Unfortunately, they demonstrated that synergism was not a universal result of combination therapy.

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[121] M. Finland and C. Wilcox, "Antibiotic Combinations and Resistance to Antibiotics: Penicillin With Other Antibiotics Against Penicillin Resistant Staphylococci," Proc. Soc. Exper. Biol. Med., 83 (1953), 605.

More investigations with various agents, paired in different permutations, led to the recognition that the results of such therapy varied considerably. After several years of combination therapy the reason for the variations was explained in the literature. Four results were possible when using a given combination. The combination could be indifferent, additive, synergistic, or antagonistic. When indifferent the total effect was not greater than the effect of the more potent member alone. If additive the total effect equaled the mathematic sum of both drugs' percentage efficacy when used singly. The synergistic action exceeded the mathematic sum expected. If antagonistic the total effect was less than that expected from the more potent member when used alone.[122]

A partial explanation for those findings lay with variations in strains of the staphylococci themselves. Although it had been shown that the penicillin-erythromycin combination (in a 1:1 ratio) was often of no utility, with some strains a different ratio of the two agents did produce positive results.[123] The ratio varied widely with the strain and to determine which ratio was most efficacious on a given strain, sometimes required extensive testing in vitro. The physician

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[122]Hussar, Use, p. 34.

[123]E. M. Purcell, et al., "Antibiotic Combinations and Resistance to Antibiotics: Penicillin-Erythromycin and Streptomycin-Erythromycin Combinations in vitro," Proc. Soc. Exper. Biol. Med., 82 (1953): 124-313.

attending a moribund patient, of course, had no time to vary the ratio of a combination whose effective ratio may not be known against a given strain. It may not have even been determinable given the time involved.

Erythromycin was commonly used as one member in combined therapy against Staphylococcus aureus. But other combinations were tried, too. When streptomycin-penicillin ratios were varied away from a 1:1 ratio, an increase in efficacy was sometimes possible. But an increased resistance was easily demonstrated with pairs containing members of the tetracycline group. Some hope was generated by those various reports, but the mechanisms by which combined antibiotics worked remained unknown and useful combinations unpredictable.[124]

The recognition of antagonism did little to boost morale among the physicians. Chloromycetin, aureomycin, terramycin, and some sulfas were all shown to antagonize penicillin and streptomycin under certain conditions, but such antagonism varied in an "unpredictable fashion."[125] A clinician had no real referent upon which to base any intended combination therapy. Only if all singly-used antibiotics

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[124] S.S. Wright, et al., "Antibiotic Combinations and Resistance to Antibiotics," J. Lab. Clin. Med., 42 (1953), 891.

[125] Klein, "The Role," 454 and 462.

were without positive results would he choose combination therapy. There were situations where combination therapy was actually contraindicated. Under any circumstances the two members of a combination must have different modes of action. Two tetracycline antibiotics used together, for example, would be useless in light of the rationale of combination therapy and could even produce additional cross-resistance.[126]

The organization of antibiotics into three families of cross-resistance had occurred in the literature by 1953. Erythromycin and carbomycin formed one group, while streptomycin, dihydrostreptomycin, neomycin, and streptothricin (not commonly used) formed a second family. Chloramphenicol, chlortetracycline, oxytetracycline, tetracycline, and possibly penicillin fell into the third.[127] The clinician would expect possible positive responses only if he employed no combination in which both members of the pair were from the same cross-resistance family. It seemed clear, too, that no combination would prevent the appearance of resistant staphylococci, but could only delay such appearance.[128]

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[126] Ibid., 463.

[127] H. F. Dowling, "The Effect of the Emergence of Resistant Strains on the Future of Antibiotic Therapy," Antibiotics Annual, 1953-1954, p. 27.

[128] Ibid., p. 29.

There was only one suggestion beyond combination therapy to treat the resistant staphylococci in 1953, it seemed. A complete withholding of an antibiotic during therapy once resistance emerged appeared of some benefit in reducing the resistance.[129] In the moribund patient that would not seem entirely advisable. However, in such a situation the moribund patient did not likely have a change anyway.

In 1953, the wheel of optimism, then wariness, and finally pessimism, had once again made a complete cycle. The resistant staphylococci had not diminished as a threat. The organisms pursued their refractory ways.

It is here that one very important point must be made. Although the threat of resistant Staphylococcus aureus was clear, not all staphylococci were resistant to all antibiotics under all conditions. Many strains were (and are) sensitive to many different antibiotics both in and out of the hospital. The threat arises, however, from a select group that remain refractory to all antibiotics. The percentage of resistant strains may vary widely (as discussed earlier) and will be most threatening primarily in the hospital environment. It was the otherwise totally refractory strains of staphylococci that vancomycin best dealt (and deals) with.

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[129] Ibid., p. 31.

The central story of the discovery and development of vancomycin can now be appreciated, having been given the scene-setting discussion above.

5. THE DEVELOPMENT OF VANCOMYCIN

5.1. The Soils of Borneo

The Christian and Missionary Alliance of New York City was active in its work in many places throughout the world. Busy on the Indonesian mission was the Reverend William W. Conley (b. 1917). In 1944 he joined the Army as a chaplain and in 1948 he and his wife went to Indonesia. They served on a mission there until 1961 when Mrs. Conley's health forced a return to the United States.[130] It was during those years in Indonesia that the Reverend Conley was engaged in collecting plant and soil specimens for Eli Lilly and Co.

On June 7, 1951 Edmund Kornfeld (b. 1919), a chemist at Lilly, wrote the Reverend Conley thanking him for some native plant remedies the latter had sent sometime previously. The botanical screening program at Lilly was actively pursued at that time, having been spurred on by the discoveries earlier of the efficacy of quinine and of reserpine.[131] Although Kornfeld did not personally know Conley, they had mutual connections through their church activities.

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[130] A. Shareski (Christian and Missionary Alliance), letter to D. J. McGraw, January 11, 1974. The original letter is in the author's files.

[131] E. Kornfeld, transcript of a tape-recorded interview, January 11, 1974, p. 1. Hereinafter all such recorded interviews will be abbreviated as TRI. Pagination refers to this author's typescript of the interview.

At the time of Kornfeld's letter of thanks to Conley, the Lilly Company was rapidly expanding its soil screening program, a critical element of the antibiotic discovery era. It was later in the letter that Kornfeld asked for the further help of Conley in the new soil collection endeavor. Having sent Conley several sterile vials, Kornfeld explained their proper use and method of labelling. As Kornfeld said, one need just put on the vial something like

> Soil from shady part of vegetable garden on bank of Hocus Pocus River, Tim Buc Too, U.S.A.[132]

As it turned out the vials were not much more informative than that. They merely noted that the soil samples were from some remote place in Indonesia. As Conley said in response to Kornfeld's humorous instructions,

> Certainly hope there is something promising in our dirt out here. From our past attempts at gardens I cannot say it has been promising to us.[133]

There was something promising in those soil samples. It was from that sample that a streptomycete which produced chloromycetin, would later be isolated. But chloromycetin was already a well-known antibiotic by then. This finding was not surprising, though, for repeatedly in soil sampling programs known antibiotics continued to

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[132]Letter from Kornfeld to Conley, June 7, 1951. This and all following Kornfeld correspondences are unpublished. The originals are in Kornfeld's personal files. Copies are in the author's files.

[133]Letter from Conley to Kornfeld, July 19, 1951.

be rediscovered. This rediscovery has been mentioned in Chapter 1. It played a significant role in bringing about the close of the discovery era. But the Lilly microbiologists felt that "as a group [the Indonesian soil samples] are more interesting than the usual batch of samples" received.[134] Indeed, the many microbial producers of antibiotics found in the Indonesian soils were quite unusual. For that reason Kornfeld sent more vials to Conley asking him to collect more samples from "off the beaten track . . . away from the towns and villages."[135]

Nearly a year passed before soil samples were again forthcoming. Conley had left Indonesia for a furlough from his missionary labors. But before doing so he turned over the empty vials to the Reverend William M. Bouw (b. 1918). Bouw arrived in Indonesia in 1950, two years after Conley. He and his wife, like the Conleys, remained there until 1961; after which time the former departed for further missionary service in Holland.[136] Bouw wrote Kornfeld in February of 1953 stating that he had collected soil samples in various areas throughout his district.[137] It was always the Lilly Company policy that the air freight charges were to be assumed by them. This

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[134] Letter from Kornfeld to Conley, Feb. 25, 1952.

[135] Ibid.

[136] Shareski, letter.

[137] Letter from Bouw to Kornfeld, February 7, 1953.

insured good relations with the missionary-Lilly collection agreement. The soil samples arrived in Indianapolis in good condition. And, as was customary, they were addressed to William Daily (b. 1912), long in charge of the first steps of handling a new soil sample. The sample vial collected near Tengeng, Borneo contained a few grams of soil in which a peculiar new streptomycete was found. Several years later Streptomyces orientalis, as it was named, would be the producer of one of the most singular antibiotics in the history of chemotherapeutics.[138]

5.2. Screening for New Antibiotics

The soil screening program begun with the help of the Indonesian missionaries led later to the discovery and eventual marketing of two

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[138] Much later Kornfeld wrote Bouw, a man he knew only through correspondence, of the discovery of vancomycin. Kornfeld said,

> You have probably long since forgotton about the soil samples which were sent to us from your district in 1952 . . . [but] I believe that a significant contribution to human medicine has been made beginning with that little vial of dirt years ago. (Letter from Kornfeld to Bouw, March 26, 1959).

At the same time the Lilly Co. donated $1,000 to the Christian and Missionary Alliance and expressed a desire to continue the fruitful relationship between the two groups. The Alliance used this money, in addition to other funds, to build a new school. (Letter from Louis L. King (Christian and Missionary Alliance) to Kornfeld, March 25, 1959.) Bouw, himself was very gratified to hear of the discovery in which he had had a small, but crucial part. (Letter from Bouw to Kornfeld, June 4, 1959.)

highly effective antibiotics. Tylan (tylosin, Lilly) and Capastat (capreomycin, Lilly) are both discoveries of that period. Tylosin is a feed additive (for growth promotion) in domestic animals, and capreomycin is an antituberculosis agent. Hygromycin, also an agricultural antibiotic, was a product of the 1950's world-wide soil campaign, as well. The most well known, however, is Ilotysin (erythromycin, Lilly) isolated from a soil in the Philippines. As was discussed in the previous chapter erythromycin was (and remains) a useful antistaphylococcal agent.

Edmund Kornfeld had no more involvement with the vancomycin project after his initial letters to Conley and Bouw (and the 1959 correspondence to them). The crate from Borneo was received in 1953 by William Daily, "the custodian of the soils," and from there it began its more than half decade of maturation.[139]

The Daily team seeded agar plates with samples from the various vials of soil and later transferred the grown microbial colonies to agar slant cultures for safe keeping. This culture technique had been used since about 1946, the year of Waksman's streptomycin triumph. It had become apparent by the mid-1940's that the soil was a major source of antibiotic-producing microorganisms. Pharmaceutical companies recognized this and began screening

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[139]Kornfeld, TRI, p. 2.

programs to isolate potential antibiotic producers.

From 1946 until July of 1949 about 5,000 soil samples had been examined at Lilly, following procedures that had been worked out and administered by James M. McGuire (b. 1908 and now retired).[140] At that later date Marvin Hoehn (b. 1920) came to the company. Hoehn was only the second man at Lilly in 1949, working under McGuire, to be dealing with the screening program.[141]

Daily's workers in the early 1950's employed the screening method pioneered by Waksman. This involved streaking an unknown actinomycete (usually a Streptomyces species) along one edge of a standard Petri plate. At right angles to this several streaks of various known bacteria and fungi (both pathogenic and non-pathogenic) were made. After a suitable incubation period had elapsed, the cultures were examined for evidence of antimicrobial activity. If such activity was present the growth of the test organism(s) was inhibited in the vicinity of colonies of the unknown organism. When Daily found a positive unknown he gave the organism a number, lyophilized some of the growth for purposes of preservation, and sent a fresh slant culture to Hoehn's laboratory.

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[140] Marvin Hoehn, TRI, January 17, 1974, p. 5, and personal communication with Everet Smith, April 2, 1974.

[141] Hoehn, TRI, p. 1. The patent (U.S. Pat. 3,067,099) for vancomycin is jointly held by James M. McGuire and Mack H. McCormick. The important role of McCormick is considered in more detail later in this chapter.

Hoehn cultured the organisms in broth media in hopes of duplicating the activity demonstrated by Daily. Only 4-6% of all cultures Daily found to be active were shown to be so by Hoehn. This seemed to represent a major step in screening. This was the standard approach to the initial phases of screening in its first application at Lilly.

In 1950 R. C. Pittenger (b. 1920) came to the company. Dissatisfied by the low percentages being found when beginning with the Waksman plate, Pittenger sought a new approach. The plate was abandoned and the initial screening begun with liquid tube culture. Although the aeration of such tubes on the available rotary shakers was poor, it quickly became apparent that, as a first step, the plate method was missing a lot of potential antibiotic-producers.[142] Following Pittenger's approach, however, the number of potential producers jumped from 4-6% to 40-50% of organisms examined. Hoehn followed Pittengers lead and began the same approach in his laboratory. Just why the liquid technique was superior to the plate as the initial screen was never elucidated.[143] The Waksman streak plate was dropped entirely.

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[142] Ibid., p. 3.

[143] Ibid., p. 4. But see footnote 152 below for further clarification on this topic.

One of the cultures that emanated from the Pittenger laboratory was numbered M43-05865. Daily was phasing-out the plate method and Pittenger was phasing-in the tube technique. Prior to the tube method all cultures were numbered with the prefix M5, or similar, indicating they had been tested on a streak plate. When the tube method became established all cultures then were prefixed with M43. This indicated that the culture emanated from Pittenger's laboratory and had been tested by the tube method only. The remainder of the culture number (05865) indicated that it was the 5,865th isolate screened by the new technique. It was that isolate that would become the initial producing strain of vancomycin.

The actual date that Pittenger recognized antibiotic activity manifested by M43-05865 (or familiarly termed 05865) is not recorded.[144] It is known, however, that on June 18, 1953, Marvin Hoehn recognized, by paper chromatography, that a radically new antibiotic occurred in the broth culture fermenting in his laboratory.[145]

It was in this second screening stage, that of paper chromatography, that a real potential was recognized. The

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[144] Ibid., p. 5.

[145] A drawing of the chromatogram showing the biochemical "fingerprint" of vancomycin is dated June, 18, 1953. This drawing is still in Hoehn's file at the Lilly Company. See text for further elaboration. Figure 1 herein is a recent chromatograph of vancomycin, but is essentially identical to the 1953 preparation.

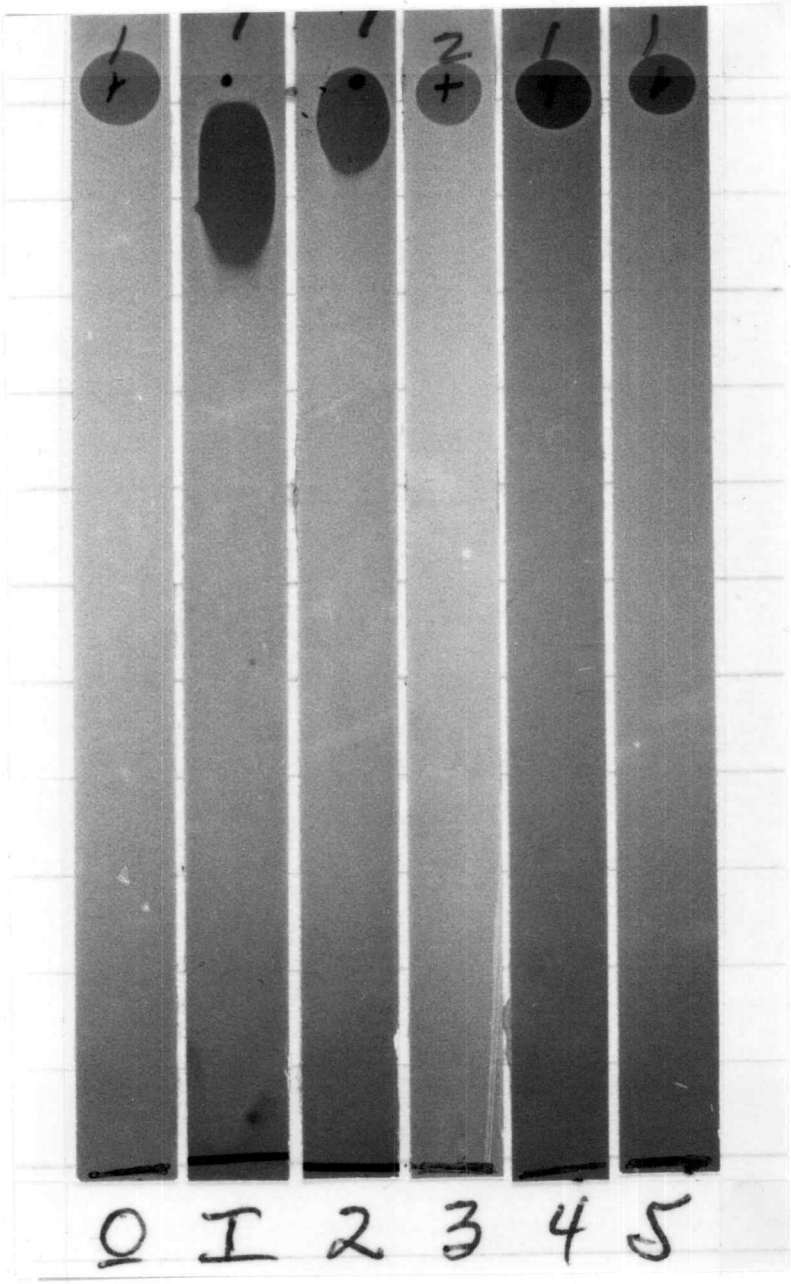

Figure 1. The chromatograpm of vancomycin. The lanes numbered 0-5 contain a solution of filtered broth, in which Streptomyces orientalis has grown, and the following: 0. Butanol saturated with water; 1. Butanol saturated with water plus 2% p-toluenesulphonic acid (p-TSA); 2. Butanol saturated with water plus 2% p-TSA and 2% piperidine; 3. Methylisobutylketone (MIBK) saturated with water; 4. MIBK saturated with water plus 2% p-TSA; 5. MIBK saturated with water plus 2% piperidine.

chromatographic step had shown many antibiotics found by Pittenger, Daily, and others to be identical to known agents; some of which were already on the market. Chloromycetin, as noted above, was rediscovered, and aureomycin was found several times. They were recognized by their specific chromatographic patterns or, familiarly, fingerprints.

As chromatography was done by Hoehn in the early 1950's, each suspected antibiotic (as a crude culture broth) was subjected to a given series of solvents and separated based upon partition coefficient on a filter paper strip. This method is one of the techniques alluded to by Conover (see Chapter 1). The resulting lanes (six in all) would indicate relative solubility and travel speed for each antibiotic. The pattern, or fingerprint, was characteristic for a given antibiotic. The patterns were not always precisely repeatable, however, for a variety of reasons. Not least among them was the fact that crude broth filtrates were being differentially adsorbed on the paper strips.

Hoehn's experienced eye could rapidly recognize a known antibiotic's fingerprint, and further work with that sample would cease. This was not so with 05865, however. On that June 18, 1953, Hoehn fingerprinted a new antibiotic (Fig. 1). Its pattern had never been seen in the Lilly laboratory before. One trait that was immediately recognized from the chromatogram was that 05865 was water-soluble, and virtually insoluble in organic solvents, save aqueous butanol.

That fact was to become of prime importance in the course of its development. Unlike penicillin, which is easily extracted by organic solvents, 05865 would require ion exchange extraction.

From 1947 onward paper chromatography was of premier importance at Lilly. The researchers at Lilly made intensive use of the technique from its modern rebirth in 1944. Only later did other companies increase their emphasis on paper chromatography.[146] Hoehn recognized the need to pursue the potential of 05865 from its chromatographic pattern. He recorded on a card in his filing system the fingerprint of the new agent.

The antimicrobial spectrum of 05865 had been examined by this time. It had been shown by Linville Baker (b. 1908 and now retired), in the microbiology laboratory, that the antibiotic was active against many diverse organisms, but of the eubacteriales only the gram-positive ones were affected. More will be said of its spectrum in a succeeding section, but one point is of interest here. In that initial antimicrobial screen Staphylococcus aureus was not included. Though it usually was included, for unknown reasons it was excluded in the first 05865 tests. Only later in a much more exacting study was that organism tested.

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[146] Hoehn, TRI, p. 7.

In order that Hoehn provide the microbiologists with crude broth filtrates for their study he had to grow more of the organism. Hoehn was able to garner just the barest idea of the nutritional requirements of 05865. From mid-June, 1953, to the end of December of that year he studied the nutrient needs of the streptomycete. One of the first sources tried was corn steep liquor. The idea to use this concentrated by-product of the corn starch industry came from the fact that it had been used to such advantage in penicillin production.[147] It was after Florey went to the Northern Regional Research Laboratory at Peoria, Illinois, that corn steep liquor, hitherto an unused by-product, gained fame as essential to high-yield penicillin production.[148] This chemical became a commonly used nutrient throughout the discovery period.

By the new year of 1954 culture 05865 had gone from a Borneo soil sample to a producer of a totally different, possibly useful new antibiotic. It was shown to be fastidious in its nutritional needs, however, and just how fastidious was a matter that required extensive research.

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[147] Hoehn, TRI, p. 24.

[148] See the many historical studies on penicillin. Florey's biography by Bickel is, however, the best source. See p. 147 ff, therein.

5.3. The Nutrition of *Streptomyces orientalis*

There was a hiatus of 10 weeks from the close of Marvin Hoehn's work to the beginning of the studies done by W. Max Stark (b. 1914). That hiatus closed with the opening of Stark's studies on the nutritional requirements of the newly-found organism.

On March 19, 1954, Stark began a series of studies that were at their inception tedious and singularly unexciting. But by the close of his involvement with the project during the year of 1957, he would have made one of the most startling discoveries in vancomycin's developmental history.

Over the first portion of 1954, the work on 05865 was confined mostly to Stark's laboratory. There were no great expectations of the potential of 05865 at that time. It was true that Hoen had definitely shown that the organism produced a very different antibiotic with a rather wide spectrum of antimicrobial activity. Nevertheless it was still, as Stark put it, "a routine type of thing."[149]

One of Stark's earliest findings was the utility of medium A9 for the growth of the streptomycete.[150] That Lilly in-house medium was composed of very complex organic materials. Not unlike *Penicillium* species medium, that for 05865 had peptones, milk derivatives,

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[149] Max Stark, TRI, January 17, 1974, p. 3.

[150] *Ibid*.

sugars, and other chemically undefined components. Among that organic maze was found one very quaint ingredient. Brer Rabbit brand molasses (of undefined composition) seemed to be very amenable to the needs of the organism. During April of 1954 a great many variations of A9 medium were tried, but always containing the efficacious Brer Rabbit Molasses. Somewhat later that was replaced by other materials, however. This empirical search for media design was a common approach throughout the early portion of the discovery era.

During that same period 05865 was renamed C-260. Although throughout its history the organism was known by either designation the latter term was sometimes stressed. That new handle was given it because all cultures that were sent from Stark's small laboratory-level fermentation experiments to larger pilot plant studies were given a C number. It was a matter of in-house convenience (C has no specific meaning). Since Stark was getting poor yields in his flasks it was decided to attempt fermentation in larger tanks of 250 gallons capacity.[151] Stark supplied cultures to Peter Hosler (b. 1921, and

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[151] Most research and virtually all of the administration of Lilly occurs at its McCarty Street (Indianapolis) headquarters. Most pilot scale and virtually all production scale fermentation occurs at the company's Kentucky Ave. plant within the city. Other similar plants, however, exist both in Indiana and foreign countries. Throughout the history of most Lilly antibiotics there is seen much communication back and forth between the two nearby facilities. Indeed, part of the vancomycin production of today occurs at Kentucky Ave. and part at the laboratories and plant at Lafayette, Indiana.

who soon thereafter retired) at the fermentation facilities and, though the latter made some attempts at better yields under different physical conditions, it was apparent that a better medium was necessary.

Also during those preliminary cultural studies a few pure chemical studies were done and crude broth filtrates were assayed by turbidimetric techniques. That was done by making dilutions of the filtrates and testing them against Staphylococcus aureus. After incubation, optical density measurements were made. A response curve of microbial kill versus concentration of active substance then obtained. Such a turbidimetric assay was standard within Lilly (though not so in most other firms) and was used throughout vancomycin's history.[152]

At the same time these assays were being carried out, very limited toxicity testing was done in animals. That aspect assumed little importance until yield could be increased. Unless greater yields were possible the whole project might have never progressed.

Many further A9 modifications were made, but still yield was low. Various sugars, nitrogen sources, and other components were manipulated into a profusion of permutations. On May 12, 1954 a new

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[152]Plate assay techniques were more common than turbidimetric methods throughout the industry at that time. However, the former approach is less desirable for it requires small concentrations of the antibiotic to diffuse through the solid agar. In the case of vancomycin, a large molecule, it was fortunate that the Lilly researchers did not use the agar plate technique. Stark, TRI, p. 8.

ingredient was examined.[153] Soy bean oil meal (a chemically undefined mixture) was added to the medium. The fastidious streptomycete would not utilize it as a fine powder suspended in the liquid medium, however. Stark said, "this was different from most organisms [streptomyces] we've had to work with."[154]

For 05865 the soy meal was acid-hydrolized. Where potencies of the antibiotic were in the range of 250 units per milliliter without soy in the medium, they were often in excess of 500 units with the new material.[155]

During the first half of 1954, other research groups tentatively examined the new antibiotic. A chronic toxicity test of several months duration was being conducted on mice and had suggested by June, 1954 that the new substance was only moderately toxic. Certain things concerning the chemical structure of the molecule had been learned. Chromatography and microbial assays were continually done on material prepared by Stark. The former technique confirmed that manipulations of nutrition and physical parameters during culturing

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[153]Stark, Laboratory Notebook 193B, p. 254. The original notebooks are in Stark's files. Copies of pertinent passages are in the author's files.

[154]Stark, TRI, p. 10.

[155]Stark, Notebook 193B, p. 252 and 267. The unit is an arbitrary measurement without precise definition for vancomycin. It was not comparable to the penicillin Oxford unit.

did not change the fingerprint. The latter confirmed that the potency was either improving or remaining static under the varying experimental conditions.

By that June the researchers had shown that the addition of aspartic acid increased yield and that sufficient aeration during fermentation was important. Indeed, a variety of tentative data had been gathered. The data was sufficient, in fact, to precipitate the formation of a steering committee to attend the development of the new antibiotic.

Stark continued his studies for the next two and a half years. But at mid-1954, efforts on the research into the new agent began to proliferate and ramify. Straight-line evolution of what was to become a viable new medical tool was no longer the case. The team approach was in the making. Not until several years later would the history of vancomycin again be amenable to topical treatment.

5.4. The Diary of 1955

Several things precipitated the increased interest in 05865. They included the spectrum of the agent and the fact that it seemed singular in its structure. It had been tested in animals and, by June 1954, in one human being.[156] But one trait of 05865 more than any

[156] Clinical applications are left to the discussion in the following chapter.

other stood out. It was found that a concentration of active principle in the crude filtrates sufficient to be bactericidal was only a minute amount more than that which was merely bacteriostatic. The implications of this were of great moment. It indicated that resistance to vancomycin was not likely to occur. If a bacterium requires a large amount of antibiotic to kill it, but is somewhat inhibited by a much smaller amount, it has an opportunity to acquire resistance within this in-between range. If the host need be infused with only a small dose, and if that dose be bactericidal, resistance is not encountered.

In the Antibiotics Annual for 1955-1956, Mack H. McCormick (b. 1921) and Max Stark and others made this point very clearly.[157] They noted that this bactericidal capacity, at only a little higher concentration than the bacteriostatic level, was "one of the most striking properties of vancomycin."[158] This had been observed by Stark and others early in 1954 and was instrumental in raising much interest within the company by the middle of that year.

The events of 1955 are firmly rooted in the events of mid-1954. On June 15, 1954 a steering committee was set up to guide research on the new antibiotic. At that time several of the researchers

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[157] M. H. McCormick, et al., "Vancomycin, A New Antibiotic. I. Chemical and Biologic Properties," Antibiotics Annual, 1955-1956, 606-611.

[158] Ibid., 606.

reported on the status of knowledge gained to date.[159]

Stark, opening that meeting, apprised the 17 members present of his progress on cultural studies. He had raised the potency to 1,000 units per milliliter in 250 ml. flasks and was pursuing the role of amino acids as growth stimulators. His colleague, William H. Jackson (b. 1918), had gotten yields of only 150-200 units per ml. in tank fermenters of 45 liter capacity. (The tank fermentation technique had been created during the early penicillin days.) This was due to a problem related to the use of an antifoaming agent. This was adjusted, but within a month contamination would be of central importance. R.C. Pittenger and R.B. Brigham (b. 1925) had examined the organism itself and had bestowed the name Streptomyces

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[159] Minutes of a Research Meeting on Antibiotic 05865, June 15, 1954, chaired by Dr. C.W. Pettinga. Copy in the author's files. The committee's 17 members included toxicologists, clinicians, chemists, biologists, chromatographers. They represented those who, over the next several years, would be the principal contributors to the development of vancomycin. Although some would later become inactive and, others would be added, they included: R.C. Anderson, O.K. Behrens (b. 1911), C.G. Culbertson (b. 1906), R.S. Griffith, H. Higgins, P. Hosler, M.M. Hoehn, W.H. Jackson, M.H. McCormick, R.C. Pittenger, G. Pittenger, H.M. Powell (b. 1884), E. Rohrmann (1912-1960), W.M. Stark, D.W. Zeigler, and F.R. Van Abeele (b. 1916). Copies of this and other Lilly in-house documents are seldom found in the Lilly Archives. Most commonly copies of these reports exist in the personal files of the men who worked on vancomycin. The locations of copies, and sometimes originals, for all such documents are cited in the appropriate footnotes throughout this and the following chapter.

orientalis upon it. On this they soon published.[160] M. H. McCormick and G. Pittenger (b. 1924, and not to be confused with R. C. Pittenger, to whom he is unrelated) offered a tentative chemical description. They had shown that the antibiotic from 05865 was ion resin-adsorbable giving 80% yields of 600-800 units of activity per milligram. Since only small amounts of the antibiotic were available from Stark's laboratory, little further was known of the structure. They did show, though, that it seemed to be a polypeptide in nature,[161] and that its molecular weight was of the magnitude of several thousands.[162] The bactericidal and anti-staphylococcal activity was considered of prime interest. The first human subject had, however, suffered a severe phlebitic reaction. This reaction, along with certain others, later played central-most roles in vancomycin's development.

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[160] R. C. Pittenger and R. B. Brigham, "Streptomyces orientalis, n. sp., the Source of Vancomycin," Antibiotics and Chemotherapy, 11 (1956): 642-647. They not only described M43-05865, but two other similar cultures isolated in India (discussed later).

[161] Harvey Higgins, who was responsible later for purification of vancomycin, objects to the use of a term polypeptide in connection with vancomycin. Although amino acids compose vancomycin, peptide bonds per se have not been demonstrated to occur in its, as yet incompletely known, structure. Higgins prefers to regard the agent more as an aminoglycoside, though Kucers (The Use of Antibiotics, p. v-vi) does not consider it so. Aminoglycoside antibiotics include streptomycin, kanamycin, neomycin, and others. Personal communication, Feb. 5, 1974.

[162] This matter has received much attention. Current findings suggest a weight of 1600-1800. Early estimates ranged to 4000. This is discussed fully later.

That first exploratory meeting closed on a non-commital note of caution. Assignments were made in regard to animal toxicity testing and chemical characterization. Simultaneously the work on media composition and fermentation conditions continued to expand.

Stark's associate Jackson, in his early small tank (45 liters) laboratory-level fermentations, ran into severe difficulties. In attempts to gain culturing information that would help translate shake-flask (250 ml.) fermentation to small tanks, contamination was encountered. Laboratory notes for August, 1954 were replete with comments such as, "contaminated, yeast and bacteria," for virtually all tanks.[163] With a small flask sterile technique was easy. With 45 liter tanks it was considerably more difficult to maintain sterility, given such things as large ports and openings, bubbling air which was presumably sterile, and other mechanical problems. Most significant was the fact that the medium was far from ideal. Allowing only slow growth by Streptomyces orientalis, any less fastidious contaminants rapidly overgrew the desired organism.

As has been mentioned, Stark and other early workers monitored their research activities by continually chromatographing fermented broths. On August 9, 1954 a new fingerprint was seen.[164] It

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[163] Stark, notebook 231 C, p. 170, 171.

[164] Stark, notebook 231 B, p. 59.

indicated that the culture was not producing one, but at least two closely related active fractions. This did not come as a great surprise either to Stark or McCormick.[165] By the mid-1950's they had experienced this before. The 1940's was also full of similar circumstances. Antibiotics are seldom produced by microorganisms in one chemical form, but usually a group of similar, yet chromatographically-separable fractions, are elaborated. This was true for penicillin and was, as Stark believes, probably true for all antibiotics known.[166] It was immediately clear to all concerned, however, that it presented problems of purification. This eventually became another of the major problems in vancomycin's history.

Media studies continued uneventfully through the remainder of 1954. Stark continued to test other strains of vancomycin-producing organisms that were sent to him after having passed the Hoehn screening barrier. These studies only much later gained importance by illuminating a problem as great as that of the problem of multiple fractions (see below). Indeed, by this time the roots of two, soon to be foremost, problems were encountered. Human toxicity, and the complex of fractions of vancomycin had to be resolved in order to successfully bring the new agent to clinical usefulness.

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[165] Stark, TRI, p. 11-12; McCormick, TRI, February 4, 1974, p. 13.

[166] Stark, TRI, p. 12.

R.C. Anderson (b. 1911), a toxicologist, had been assigned in June the task of continuing and expanding animal testing. Like the other researchers during 1954, he was using the dried, brown, amorphous powder gleaned from ion-exchanged fermentation broths (see below). By the January 11, 1955 steering committee meeting, he was able to report an intravenous testing on dogs. Direct single injections of 10 milligrams per kilogram of body weight (mg/kg) of that crude, hydrated powder produced no toxic effects. A two-hour long intravenous infusion of 100 mg/kg likewise produced no observable difficulties.[167] That use of material during 1954 left only a total of less than 4 grams available as of January 1955. Difficulties in obtaining higher yields and problems with contamination were continuing.

By January 1955, the research clinician, R.S. Griffith (b. 1920), had injected two more human subjects with aqueous solutions of 05865, one containing an analgesic (digammacaine). Phlebitis and pain upon injection were noted.[168]

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[167] Project Meeting: Antibiotic 05865, January 11, 1955, chaired by E. Rohrmann. Copy in author's files.

[168] The apparent inconsistency between humans and dogs regarding pain is easily explained. A technique for detecting and measuring pain in dogs under these conditions is lacking. K.B. Kohlsteadt, personal communication, Jan. 21, 1974.

At that same meeting McCormick, reporting on the chemistry of 05865, indicated he had demonstrated the presence of yet more fractions. He felt that he could detect from 4 to 7 such components. (Much later it was established that only two fractions make up vancomycin.) One fraction did predominate, so it seemed then. This was labeled fraction B.

Feelings in early 1955 that 05865 was a significant new antibiotic were beginning to be seen in the otherwise often dry notes of the steering committee. A bold and, as it happened, very premature step was taken then. The committee "felt that Dr. Griffith should go ahead and run at least one more patient in order to get additional data in support of our patent application."[169]

That was very premature, for as it turned out each time the subject of the patent application seemed to be progressing it would need to be reconsidered. Over the next few years 05865 offered many surprises. Only after a large clinical trial was it possible again to consider seriously a patent application. It is to the lasting credit of the Lilly researchers that they proceeded in 1957 and also in 1958 (the year of the commercial availability of vancomycin) with extreme caution. The steering committee felt it proper to run a large clinical trial (though not then required by federal law) because of the

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[169] Meeting, January 11, 1955.

inconsistencies seen in various batches of vancomycin. However, in 1955, the excitement surrounding a new, revolutionary antistaphylococcal agent would not be muffled.

Such excitement was clearly at a high level when Griffith reported a 24-hour cure of a "rather serious strep[tococcal]-throat."[170] Thus, February was off to an auspicious beginning. Others were pursuing the patent possibilities; especially when it was felt that side-effects seemed lower with 05865 than with the recently marketed erythromycin. It was probably pure coincidence that the few patients up to that point showed limited toxic reactions. Over the next few years side-effects were probably the central-most problems in the development of vancomycin.

Thoughts on the possibilities of using 05865 for veterinary medicine were expressed in February and, although that usage was considered off and on for some time thereafter, it never gained prominence.

McCormick sent off 4.5 grams of crude amorphous powder to be filled into ampoules for Griffith's clinical investigations.[171] Its

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[170] Project Meeting: Antibiotic 05865, February 7, 1955, Chaired by E. Rohrmann. Copy in the author's files.

[171] The Lilly Research Clinic occupies two floors of the Marion County (Indiana) General Hospital. It went into operation in 1926, but was formally dedicated in 1930. K. G. Kohlsteadt, former vice-president for medical research indicates (TRI, Feb. 4, 1974, p. 8)

activity was 700 units/mg.; whereas, Griffith had been using 800 unit potency material the month before. There was great difficulty in maintaining a given level of potency at that time. The 4.5 grams came from the fermentation operations that were proceeding on a small scale at the Kentucky Avenue Pilot plant where a 250 gallon fermenter was in operation. A medium which contained several complex organic compounds reflected, in part, research that Stark had done up to that point.[172] However, even after 96 hours of fermentation, the potency did not exceed 225 units/ml., which was considered low at best. Within two months the yield was increased by changing certain conditions of fermentation. The nature of the changes went unrecorded.

In early March, at the next meeting of the steering committee, the importance of a proper level of aeration of the submerged fermentation was made apparent. The following month would show that yields

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that Eli Lilly is the only pharmaceutical company in America that has such a facility even today. The first clinical trials for all new Lilly human drugs are tested at the Clinic prior to the larger trials by chosen outside clinicians.

[172]The medium and physical conditions are worth mentioning here, for much later a comparison of these same two points would indicate something of the technical evolution of this type of thing. The medium consisted of: 2% white dextrin; 1.5% cerelose; 0.25% Stamino A; 1.5% Wilson's Peptone # 159; 0.5% B-Y Fermentable solubles. The first two and the last ingredient were carbohydrate sources and the remaining two were amino acid (nitrogen) sources. Physical conditions included 150 RPM agitation, 5 pounds tank back-pressure, anti-foam on demand, aeration at 12 cfm and a temperature of 28-30°C.

were increased if the previous minimal temperature of 28°C was not exceeded. The importance of temperature was not recognized prior to April.

The generally low yields made Griffith's work difficult. The potency had increased to 1000 units/ml. under the increased aeration, but the total gram volume of material was disappointing. The symptoms seen in the one patient treated that previous month did not bode well for 05865, as distinct toxicity seemed increased over that seen in using previous lots.

The status of the patent application came into doubt in early March, too. For "the identification of antibiotic 05865 for patent purposes is becoming very difficult."[173] That difficulty arose from the problem of the multiple fractions. They had not been separated and thus further identification of the chemical structure of 05865 was impossible. The variation of the material coming from fermentation was great.

> The last preparation of the antibiotic looks like it is a one component material, but it appears to be different from any which had been obtained in previous preparations.[174]

The almost arbitrary change in aeration had evidently led to the increase of one fraction over others. What had been termed the B

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[173] Project Meeting: Antibiotic 05865, March 11, 1955, Chaired by E. Rohrmann. Copy in the author's files.

[174] Ibid.

fraction earlier was less apparent in the new batches, some other component having displaced it.

These unexpected vacillations were a matter of concern, of course, but not of real threat. In fact, the meeting ended on an optimistic note when one member was assigned the task of finding a name for antibiotic 05865. Originally, the culture of Streptomyces orientalis was termed 05865. When it became significant as a producer of an antibiotic, the substance itself came to be termed 05865.[175] By March the steering committee felt the need for a formal name. But it was not until the following May that a name was proposed.

The date of June 15, 1954 was an important date in vancomycin's history as the first steering committee was set up then, but on March 16, 1955 another higher eschelon committee was established. The Product Development Committee (PDC) for Antibiotic 05865 was composed of about 10 individuals. The members of the committee varied but little over the following three years. It was the purpose of the PDC, as it was familiarly called, to guide in the development of the antibiotic from the standpoints of fermentation, purification, clinical studies, and so forth.

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[175] The matter of terming an antibiotic or its producing culture by its number is a typical in-house practice.

During that first meeting several points were stressed. The immediate needs of determining a dosage form and selecting a generic and trade name were noted. With few exceptions, the PDC met every two weeks from mid-March, 1956 on into 1960. The body summarized progress and more importantly made management decisions which directly affected the genesis of the product vancomycin itself.

The earlier steering committee met thereafter as well, but later in 1955 became known as the Development Committee. Many other reports came in during 1955 from various laboratories too. And at the laboratory research level much had been accomplished on purification by April of 1955.

The work of McCormick and G. Pittenger had begun in 1954 when they first attacked the problem of extracting the active principle from the fermentation broths prepared by Stark. On a purely empirical level they began to search for a solvent system that, by counter-current distribution in a liquid-liquid format, would extract 05865's active fraction.[176] McCormick knew that 05865 was water-soluble and amphoteric and therefore he had at least some ideal of how to extract it.

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[176] A discussion of the technique of counter-current distribution is given on page 29 in I. M. Hais and K. Macek, Paper Chromatography: A Comprehensive Treatise (New York: Academic Press, 1963).

A great many solvent and water systems were tried, but during late 1954 a mixture of carbon tetrachloride as one solvent and phenol dissolved in water as the other seemed most efficacious. McCormick later said that that solvent system was difficult to handle.[177] The problem was that the phenol needed to be distilled to be used. The distillation was required because of the presence of a phosphonic acid stabilizer in the phenol which interferred with the extraction of 05865.

Once the active ingredient was dissolved in the water phase it required passage over an ion exchange resin, subsequent elution, decolorization over carbon, and finally precipitation to remove it. Several precipitating agents were tried empirically. Two were of real value, helianthate and picric acid. The former is a derivative of the dye methyl orange. The latter gave the best results, however, and was chosen as the precipitating agent. Picric acid had one severe drawback. It is highly explosive. It had been an integral ingredient in many bombs and related devices used during World War I. The complex of 05865 and picrate was decomposed in hydrochloric acid and the 05865-HCl complex was then dried. It was the final dried, amorphous, often brownish, powder that was used in 1954 by Anderson in toxicology and by Griffith at the clinic.

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[177] McCormick, TRI, p. 6.

By February 1955, the choice of an efficient carbon filter led to making tan preparations whereas they had been brown before. By March it was approaching a yellow color and was being prepared in hundreds of grams at a time. In April the committee noted a projected production of 300 grams every two weeks. Two 250 gallon fermenters were then in operation and at the same time an apparently stable chromatogram of the material led the researchers to hope again that the patent application was not far off. The material was then being prepared not only in the HCl format, but also in the sulfate for clinical testing. This was to see if one seemed better than the other in human use.

It was abundantly clear at that time that even though 05865 demonstrated a spectrum not unlike that of penicillin, it seemed that the agent was most especially efficacious as an anti-staphylococcal antibiotic. The Committee, realizing the potential of 05865, said in April 1955

> It was suggested that we make some of the antibiotic available for intravenous use in the case of emergency where people are dying of staphylococcal septicemia.[178]

A very significant situation is apparent in these words. From at least the date of that proposal onward, vancomycin (05865) became,

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[178] Project Meeting: Antibiotic 05865, April 12, 1955, chaired by E. Rohrmann. Copy in the author's files.

in the minds of all concerned with it, a specific cure for a specific ill--staphylococcal disease.

Having found a good decolorizing carbon, McCormick and G. Pittenger were able to produce the antibiotic as an amorphous white powder. Chromatograms continued to indicate a "one-spot" solution. Such chromatograms seemed to suggest only one fraction was being produced during fermentation; hence, the clinical trials would not be threatened by widely varying material. But two months later, on July 28, G. Pittenger's notebook read, "Paper chromatography reports [05865] to contain a trace of a second factor."[179] Again the multiplicity of factors threatened progress. But in May, Pittenger's discovery was still two months in the future.

Griffith sought an intramuscular format for 05865, but over the following years such a format was never achieved (see below). As it is given today only intravenously, it remains a hospital antibiotic. This is an advantage because, unlike penicillin and many other antibiotics, it cannot be so promiscuously used; hence bacterial resistance is less likely to build up.

On May 17 the first proposal of a name for 05865 was made. The name Tengacin was suggested. It was not recorded, nor did any

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[179] G. Pittenger, laboratory notebook number 270 B, p. 48. The original is no longer extant. A microfilm of it is in the Lilly Microfilm vault. A copy of pertinent passages is in the author's files.

of those interviewed remember, who suggested the name Tengacin. That name reflected the fact that culture 05865 had been collected near Tengeng, Borneo. That epithet was never again seen. It had been pointed out by the American Society for Microbiology's committee on the nomenclature of antibiotics that geographic names for antibiotics are not desirable.[180] The term terramycin, for example, is uninformative; whereas chlortetracycline indicates the fact that, at least, chlorine and four aromatic rings exist in that compound. The name vancomycin (which is not informative) finally emerged late in 1955 without fanfare. It was first used in September of 1955, but who coined the term and when remains unrecorded. It is the approach at the Lilly Company to have individuals in the marketing department create a name. It seems that is how the term vancomycin came into existence. The term was derived from the Latin vanesco which means to vanish or disappear. No one knows why the unknown coiner of the name picked the Latin term.[181] The choice of it, then, was purely arbitrary. The "-mycin" suffix was suggested by the nomenclature committee of the American Society for Microbiology for all products

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[180] Waksman, Actinomycetes, p. 210 ff.

[181] Memo from B. F. DeHays to C. W. Pettinga, October 31, 1973. Original in the author's files.

derived from actinomycetes, and the Lilly management respected that suggestion.[182]

By May, the chemistry group had shown 05865 to contain an undetermined amount of chlorine, 8% nitrogen, no sulphur, six to eight phenolic groups, and had a molecular weight of 3,200. That early estimate of the weight has since been shown to be much in error. The infrared spectrum indicated the presence of amide and hydroxyl groups and the compound had not yet been crystallized. The discovery of the presence of phenolic groups on the molecule in part helped to explain why 05865 was so toxic. It was general knowledge at that time that such chemical groups in an antibiotic made it likely that the agent would be to some degree toxic, no matter how well purified.

The knowledge of the chemical structure virtually never increased from that time onward in the vancomycin project. The pure chemistry of vancomycin was never further investigated at Lilly. Indeed, one month later "Dr. McCormick mentioned that since the last meeting very little work has been done on the chemistry of antibiotic 05865."[183] The current knowledge has been accumulated in other laboratories in both the U.S. and abroad (discussed below). The reason for this was simple. Again it was a pragmatic decision.

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[182] Waksman, Actinomycetes, p. 210.

[183] Project Meeting: Antibiotic 05865, June 14, 1955, chaired by E. Rohrmann. Copy in the author's files.

The rise of staphylococcal resistance during the 1940's and 1950's to antibiotics was such that the aim at Lilly was to make available the new agent as soon as possible to the medical community.[184]

By June, fermentation in 1,000 gallon tanks was in progress, but the researchers encountered "numerous difficulties" of an unrecorded technical nature. Griffith was able to maintain bactericidal blood levels for six hours with 100 mg. dosages. The hydrochloride salt of 05865 was judged better than the sulfate and diluents other than water had been given some consideration. The purpose of that study was to find some carrying agent which would lessen the pain experienced upon injection. Pantothenic acid, di- and triglycine, and 10% nicotinamide were all tried.

Veterinary applications were shelved, but attempts at combining erythromycin and 05865 were made. The middle of the decade was characterized by the intensive use of antibiotic combinations. The thoughts of the vancomycin team tend to indicate that even they believed that resistance would develop to 05865. In an attempt to forestall such an eventuality they studied the combination of 05865 with erythromycin, but found that the combination was antagonistic.[185]

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[184] McCormick, TRI, p. 15 ff.

[185] Ibid.

In August certain new findings increased the general knowledge of the needs of Streptomyces orientalis in culture. A great deal of aeration was shown to be deleterious. As well, absolute sterility in the fermentation was requisite, for the antibiotic was not elaborated until full mycelial growth had occurred. Thus competition from contaminants was a severe threat to yields. R.W. Squires (b. 1921) was moving into pilot plant fermentation and simultaneously P. Hosler was moving away from his involvement with 05865. Squires and F.W. Kavanaugh (b. 1908) had shown that use of a new anti-foaming agent could raise yields from a few hundred to 500-600 units/ml. H.M. Higgins (b. 1921), like Squires, was a new-comer to the team. He noted that the picrate process would be difficult to scale-up to commercial production levels. Later that process was replaced by better methods (see below). It was the team of Squires and Higgins who would begin to play a substantial role over the next several years in vancomycin's history in their respective arenas of fermentation and purification.

In closing the later summer activities of 1955, the committee noted that "no strain [organism] selection has been made as yet."[186] Although Stark was upon occasion examining new strains of Streptomyces orientalis in hopes of finding higher-yield producers, this

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[186] Project Meeting: Antibiotic 05865, August 16, 1955, Chaired by H.W. Rhodehamel. Copy in author's files.

aspect of the project had been up to that time unimportant. It was very common with other discovery era antibiotics, however (e.g. streptomycin). It was not until Stepmber that the overwhelming importance of employment of the proper strain in fermentation was realized. That was probably the only major difficulty in vancomycin's development, the roots of which were not clearly apparent in 1954.

Why strain selection was not emphasized in regard to vancomycin's history until late presents an interesting problem. An answer to it seems double-edged. In the first place culture 05865 was not a low-yield producer, unlike Fleming's Penicillium species. Therefore there seemed little necessity of seeking its replacement. At the same time most developmental emphasis was on media composition. Therefore it must have seemed that by proper manipulation of the fermentation medium the stability of the product (as to fraction ratios) could be assured. There is evidence to support this suggestion.[187]

In September the above situation changed for,

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[187] Kavanaugh "stated that the medium is not considered good" just prior to mentioning that strain selection had not been done. And earlier in the same paragraph he enumerated what conditions were known to give better fermentation (i.e., temperature, aeration, etc). Meeting August 16, 1955.

> The culture [05865] used in producing vancomycin in laboratory and pilot plant was shown to be a mixture of strains at least one of which is worthless as a producing organism.[188]

Other findings in September contributed to increases in the efficacy of the medium. Calcium chloride would increase potency it was found. October saw continuing, though unproductive, research on medium composition. By November it was suspected that varying lots of the components which went into the medium played some role in the variations in vancomycin's composition.[189] The matter of strains was of continuing high interest.

In December of 1955 an amount of vancomycin hydrochloride was given to several clinicians outside of the Lilly Company, thus a viable program of further clinical trials was underway.

5.5. News of a New Antibiotic

The first month of 1956 opened with an enthusiastic note. Three clinicians across the country had been given over 100 ampoules among them in order to pursue clinical trials. The PDC in January noted that "a rather enthusiastic report was received from one clinician."[190]

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[188] Laboratory Report No. 22: Vancomycin--C260, September 29, 1955. Copy in author's files.

[189] Laboratory Report No. 26: Vancomycin--C260, November 10, 1955. Copy in author's files.

[190] Product Development Committee, January 24, 1956 Series 1570. Hereinafter this committee is referred to by the designation

The fermentation and purification studies continued during 1956. But during that year the preparation of several publications absorbed the time of quite a few members of the vancomycin team. A series of eight such publications (cited throughout this chapter) emanated from Lilly during 1955-1957; the majority being prepared during the year of 1956. Those papers, though terse and technical, remain the primary sources of information for that year. No further publications beyond these eight were forthcoming from Lilly researchers on vancomycin after that time. Until recently the literature on the subject was limited almost entirely to clinical applications (next chapter). Recent renewed interest outside of Lilly in vancomycin has led to an understanding of its mode of action and its chemical structure (in part). (This is discussed more fully below.)

The PDC continued to note certain dismal facts among scattered moments of enthusiasm. The enthusiasm was clear cut, for vancomycin seldom ever failed during treatment of chronically-ill patients. The disturbing notes were struck largely due to one recurrent problem--side effects. As shall be seen, purity had much to do with that matter of toxic reactions, but during most of 1956 reports remained distressing.

PDC. Copy of this report is in the author's files. The clinician making the enthusiastic report was not named.

In February several hundred more ampoules went to one clinician, but such were "said to be pyrogenic [fever-producing]."[191] Such reports continued over the months. The attempts at intramuscular injections continued to be painful; thus reminding the development team that such a format for the antibiotic looked untenable. Cures remained excellent, however, for "the results have been rather dramatic."[192]

By mid-year production of clinical-testing lots had increased to 4,000 liters per order. During the same period another one of the various carrying agents tried, i.e., polyethyleneglycol, seemed of great efficacy. Less pain was experienced on injection with that agent than with any other tried up to that time.[193] As well, the vancomycin itself remained "one-spot" on chromatographic analysis (i.e., apparently rather pure in regard to fractions).

In the late summer of the year the so-called average dose (see next chapter) had been tentatively set at one gram every six hours by infusion or slow injection. At an antibiotics symposium there was much clinical discussion of vancomycin. Researchers at the Mayo Clinic reported on their studies. Soon after the end of the conference

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[191] Ibid., p. 3.

[192] Ibid., p. 4.

[193] Ibid.

the PDC said that the Mayo researchers had reported very favorably on vancomycin. That report was so favorable that "an FDA [U.S. Food and Drug Administration] official suggested that we should be in a position to file a new drug application."[194] But, for the first time, Lilly researchers were very cautious in that regard. As they said:

> After reviewing the status of all phases of the project, however, the committee concluded that this point [of filing an application] has not been reached. The major problem remaining is uniformity from lot to lot.[195]

That conclusion was well-founded for many reasons. Vancomycin had not yet been crystallized by November of 1956, nor had "processing methods and tests for identity, purity, and potency," yet been formalized.[196] The committee felt it necessary to face the possibility that vancomycin might never by crystallized. So at minimum, they felt, in order even to conceive of filing a new drug application with the Food and Drug Administration at least three comparable lots produced by the same organism, processed by the same method, meeting the same chemical standards, and giving satisfactory clinical results were required.

On December 11, 1956, the PDC felt that its minimum requirements had been met sufficiently to consider filing the new drug

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[194] Ibid., p. 5.

[195] Ibid.

[196] Ibid., p. 6.

application.[197] By that date "a series of assays on the last six lots produced show the materials to be identical."[198] There were approximately 50 patients on record then who had been treated with vancomycin. Though that was under the average number normally included on a new drug application, another three months would have been needed to build the record up to 100 cases. The clinicians felt that vancomycin was so important in treating staphylococcal infections resistant to other antibiotics that the three months delay was unconscionable.[199]

Most pharmacological work and all microbiological work had been completed. The stability of the antibiotic in dry form held for

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[197]Prior to 1963, an antibiotic could be approved by the Food and Drug Administration under two alternative plans. One was to file for approval as a new drug. The other was to file the agent as a "certifiable antibiotic." All drugs, antibiotics included could be approved under the first class, but only antibiotics under the second class. Filing under the first class was less expensive and allowed for lot variation from one lot to the next. "Certifiable antibiotics," on the other hand, must be approved lot by lot by the Food and Drug Administration. Vancomycin would not likely fit that category. After 1963, all antibiotics filed originally under either class by any manufacturer automatically reverted to the "certifiable" status. This, of course, protects both physician and patients from dangers inherent in lot variation. After November, 1958, when vancomycin became commercially available, but before 1963, Lilly discoveries eliminated lot to lot variation in vancomycin anyway (see later below). So by the time vancomycin, and all other antibiotics became routinely "certifiable," the former had been stabilized from lot variation. See Federal Food, Drug and Cosmetic Act Including Drug Amendments of 1962 with Explanation (New York; Commerce Clearing House, 1962) for further clarification on certification laws.

[198]PDC, p. 7.

[199]Ibid.

over a year in storage had been demonstrated and the deadline for preparing the new drug application had been set for January 10, 1957. On January 15, however, the data that had been carefully gathered since mid-1954, seemed invalidated. A new high-yielding strain recently put into production was shown by Max Stark to produce predominantly a different fraction from that produced by C-260, thus making useless clinical data gathered up to that time. (Ramifications of that problem are considered in the next section.)

The PDC was thus beset with its ups and downs during 1956, but the purely scientific side of 05865 had been successful on many fronts. Not least among them were the findings made in the microbiology laboratories.

The actinomycete producing vancomycin had been identified. The microorganisms toward which the antibiotic was antagonistic were demonstrated, as well. And in so doing the researchers showed just what a wide spectrum vancomycin possessed. With the exception of gram-negative bacteria, vancomycin was shown to be active against an intriguing variety of microbial forms.

McCormick and his colleagues had shown that vancomycin was active against such disparate microorganisms as Bacillus species (non-pathogens), Corynebacterium diptheriae, various staphylococci,

and Streptococcus pyogenes.[200] D. W. Ziegler (b. 1920) and his colleagues pursued research specifically on the last named two genera.[201] They found that activity of vancomycin against those organisms was pH-independent. Where streptomycin was about 12-fold more active at pH 8 than pH 6.5, vancomycin's activity was altered less than two-fold over that same range. None of a variety of inorganic and organic compounds had adverse effects on the activity of vancomycin in culture broths or serum. More importantly they showed that a lag phase preceding a bactericidal phase did not occur with the antibiotic, as was the case for antibiotics considered bacteriostatic only. Vancomycin was clearly bactericidal. Thus 2.0 micrograms of vancomycin per milliliter of broth completely sterilized a culture (in vitro) of a strain of staphylococcus in 11 hours. Like many antibiotics it was shown to be active against multiplying cells only, but not against either resting or merely respiring cells. That suggested it interferred with cell-wall production. Basic research on that subject was not done until many years later (see below).

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[200] McCormick, "Vancomycin: I.," 610.

[201] D. W. Zeigler, et al., "Vancomycin, A New Antibiotic II. In Vitro Antibacterial Studies," Antibiotics Annual, 1955-1956, 612-618.

The research on the rate at which bacterial resistance could build against vancomycin was the most significant portion of Ziegler's contribution. Three strains of staphylococci were grown in broth for a minimum of 25 serial exposures to vancomycin and also to penicillin (for comparison). After 25 exposures to penicillin one of the staphylococci exhibited a 131,056-fold increase in resistance to that agent, but with vancomycin the resistance did not exceed 4 to 8-fold. The authors noted that such studies were done in vitro only. But an examination of the literature on vancomycin for the past nearly two decades for both in vitro and in vivo use, shows a 16-fold resistance acquisition has never been surpassed (see Chapter 6).

During that same year pharmacological and toxicological studies were accomplished.[202] Several points including acute and chronic toxicity, anaphylaxis, effects on vital signs, and effects on isolated muscles were examined. Mice, rats, guinea pigs, dogs and rhesus monkeys were utilized in the studies. The findings again confirmed the already established difficulties. As with humans vancomycin was shown to produce phlebitis in monkeys. No tissue or hematopoietic damage was demonstrable in any animal, nor was anaphylaxis seen and no other toxicity (other than phlebitis) was shown in monkeys even

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[202] R. C. Anderson, et al., "Vancomycin, A New Antibiotic. IV. Pharmacologic and Toxicologic Studies," Antibiotics Annual, 1956-1957, 75-81.

after 187 consecutive daily intravenous doses. Damage to various inner-ear functions could not be demonstrated in animals, although in man ototoxicity had been reported (see Chapter 6).

The same researchers (i.e., Anderson and his colleagues) were interested in the distribution, excretion, and renal clearance of vancomycin. The studies performed in these regards led to the cautious stand on the use of vancomycin taken by several physicians during the 1950's and early 1960's. The question of ototoxicity as well as phlebitis was, from the beginning, a problem for vancomycin. Although the danger of phlebitis has never been entirely eliminated with respect to vancomycin, the matter of ototoxicity, long considered a danger, may, in fact, not be so (see Chapter 6).

The toxicology team demonstrated that vancomycin's exit from the body was almost entirely via renal excretion. Serum levels were shown to drop rapidly 15 minutes after intravenous injection and to continue dropping more slowly after that. The agent was half-excreted (half-life) in 105 minutes. Although Anderson dealt only with healthy animals, the implication was clear to those in human medicine that if animals with impaired renal function were tested, elevated serum levels of vancomycin could undoubtedly be demonstrated. That elevation might prove injurious to inner-ear functions. Streptomycin and other aminoglycoside antibiotics had a distinct history of

ototoxicity,[203] and vancomycin was not free of such notice. As noted above, this facet may well be unfounded, for extremely strong evidence militates against the reality of ototoxicity being necessarily incurred with use of vancomycin (see Chapter 6).

Work on purification and other chemical aspects of vancomycin had been intensive during 1956. From September 7, 1953, when the first crude filtrate of vancomycin became available, until November 1, 1956, when the antibiotic became a formal project, a total of 8,522 man-hours had been expended on it.[204]

Thus vancomycin had undergone a good deal of study over the more than three years of its recognition. During such time the researchers, the clinicians, and those responsible for its development as a formal product had become aware of its quirks.

Three major problem foci had become apparent by the close of 1956. The multiple fractions and the choice of producing strain, as two of the three problems, represent an organic whole. Even though the presence of more than one fraction had been recognized in mid-1954, its cause was not fully understood. The composition of the medium came under most scrutiny in attempts to stabilize the varying

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[203] Kucers, Use of Antibiotics, p. 116 ff.

[204] Memo from O. K. Behrens to D. J. Shimer, July 10, 1959. Copy in the author's files.

fraction ratios. With an understanding of the significance of utilization of a single strain in fermentation the problem of varying fractions was eliminated. Thus the two problems came to be seen as but two sides of the same coin.

The third problem was the continuing reports of toxic reactions in man. In part the success which emanated from the choice of a more productive strain cleared up certain of the toxic manifestations. More significant to ridding the antibiotic of pyrogens particularly was the employment of better purification techniques.

Chronologically the above noted two-sided problem occurs first and is thus treated now.

5.6. Microbial Strains and Antibiotic Factors

Marvin Hoehn had originally demonstrated the existence of a new antibiotic from the culture from Borneo, M43-05865. During those same early years of the decade the soil sampling program was receiving soil from countries all over the world. From India came at least two other vancomycin-producing cultures, M5-18215 and M5-18260. All three of these cultures were used in the taxonomic diagnosis by R. C. Pittenger and R. B. Brigham when they assigned the specific epithet to the 05865 culture.[205]

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[205] Pittenger, "Streptomyces orientalis," 647. The paper was received for publication on May 14, 1956.

Production of vancomycin from 05865 (or C-260) had begun sometime after August 31, 1955 by the development group at the Kentucky Avenue plant.[206] It had, of course, been in use for over a year in pilot studies at the main McCarty Street plant.

During 1954, 1955, and 1956 Stark continued to examine new cultures sent to him by Hoehn. Squires was also examining new cultures during that time. On October 8, 1956 Squires sent Stark some tubes of culture number M5-18260 for nutrient studies, one of the vancomycin producers from India. Squires had originally gotten that culture from Hoehn about June 21, 1956.[207] At that time, Stark's assistant Rosalie Tetrault (b. 1934) was studying the effects of various alterations in nutrient content on cultures of 05865 (C-260). She added cultures of 18260 in her next study on October 16, which was an investigation of nitrogen requirements. In November she included the two cultures in a study on the effect of the chloride ion on vancomycin production. Those studies ended on December 7, 1956. At that time Tetrault recorded the observation that

> The M5-18260 culture [from India] . . . produced material that appeared to be different than that produced by C260.[208]

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[206] Memo from M. Onofrey to D. J. McGraw, January 22, 1974. Original in the author's files.

[207] Stark, notebook 333B, p. 61.

[208] Stark, notebook 333B, p. 78.

By this period the entire situation had become very confusing. Late in 1955 the Kentucky Avenue group had found that 05865 consisted of several strains. Thus during early 1956 all material produced exhibited varying fraction ratios. During that year a different, purer strain was sought, and when found, employed. That was the Indian culture 18260. It was evidently used in production quite some time after June of 1956 in preference to the original 05865 culture, for it gave more satisfactory yields.[209]

Just prior to Rosalie Tetrault's discovery in December, Stark had assigned a new number to M5-18260. He termed it C329.1, in keeping with the C series of cultures routinely sent to the production group of Kentucky Avenue.

Tetrault's and Stark's discovery came as a surprise to the PDC. At their January 15, 1957 meeting they reviewed the state of affairs to date.

> Materials produced by the new high yield strain [i.e., 18260 from India] recently adopted have been found by a new system of chromatography to be a different substance than produced by the old strain [from Borneo]. The old system showed the compounds to be identical.[210]

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[209] Certain laboratory reports for 1956 are unavailable and by inference from various statements made in the minutes of the PDC, Stark's notebooks, and other sources, the above chronology seems correct.

[210] PDC, p. 8.

The new compound had been termed fraction C; whereas the one produced in most abundance by the Borneo culture had been termed fraction B. Each of the cultures produced both fractions to some extent, however.

The Indian culture was higher yielding than that from Borneo. When that was established, it seemed only natural to employ it. During the Fall of 1956, all materials produced for clinical trial were derived from the new strain from India which produced predominantly vancomycin C. Thus many patients were treated with the new material prior to Stark's and Tetrault's discovery. This was clinical data aimed directly at completing the upcoming new drug application. As Stark said of the unexpected finding of December 7,

> This was disastrous, because it meant our clinical trial had been done with one [fraction] and we were trying to produce the other.[211]

Under the circumstances the PDC had to rescind its intentions of a new drug application. The fermented broths then in production lines were disposed of and production reverted to the old strain, i.e., C-260, on January 24, 1957.

Nevertheless, it had been shown that the potency and even the action of fraction C was superior to that of the long established fraction B. Several fermenters were kept going with the new strain

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[211] Stark, TRI, p. 15.

C329. 1 in order to supply McCormick and G. Pittenger with material for continued study on fraction C.

Going on the already shakey assumption that C-260 material would be eventually the central production item, the manufacturing group continued production of fraction B. By early February the C component had been crystallized and was comparatively pure, thanks to much work then done by Higgins (see following section). At the same time "progress on the product [per se] will be slow for a while."[212]

The first several months of 1957 were tumultuous. The desire to pursue use of the C fraction had become evident on the part of both the clinicians and the PDC. Work on purifying the crystalline C was proceeding rapidly. The C factor could be produced at a ratio of 2 1/2-times that of the B fraction. Clinical data suggested that the high level of C was the more desirable.

In April at least some injections of C suspended in polyethyleneglycol were pain free. It was found that C was not long stable in water solution and would have to be marketed dry for reconstitution.

The following month of May then saw another turning point in the history of selections of strains. The continued good results with C,

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[212] PCD, p. 7.

especially in the purer crystalline form, led the PDC to drop fermentation by C-260 and go to full production with the new Indian strain C329. 1.[213] With that change the PDC, in essence, cleansed itself of all previous research and development, and considered the implementation of C329. 1 as the genesis of a wholly new project. What had happened, in effect, during late 1956-early 1957 was the postponement of the final new drug application for nearly two more years. The new drug application had been set for January 10, 1957, but the events of that time caused a virtual abandonment of all clinical data up to that time. In May, 1957, the committee said that,

> Since so little of the old data is transferable to the new program because of the difference in the chemical nature of Vancomycin C from what had been tested before and to eliminate confusion, the committee elected to close out [project efforts to date with C-260].[214]

Thus it seemed to all concerned that vancomycin C was a single compound and that C-329. 1 was the ideal culture producing it. Work therefore continued on purification, media improvement, and the garnering of a new set of data from patients treated with fraction C.

Higgins in his development progress report of September 12, 1957, brought news of yet further additions to the fraction mixture.

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[213] Ibid., p. 8.

[214] Ibid., p. 9.

> It appears that the older preparations containing mainly B plus some C are resolved into a third component. The importance of this material is unknown.[215]

The question of whether the third element was really just B or some hitherto unknown fraction was expressed by unnamed Product Development Committee members in mid-November. It was found that the B component was often present in as high a concentration as 50%. Some "recent and unintentional change of unknown but possibly serious magnitude" had been introduced into the fermentation process.[216] It was serious because any new drug application to be prepared was dependent upon a monofraction antibiotic, or at least one whose fraction ratios were controllable.

New improvements in paper chromatography resolved the C fraction specificially into two "discrete(?)" zones. It was "especially disturbing and defies rational explanation!"[217] At that juncture, the problem was considered distinct from that of the reappearance of the B fraction.

In October the PDC believed it impossible to ever make vancomycin a pure antibiotic. It agreed that one good lot of,

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[215] Antibiotic Purification Development: 'Vancocin' (Vancomycin, Lilly), September 12, 1957, p. 4. Emphasis added. Copy in the author's files.

[216] Antibiotic Purification Development: 'Vancocin' (Vancomycin, Lilly), November 14, 1957, p. 3. Copy in the author's files.

[217] Ibid., p. 5.

admittedly multiple fraction-filled vancomycin, would be chosen as the potency standard. Potency was arbitrarily set at 800 units/ml.[218] In late November the PDC felt that clearly the matter of multiple fractions was the "foremost" problem.

By early 1958, the Higgins team, and Squires and his colleagues, had come to regard the elusive new fraction as vancomycin X. As much as 10 to 30% of predominantly vancomycin C was actually in the new X fraction. As noted above they felt that the reappearance of B was distinct from the appearance of the new X. Eventually, refined chromatographic analyses showed B and X to be identical.[219] Evidently some change in fermentation confused the fingerprint of B giving it a new appearance.

Those difficulties alerted the development groups to possible future problems with the strains. They continued to test new strains and even attempted mutagenesis (by irradiation) on C-329.1 to increase yield.[220] Such approaches had been taken years before in Peoria with various Penicillium species and with streptomycin producers and others throughout the discovery era. Nothing positive

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[218] PDC: 1570-C, p. 4. Copy in the author's files.

[219] Antibiotic Purification Development K-737: 'Vancocin' (Vancomycin, Lilly), January 23, 1958, p. 2. Copy in the author's files.

[220] Laboratory Report No. 4: Vancomycin, January 23, 1958, p. 1. Copy in the author's files.

came from these studies with Streptomyces orientalis, however.

Further difficulties with the organism did not arise until November of 1958. At that time lowered yields in some fermentation tanks were puzzling. Several hypotheses were put forward to explain that problem. One was that the streptomycete was being attacked by an actinophage.[221]

The problem did not recur until late in 1959, a year after vancomycin went on the market. Actinophages were once again detected. This caused a renewed effort in strain selection. The researchers had shown that none of the 306 strains tested was a better producer than the then well-established C-329.1; so varietal isolates of it were made. A phage-resistant strain of C-329.1 was found. Finally in 1960 this strain (C-329.2) was incorporated into production of vancomycin. To the time of this writing it has remained the strain of choice.[222]

During the hectic period of late 1956 through early 1957, purification and media improvement were under an intense investigation as was strain choice. The pyrogen problem was in part due to

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[221] Vancomycin Fermentation Production Development, Report No. 2, November 10, 1958, p. 3. Copy in the author's files. Actinophages are viruses specific for actinomycetes, whereas the more familiar bacteriophages are bacterial viruses. Actinophages had been found in the vancomycin fermentation tanks.

[222] Memo from M. Onofrey to D. J. McGraw, undated.

fluctuations in batch composition. Such variations caused clinical data to vary. More important in these variations than the multiple fractions were impurities needing removal.

Both chronologically and topically the major advances in purification and media improvement were seen to occur last in the developmental history of vancomycin. Such discoveries as were made in those twin areas were crucial to the production of vancomycin in its purest and most potent form.

5.7. The Problem of Pyrogens

The purest form of a chemical such as an antibiotic, at least, is the crystalline form. Prior to June of 1957, all clinically-employed vancomycin had been the amorphous hydrochloride form.[223]

The earliest work on extraction and purification for vancomycin was not without a generalized precedent. In the mid-1940's, Waksman isolated streptomycin and in the most general terms the Waksman technique and the finally evolved vancomycin technique are comparable. Both required ion exchange adsorption, elution, precipitation as a toxic salt, and reprecipitation as an injectible salt. Both

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[223] H. M. Higgins, et al., "Vancomycin, A New Antibiotic. VI. Purification and Properties of Vancomycin," Antibiotics Annual, 1957-1958, 908.

processes employed the explosive picric acid,[224] though in vancomycin that was eventually circumvented.

Multiple fractions were known in the 1940's and 1950's as they occurred in many antibiotics. Waksman had in 1949 isolated neomycin, which was later shown to be a family of antibiotics. He eventually considered the agent the neomycin complex and noted it contained at least three fractions (A, B, C), two of which (B and C) were active.[225]

The above named antibiotics are all essentially aminoglycosides. Vancomycin can be considered so only reservedly for its chemical structure is yet only 75% understood (see next section). As they are amphoteric these molecules (or families thereof) are resin-adsorbable. This had been demonstrated for vancomycin (05865) by the time of the inauguration of the first steering committee in mid-1954.

As noted earlier, a series of basic steps necessary to yield a rather impure, but injectable solution, had evolved during the 1954-1955 period. The use of an ion exchange resin, Permutit DR (trademark), allowed an initial adsorption from crude, fermented broths of 14,000 units of activity per milliliter of wet resin. Much later, with

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[224] Waksman, Actinomycetes, p. 214 ff.

[225] Ibid., p. 216.

the use of a better resin (Dowex 50 [trademark], 2% cross-linked), that adsorption had increased to more than 50,000 units/ml.

The purification of vancomycin was dependent in no small part on composition of the medium. For example, in September of 1955, it was first shown that the addition of calcium salts as a nutrient boosted potency yields.[226] Those salts proved a difficulty. When the broth was to be purified over the ion exchange column, the calcium ions blocked active sites on the resin (see below).

The early general approach for purification was the following:

1. Filter fermented broth through diatomaceous earth.
2. Concentrate and decolorize the broth through Permutit DR ion exchange resin.
3. Elute by acetone-acetic acid wash.
4. Concentrate (removed acetone and some acetic acid).
5. Acidify with HCl to pH 3.2.
6. Precipitate with picrate in methanol.
7. Filter; wash; resuspend in acetone.
8. Precipitate as the hydrochloride.
9. Filter; wash; dissolve in methanol.
10. Decolorize over carbon.
11. Evaporate to amorphous vancomycin hydrochloride.[227]

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[226] Laboratory Report No. 22: Vancomycin--C-260, September 29, 1955, p. 3. Copy in the author's files.

[227] Higgins, "Vancomycin. VI.," 906-907.

The above basic protocol was the method of choice at the beginning of 1957, that is the period of confusion of strains and fractions. The desire was not only to elucidate and eliminate problems issuing from the choice of the proper organism, but also to achieve an improved purification process. Two points in particular were of immediate concern in early 1957. First, several new resins had become available since the Permutit DR resin had been incorporated into the more-or-less standard protocol. Those were attracting attention. Second, the elimination of the explosive picrate step was very attractive. Those were the most important problems Higgins and his colleagues faced in February of 1957.[228]

In order to avoid the steps using the Permutit resin and picrate precipitation which had always given a fairly low yield and involved an explosive chemical, Higgins offered three alternatives. The first was to precipitate with zinc; however, the picrate step remained obligatory under that regime. The second was to precipitate with tannic acid. In the case of zinc, vancomycin would chelate that metal and precipitate. It was known that the antibiotic was a chelating agent and that heavy metals such as manganese, cadmium, cobalt, nickel, zinc, and lead as divalent hydroxides were selectively incorporated in

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[228] Antibiotic Purification and Development K489: 'Vancocin' (Vancomycin, Lilly), February 7, 1957, p. 1. Copy in the author's files. That they were the most important problems was stated by Higgins, himself.

vancomycin. Zinc was the preferred metal of the series. It was inexpensive, non-toxic and easily removed.[229] Experimentation showed that when zinc sulfate was added to the filtered crude broth and adjusted to pH 9, a zinc hydroxide-vancomycin complex would precipitate. That complex could be purified by forming an oxalate during the later steps of the basic procedure outlined above. Neither the zinc nor tannic acid methods were ever seriously employed. The third method suggested and soon adopted was use of the Dowex resin. The reason for this is cited in the quotation below.

Dowex was employed by April of 1954 predicated upon the smaller total amount of pyrogens escaping through purification. This was due to the polymeric structure of the resin, the structure of vancomycin itself, and the structure of the pyrogenic factors. Such factors were polysaccharides from Streptomyces orientalis' cell walls, and various proteins. Not only did it help to reduce pyrogens but the total yield was much increased over that from Permutit-treated broths. Also attractive, was its elimination of the picrate precipitation step. That step had been included in the early project history as one of several steps (together with the Permutit step) to purify and remove pyrogens. Employment of Dowex, then, eliminated two undesirable procedural steps. Higgins said of Dowex that,

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[229] Higgins, "Vancomycin. VI," 908.

> In contrast to the permutit process, the capacity, elution yield, concentration factor, and purification obtained are more than adequate to ensure the elimination of the troublesome picrate precipitation.[230]

At approximately the same time vancomycin C was crystallized by Edwin Flynn (b. 1920), who somewhat later gained international fame with his work on the family of cephalosporin antibiotics. Crystallization assures much higher purity, since pyrogens can be selectively excluded during the crystallization procedure.

That first successful crystallization of comparatively pure vancomycin C (with a percentage of active, non-pyrogenic B in it) was accomplished using dimethylsulphoxide (DMSO).[231] Such a crystallization was not included in the regular processing steps for injectable vancomycin, however, but used primarily to produce crystals for x-ray diffraction investigations. The Dowex process yielded a rather pure crystal itself. Though pyrogens were decreased thereby, still a

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[230] Antibiotic Purification and Development K489: 'Vancocin' (Vancomycin, Lilly), April 18, 1957, p. 2. Copy in the author's files. An interesting finding was made by Higgins at that period. Even though the Dowex was employed with ion exchange in mind, such exchange did not obtain! In fact, the mechanism in operation was solely related to pore size of the resin and the diffusion rates of the broths and fluids passed (p. 9).

[231] DMSO has been known for some years and much has been said concerning its efficacy as a therapeutic agent in its own right. It has come under heavy criticism, as well. A recent book examines its stormy history, particularly from a journalistic point of view. See. P. McGrady, The Persecuted Drug: The Story of DMSO (New York: Doubleday, 1973).

better final purification process was sought. (That was forthcoming only sometime later, after vancomycin was commercially released [i.e., November, 1958]. See below.)

The new purification process was much simpler than that outlined above using Permutit-picrate. In short the new Dowex process included initial adsorption on Dowex, elution by an acetone-triethylamine solution, concentration, HCl acidification (to pH 3.2) and purification by acetone (as in step 7 and following, above.)[232] The Dowex process was a great success, for as Higgins noted,

> The Dowex-50 processed material has been reported [as free from pain] as the most extensively purified preparation yet tested.[233]

The calcium salts added in the fermentation (see above) were eventually eliminated by oxalate precipitation; though it was not a desirable process and was eventually replaced by another method. At the same time much color was eliminated even prior to a carbon decolorization step.

The question of the multiple fractions, in preparations containing predominantly C, continued to plague the investigators. At least 5% (usually more than 10%) of B was present no matter what Higgins'

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[232] Higgins, "Vancomycin. VI," 908-909.

[233] Antibiotic Purification Development K489: 'Vancocin' (Vancomycin, Lilly), June 27, 1957, p. 1. Copy in the author's files.

colleague Edwin Davisson (b. 1923) did in attempts to isolate pure vancomycin C. Electrophoretic techniques were useless. They had been found so with earlier and cruder materials. On October 10, 1956 G. Pittenger had found "no polar movement. Voltage limit 250 v, using rheostat--higher voltages. Blew fuses."[234] The vancomycin molecule, large and heavy, would not migrate in the electrophoretic field. Even after the antibiotic was thrice recrystallized by the Dowex process (not available to G. Pittenger), Davisson still could not separate the B and C fractions. Although research on the fractions continued through the following year, their separation was never effected. It remained for the PDC to make a decision on the matter.

On December 17, 1957, the PDC was forced to admit that if the live-saving ability of vancomycin was ever to be made commercially available it would be so as a mixture, for

> We have reached the point where marketing the product is feasible. [Dr. Griffith] recommended such an action, bearing in mind that we are dealing with a mixture and from time to time facts will appear which will complicate the situation.[235]

The facts which would complicate the situation were variables brought into the clinical picture by the variations in lots of vancomycin.

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[234] Pittenger, notebook 270 B, p. 274. The original notebook is no longer extant, but exists in toto as microfilm in the Lilly microfilm vault. A copy of pertinent passages is in the author's files.

[235] PDC, 1570-C, p. 6. Copy in the author's files.

Several members of the committee were set to organizing and classifying the clinical and scientific data toward the end of preparing the new drug application. In so doing it became apparent that lot variation was of less significance than previously imagined, for the Dowex process had helped greatly to stabilize fraction ratios once the broths were crystallized. Furthermore the pyrogens had been greatly reduced.

In view of the fact that vancomycin was destined to be a mixture, the committee recommended in January, 1958, that the ratio of C to B be set at 85% to 15%. Even if Streptomyces orientalis did not produce these ratios in fermentation, which would be virtually unexpectable, batchs could be and were mixed to yield the desired proportions. The researchers had also shown that vancomycin containing 30% of B could not be crystallized.[236] By February the researchers demonstrated that using all the technical knowledge gained to date they could routinely produce vancomycin C in concentrations approximating 90%. Thus the patent department set the definitions at not less than 85% vancomycin (without letter designation) with not more than 15% of other fractions (arbitrarily designated A).[237] The submission of the new drug application was set for May 1, 1958.

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[236] Ibid., p. 7.

[237] Ibid.

During mid-1958, many reports came in from the clinical trails describing ototoxic reactions; however, it was found after that these patients often had reduced renal function. Hence, vancomycin concentrated in their blood. This is considered extensively in Chapter 6. The positive data to be included (circa 57 cases) in the new drug application were very positive, indeed. One investigator was "very enthusiastic" as he had obtained "twelve remarkable cures."[238] Due to reports of ototoxicity, it was decided to include precautions into the literature for the new drug application on that regard. The ramifications on that particular subject are important and are hence considered in extenso in the following chapter.

During the middle and later parts of 1958, the central concern of the PDC was on two points. The question of how to lyophilize the material in order to have dry powder for rehydration in the ampoules was a problem. Two systems existed: lyophilization either in the ampoule, or in bulk prior to distribution into the ampoules. The genesis for the problem lay in the fact that it seemed bulk-lyophilized material was more toxic than that lyophilized in the ampoule. For that reason it was felt necessary to purchase certain very expensive machinery for ampoule-lyophilization. Other antibiotics in production could benefit from such machinery, but it turned out later that year

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[238] Ibid., p. 8. The investigator was not named.

that bulk-lyophilized vancomycin was not toxic due to that process, but to unrelated purification problems. The less expensive bulk method was finally decided upon by September.[239]

The second concern of the PDC during 1958 was the use of polyethyleneglycol (mentioned earlier) as the diluent or carrier for the injectible rehydrated vancomycin. As late as September the PDC was considering marketing a dual package, one ampoule containing the dried antibiotic, the other a 50% sterile solution of the polyethyleneglycol. That carrier, as opposed to water, had been used much earlier because it eliminated much of the pain upon injection. However, because improvements in purification of vancomycin and also because the glycol was very slow to dissolve the powder, this was abandoned. Sterile water, to be provided at the hospital, was finally chosen as the diluent of choice.[240] Vancomycin was released on November 24, 1958.

From a purely scientific standpoint the purification procedure was not so fixed that research on improvement was not continued. On the contrary, Davisson was pursuing a better method to achieve even greater purity than was possible in 1957 and much of 1958. The very day after vancomycin's commercial release Higgins said,

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[239] Ibid., p. 9-12.

[240] Ibid., p. 12.

> Dr. Davisson has reported the crystallization of a copper complex that is believed to be specific for vancomycin.[241]

Since vancomycin was a chelating agent, it readily took up copper, and with such great specificity that very pure crystals could be produced by a precipitation procedure. That process was highly efficient for,

> We are fortunate, I think, in the fact that most of the material that may be associated with pyrogens do not readily form chelates with the heavy metals such as copper.[242]

and,

> It was immediately obvious to us that we had certainly improved the quality of our product.[243]

Thus with the advent of the copper precipitation step no further significant advances in purification were made in the production of vancomycin. From 1959 to at least 1974, no changes have been made in this field. The production of vancomycin is very conservative in comparison to other antibiotics in this regard.[244] Most other antibiotics have seen additional improvements over time.[245]

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[241] Antibiotic Purification Development K737: Vancomycin, November 25, 1958, p. 1.

[242] Harvey Higgins, TRI, January 21, 1974, p. 13.

[243] Ibid., p. 14.

[244] H. Higgins, personal communication, Feb. 5, 1974.

[245] Ibid.

One other very significant improvement was made in media composition, however, after November of 1958. This will be discussed in the following section.

5.8. Recent History

Before entering upon a discussion of the medical uses of vancomycin, in the next chapter, several purely scientific matters remain to be discussed. Certain other features of product development history are of interest. From the chronological standpoint, five subject areas must be examined in order: (1) product formats other than the intravenous injectable; (2) non-human uses; (3) media development after 1957; (4) mode of action; and (5) pure chemistry.

Vancomycin is today given solely as a hospital antibiotic and is administered by intravenous injection or infusion.[246] The intramuscular route long-sought as more desirable by the PDC was never achievable due to undue pain upon injection into the muscle directly.[247]

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[246] One other commercial format does exist, although produced on a very limited scale. Since vancomycin is not absorbed from the gastrointestinal tract, an oral form is useless for systemic infections. In the case of staphylococcal enterocolitis and prebowel surgery bowel-"sterilization" (only limited sterilization), vancomycin is extremely useful. This is discussed somewhat in the next chapter.

[247] K. G. Kohlsteadt who was in charge of much of the PDC clinical investigations and who was Griffith's superior noted that the matter of an explanation of pain upon injection with vancomycin is not forthcoming. Certainly molecular size and pH of the HCl format may be involved. Beyond that it is not well understood. Kohlsteadt, TRI, February 4, 1974, p. 3.

But, starting as early as November of 1956, the PDC branched out into examining various possible formats for vancomycin, other than intravenous. Such short-lived projects included combinations of vancomycin with neomycin as an oral capsule and oral suspension. The aim of those studies was to provide a gastro-intestinal tract sterilizer, especially for the treatment of dysentery (not to be available in the United States). The clinical interest in such a combination was never high and all such projects were discontinued, the last being in mid-1959.[248]

Another early use of vancomycin was a non-human application. It has been mentioned that veterinary uses were never notable after some initial investigation in 1954-1955. However, one successful use in plants had appeared in the literature in 1959.[249] It was demonstrated that the antibiotic inhibited the growth of many phytopathogenic bacteria. Vancomycin was readily absorbed, even against a concentration gradient, by both leaves and roots. Since it was also readily translocated both up and down, its use as a systemic antibacterial agent appeared promising. But an examination of the literature since

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[248] PDC Series 1570-A, -B, -D, -E. Copies in the author's files. There had been a continuing interest in an ointment for dental use over the past decade, although no very extensive research had been done and it was dropped entirely by 1975.

[249] P. P. Mehta, et al., "Vancomycin, A Potential Agent for Plant Disease Prevention," Phytopathology, 49 (1959): 177-183.

that time does not confirm that vancomycin has ever achieved use as an agricultural antibiotic.

With the exception of the copper complex purification technique the only other notable advance in vancomycin's history, after 1958, was in the area of the composition of the fermentation medium. Little has been said of modifications of the composition of the medium in the past several pages. Because of the empirical approach, evolution was slow, piecemeal and presented few historically notable moments. This was not so after 1959.

By late 1959 the original medium had evolved into an even more complex one.[250] It was considered desirable to substitute a chemically-defined synthetic medium for the complex undefined media used for so long in vancomycin production.[251] That would allow more controlled conditions during fermentation and allow stabilization of the varying fraction ratios within each batch. Laboratory experimentation by Squires and his colleagues on a new medium was conducted in 1959. By early 1960 a synthetic medium was developed that gave suitable

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[250] By September 1, 1959 the medium was composed of: 3% dextrin #700; 2% cerelose; 1.5% peptone #159; 1% CaCl; 1% PACO; and 0.5% Stamino "A." (PACO is distillers soluable.) The increased dextrin and cerelose, and the added CaCl and PACO increased the complexity of the medium. Physical conditions also evolved. Agitation rose to 225 rpm and aeration dropped considerably.

[251] Squires, personal communication, January 22, 1974.

yields and has remained the choice ever since.[252]

In much later developments the mode of action of vancomycin against bacterial cell wall structure was elucidated. Although such studies are not particularly amenable to historical consideration, an abbreviated review of the findings is appropriate to an appreciation of vancomycin's bactericidal properties.

Prior to 1958, Lilly workers had demonstrated that vancomycin was detrimental to dividing bacteria only (see above). In December of 1959, two Canadian researchers found that although cell wall synthesis was inhibited, protoplasmic synthesis continued.[253] That indicated that vancomycin was inoperative against protoplast functions. Two years later another worker at Cambridge confirmed the Canadian findings. He used Staphylococcus aureus, as had his predecessors, and virtually all his successors, in similar studies. The comparison between vancomycin and penicillin's mode of action was made. However, and in a significant finding, he pointed out that since no cross-resistance could be demonstrated for the two

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[252] As the composition of the medium is proprietary it cannot be described here.

[253] D. C. Jordan, and W. E. Inniss, "Selective Inhibition of Ribonucleic Acid Synthesis in Staphylococcus aureus by Vancomycin," Nature, 184 (1959): 1894-1895.

antibiotics their precise mode of action against cell wall synthesis must differ.[254]

Although it was recognized in the 1940's[255] that penicillin's general mode of action was upon cell wall integrity its precise action was far from known. By 1967 it had been demonstrated that the cross-linking of a linear cell wall glycoside was inhibited by penicillin.[256] Thus research on vancomycin's action was brought into focus upon that subject area. Various workers in recent years have shown that vancomycin interferes with the biosynthesis of peptidoglycan (a cell wall mucopeptide) and other peptides terminating in D-alanyl-D-alanine. Such studies continue today.[257]

Finally the pure chemistry of vancomycin needs to be briefly considered. In 1968 the chemical structure of the vancomycin was

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[254] P.E. Reynolds, "Studies on the Mode of Action of Vancomycin," Biochim. Biophys. Acta, 52 (1961): 403-405.

[255] J.P. Duguid, "The Sensitivity of Bacteria to the Action of Penicillin," Edinburgh Med. J., 53 (1946): 401-412.

[256] R. Rich and G. Weinbaum, "Antibiotics Affecting Bacterial Cell Wall," J. Albert Einstein Med. Cntr., 15 (1967): 35-43.

[257] The most recent publication is dated 1972. The interested reader is referred to M. Nieto et al., "Reversal by a Specific Peptide (Diacetyl-αγL-diaminobutyryl-D-alanyl-D-alanine) of Vancomycin Inhibition in Intact Bacteria and Cell-Free Preparations," Biochem. J., 126 (1972): 139-149, and previous workers whom they cite. None of the researchers pursuing such studies were associated with Eli Lilly and Co., at which place such research has not been actively pursued.

still poorly understood. Several workers in America noted that

> Even though vancomycin inhibition of cell wall synthesis is well established and appears to be a direct consequence of adsorption to the cell wall, detailed information on the exact mechanism by which an adsorbed vancomycin molecule can block the elongation of peptidoglycan depends on more exact knowledge of the chemical composition and structure of vancomycin.[258]

These workers succeeded in chromatographically separating the antibiotic into three factions. (Such a process is not practical industrially; hence, vancomycin for medical use remains the mixture the PDC recognized it must.)

By 1972, Soviet workers had shown the molecular weight to be about 1,600 which was much reduced from early Lilly estimates made on poorly purified materials. In both the Soviet Union and Hungary much work was in progress, but two British workers produced the most enlightening studies.[259] They fragmented vancomycin and defined the structure of the various pieces. Thus it was possible in

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[258] G. K. Best, et al., "Chromatographic Separation of the Vancomycin Complex," Antimicrobial Agents and Chemotherapy, 1968, p. 115-119. Quotation on page 115.

[259] A. J. Johnson and R. M. Smith, "Vancomycin. Hydrolysis and Oxidation Studies," J. Antibiotics, 25 (1972): 292-297. The Soviet and Hungarian work is to be found in R. Bognár, "Results of Soviet-Hungarian Cooperation in Antibiotic Research in Debrecen. Chemical Investigation of Actinoidin and Restomycin," Acta Univ. Debrecen, 1968, p. 185-200.

mid-1973 to account for 75% of vancomycin's structure.[260] Our knowledge of the antibiotic's structure remains at that juncture.

In this chapter the scientific and product development of vancomycin has been traced. But the value of an antibiotic must be measured at the bedside. The physician, as a pragmatist, wants results. What did vancomycin accomplish? That is the question to which we now turn.

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[260] P. J. Roberts, et al., "Concerning the Molecular Weight and Structure of the Antibiotic Vancomycin," J. Chem. Soc. --Chem. Comm., 7 (1973): 772-773.

6. VANCOMYCIN AND STAPHYLOCOCCUS AUREUS

6.1. Trials of a New Antibiotic

Constable Albert Alexander entered Radcliffe Infirmary in October of 1940. The Oxford bobby had scratched his face with a rose thorn. This simple accident soon led to a fulminating infection of Staphylococcus aureus. By mid-January the infection was widely metastatic, giving rise to infectious loci in the scalp and eyes and by February 12, 1941 he was moribund. Mary Florey began injections of penicillin to which Alexander rallied rapidly with a clearing of many loci, a drop in temperature, and a return of apetite. Penicillin was obviously working and all appeared well for five days. Then a note on his chart was penned which read, "penicillin supply exhausted."[261] Constable Albert Alexander died one month later.

In late 1954, a man entered the Lilly Research Clinic at the Marion County (Indiana) General Hospital (now Wishard Memorial Hospital). He had been undergoing another bout of acute and recurrent urethritis. He had no fever but had a urethral discharge and pain upon urinating. A culture of the purulent matter demonstrated a gonococcus to be present and a regime of 100 mg. of vancomycin every eight hours was instituted. That lasted for seven days--but

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[261] Bickel, Rise Up to Life, p. 123.

without improvement. On the eighth day penicillin therapy was begun and the infection cleared completely. An in vitro test of the infectious organism showed that it was not sensitive to vancomycin.[262] Both were the first human uses of a new drug, penicillin in the first case, vancomycin in the second.[263] Also both were treatment failures, but for very different reasons. In the case of the bobby the supply of penicillin ran out. In the case of the second patient the infectious microorganism was insensitive to the action of vancomycin. Fortunately for that second patient penicillin was by then in adequate supply, and it cured him.

The next three cases treated by vancomycin were successful. One patient was cured of bronchopneumonia within 24 hours, thus it was shown that pneumococci were vancomycin-sensitive. A second patient was cured of streptococcal pharyngitis in a 24-hour period and that showed that streptococci in human infections were sensitive too. The third patient had severe erysipelas, a streptococcal disease. The face was so swollen that the eyes were almost entirely shut. The

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[262] R. S. Griffith and F. B. Peck, "Vancomycin, A New Antibiotic. III. Preliminary Clinical and Laboratory Studies," Antibiotics Annual, 1955-1956, p. 619-622.

[263] Though the story of the Oxford bobby is popularly considered as the first use of penicillin in man, Bickel points out that he was actually the second. The first use was in a woman in New York dying of cancer. The event remained unknown until recently. See Bickel, p. 124.

white cell count was elevated and the temperature was 101 F. Under the same regime of vancomycin administration as in the first unsuccessful case, the erysipelas was cured--again in only 24 hours.[264]

Not unlike the penicillin story, the situation of rapidly diminishing supplies threatened treatment by vancomycin in this early period. At the March 11, 1955 meeting of the PDC, the Committee reported that "clinical work has been hampered by lack of material."[265] During that whole early period such difficulties plagued the vancomycin team. In that connection one case in the vancomycin story took on some of the drama that was so much a part of the Constable Alexander situation.

A patient at the Lilly Research Clinic had undergone surgery on his foot. Following that operation he developed a staphylococcal infection at the wound site. He was given antibiotic therapy, but with no positive results. It soon became apparent that all known antibiotics then available (1955) given alone or in combination, topically or systemically, were of no use. The surgical staff had recommended amputation, but Griffith was alerted to the patient's plight and offered the use of experimental 05865. The patient responded, "anything that

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[264] Griffith, "Vancomycin. III," 621.

[265] PDC, March 11, 1955, p. 2. Copy in the author's files.

might save my foot."[266] After five days of therapy the white count dropped, exudation slowed, and the heat decreased at the infection site. During the following seven days bacterial cultures became negative for the staphylococci. Then, not unlike the case at Radcliffe a decade and a half earlier, the supply of the antibiotic ran out. Griffith later said, "while we held our breath, he continued to improve."[267] Two months later he was released, to walk out of the hospital on two feet.

Over the next months, as developmental work proceeded on 05865 (Chapter 5), clinical investigations increased. Another half dozen cases were added to the, as yet, small clinical trial program. For example, a man with a penicillin-resistant carbuncle infection was cured by vancomycin after three days of treatment.[268]

In a more severe case another patient was not cured by the new antibiotic. A 32 year-old man had been kicked by a horse, which punctured the man's liver and caused abscesses to form subsequently. Surgical drainage and multiple antibiotic therapy were to no avail. Vancomycin, however, was effective against the Staphylococcus

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[266] R. C. Anderson, et al., "Symposium: How A Drug is Born," Cincinnati J. Med., 42 (1961): 49-60. Quotation on p. 54.

[267] Ibid.

[268] R. S. Griffith, "Vancomycin: Continued Clinical Studies," Antibiotics Annual, 1956-1957, p. 118-122.

invading the patient's system and kept the blood sterile for 11 days, but thereafter the blood cultures again became positive. Death followed 17 days later. At postmortem the findings, however, made it clear why no antibiotic could save the man. In a hole between the inferior vena cava and the liver abscess cavity, a blood clot "the size of a grapefruit" was found.[269] At the same time large masses of staphylococci were found on his heart valves. It was Griffith's opinion that continued breaking away of thrombi from the masses caused the recurrent positive blood cultures. The masses, he felt, were probably impenetrable by the various antibiotics employed.[270] Although that difficult case was reported as a failure, it was reported that the staphylococci isolated from the patient remained sensitive to vancomycin throughout the case history.

During the same period Griffith had his encouraging moments, too. J.S., a 68 year-old man, had been referred to Griffith (as are all patients at the Lilly Research Clinic, see below) and presented an advanced case of furunculosis. Lesions were to be found on his neck, back, and left forearm. Previous penicillin therapy was unproductive of positive results and so a regime of 500 mg. of vancomycin every eight hours was begun. The boils began to drain slowly and to assist

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[269] Ibid., p. 121.

[270] Ibid., p. 121-122.

that process the patient himself instituted some amusing additional therapy. He supplemented vancomycin with "alcohol, witch hazel, and bay rum massages, and liberal dusting of talcum powder to the infected areas."[271] Vancomycin was, however, the agent most likely responsible for the cure effected in the case.

The clinical trials were succeeding. Nevertheless, the lot to lot variation was a continuing problem during that early period and pain upon injection, flushing, and phlebitis were not uncommon with the still rather crude preparations. Yet it was clear to the PDC and to Griffith himself that expanded clinical trials outside of the Lilly Clinic should be considered. During the year 1955, several outside clinicians were approached in regard to beginning independent trials of antibiotic 05865.[272] Thus the period of trials per se, which was

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[271] Ibid., p. 120.

[272] The choice of the outside clinicians was left entirely up to Griffith. His choice was based upon such factors as how well he knew the other clinicians, what reputation they had in antibiotic testing, his personal rapport with them and past experience with them. When such clinicians were chosen they received two grants. One was for continuing research in areas of their own interest. How the funds were allocated was entirely up to them. The second grant was to be used solely for testing the new antibiotic (or other drug). It behooved the investigators to report all findings that they made concerning the test antibiotics. For the Lilly Co., when preparing a new drug application, does not leave out any data favorable or unfavorable for the new antibiotic (drug). As Kohlsteadt said, in comparing this approach to Jack Webb's well-known lines on the Dragnet television series, "give us the facts" (TRI, p. 7). "Positive bias is just as bad as negative bias." (ibid.) Thus, hopes are falsely raised and much

all testing prior to November, 1958, began to expand. Since the antibiotic was federally approved and released for sale in November of 1958, use of it thereafter would not properly be classed as trials.

Among the various investigators who employed antibiotic 05865 in early outside trials were Joseph E. Geraci (b. 1916) at the Mayo Foundation, N. Joel Ehrenkranz (b. 1924), University of Miami School of Medicine, and William M. M. Kirby (b. 1914) of the University of Washington School of Medicine. The latter two were also associated with nearby major hospitals where the trials actually occurred. Those were the Jackson Memorial Hospital, Miami, and the King County Hospital, Seattle, respectively. Geraci's work was performed at the Mayo facility.

A good many other investigators employed vancomycin during the years preceding November of 1958, either by requesting supplies from Griffith or by having been contacted by him. Nevertheless, the bulk of cases useable in the new drug application and the bulk of medical opinion derive from the three aforementioned physicians. Of them, Kirby and Geraci were particularly active. Although the early trials covered many and varied types of infections (primarily

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money is uselessly spent on a foredoomed project if clinicians unwisely bias their reports, giving Lilly what they think it wants to hear. For these reasons only a few of the most reputable clinicians in America are repeatedly approached by Griffith. They are named and discussed in the text above.

staphylococcal), Geraci pursued bacterial endocarditis heavily. The subject of vancomycin and endocarditis is one of the most important in the antibiotic's history. A section later in this chapter considers this area fully.

The trials, as accomplished by Griffith, may be considered the first of a tripartite program. Phase one is in-house testing at the Lilly Clinic; phase two is outside testing by selected clinicians; phase three is even broader outside testing.[273] The third phase may be used with many drugs, but phase two is the end-point for antibiotic testing. The reason for this is that results derived from antibiotic treatment are seldom equivocal. Either a cure is effected or it is not. On the other hand results emanating from trials (even phase three) on a drug such as an analgesic often remain qualitative and subjective. For these reasons the historical development, in the clinical respect,

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[273] In 1960 K. G. Kohlsteadt published an article ("Developing and Testing of New Drugs by the Pharmaceutical Industry," Clin. Pharmacol. Therapeutics, 1:192-201) which described how new drugs underwent clinical testing at Eli Lilly. The impetus for writing the paper came from a request made by the then chief of the Food and Drug Administration (FDA) who wanted said information (Kohlsteadt, TRI, p. 9). Within three years thereafter the FDA published a new set of guidelines on how clinical testing should be accomplished. The new methods described paralleled the long-standing Lilly approach, but for one exception. It was Kohlsteadt's personal opinion that the FDA "missed the concept from the very beginning," for "when you extend out to less well controlled studies you don't stop the controlled studies" (TRI, p. 9). The FDA approach (as Kohlsteadt interprets it) calls for the total end of one phase prior to initiation of the next phase. See also the 1962 FDA Drug Amendments cited earlier.

for vancomycin, and most antibacterial antibiotics, can be considered to undergo a somewhat altered phase three. The last phase is post-commercial release and lasts indefinitely. It is dependent primarily on continued general acceptance by the medical community of the new antibiotic. In some cases after a few years a new antibiotic is found to become unacceptable, either due to increased microbial resistance to it, or the appearance of too many unacceptable side-effects. Aureomycin is an example of the first instance just mentioned (see Chapter 4). There are also antibiotics which fit the second category, ristocetin, for example (see below).

The phase two trials spearheaded by Geraci, Kirby, and Ehrenkranz gave more information to the Lilly workers, both at the clinic and to the PDC. Decisions made at Lilly were often the result of findings reported by the outside researchers. The evolution of vancomycin as a clinical product reflected those various inputs. The reports themselves were of case histories and opinions emanating from evaluations of those histories. The data on the majority of the patients treated prior to November of 1958 are published in the medical literature. Some, however, and they are generally single cases derived from physicians actively seeking use of vancomycin, are not published.[274] All of those inputs were evaluated by the PDC. The

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[274] A list of about 50 are to be placed in this category. They are confidential and hence are not considered here.

PDC was very much aware of the potentials of 05865 in 1955 (Chapter 5). The reports that reached them told of a success story unlike that for any other antibiotic. For, as has been repeatedly stressed, staphylococcal resistance never increased in the presence of vancomycin as it had for all other antibiotics theretofore used, not in the 1950's, not even now.

Geraci was immersed early in vancomycin research. He made findings not only in the hospital wards, but in the research laboratories too.[275] In a large in vitro test against the staphylococci, he demonstrated that of 112 strains examined, all were susceptible to vancomycin. Of 28 strains of Streptococcus faecalis, all were killed; and seven species of Clostridium were inhibited or killed.

During that study Geraci and his colleagues induced limited resistance in two of the 112 strains of staphylococci. The resistance was only a four-fold increase and a killing concentration was still easily reached. Nowhere in vancomycin literature has induced resistance been reported wherein patient treatment with the antibiotic could not be successfully completed. Geraci had performed that experiment in vitro. In an attempt to repeat it in vivo (mice), he

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[275] J.E. Geraci, et al., "Some Laboratory and Clinical Experiences with a New Antibiotic, Vancomycin," Proc. Staff Meet. Mayo Clinic, 31 (1956): 564-582.

was unable to induce the resistance.[276]

In pharmacological tests Geraci used eight normal young men with no infectious processes. He treated 94 patients in addition. The well individuals gave no toxic signs, but did provide information regarding distribution, renal clearance and other factors theretofore tested at Lilly. In the 94 sick individuals, Geraci noted a chill in six of them and a skin rash in four. Short-term or continued phlebitis was seen in several of the cases.

The findings of Geraci were published, of course. Included in his findings he reminded his readers that he was dealing with test material of low purity as pyrogens were surely present, he felt.[277] Nevertheless, once such findings are in the literature they remain there forever and eventually find themselves included into the new drug application.

Kohlsteadt has compared the evolution of a drug to the life of a child growing into adulthood. "The things that happen in an infant [phase one of clinical trials] can have an effect throughout his life."[278] The same is true in the phase two clinical trials ("teenager"). Thus the stigma of toxic side-effects, even if partially or entirely removed

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[276] Ibid., p. 567.

[277] Ibid., p. 576.

[278] Kohlsteadt, TRI, p. 13.

later by improved purification procedures, haunts the employment of the antibiotic in later years. But at the same time, as Kohlsteadt notes, that situation is beneficial too. Every "black mark" should be recorded for the antibiotic, for if much later a patient suffers an untoward reaction, the physician employing the drug would be aware of the possibility as it has been a part of the literature from the beginning.[279]

It may be said that the discovery of penicillin first, before any other antibiotic was, in a sense, unfortunate. For today penicillin remains the most innocuous of all antibiotics. Had it been discovered later it may well be that the public and medical psyche would be accustomed to expect some side reactions as is typical for most antibiotics. It is interesting to note, however, that though the general and professional public do not often consider toxic responses from penicillin (in all its variants), the percentage of allergic reactions still runs from two to as high as seven percent of patients treated.[280]

Geraci cited nine cases from his earliest studies.[281] A positive

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[279] Ibid.

[280] R.I. Wise, "Modern Management of Severe Staphylococcal Disease," Medicine, 52 (1973): 295-304. Citation on p. 300.

[281] A second paper with the same title as that cited above (i.e., Geraci, "Some Laboratory and Clinical Experiences") appeared in Antibiotics Annual, 1956-1957, p. 90-106. The contents were the

therapeutic effect was obtained in all but one. That one was not unlike Griffith's horse-kick case. An empyema nidus in Geraci's ninth case was impenetrable to vancomycin, though the individual survived after surgical drainage. Although Geraci concluded on a note of veiled optimism for the new antibiotic, his enthusiasm was very high for one cure vancomycin had effected in a case of endocarditis (see below).

During the same period Ehrenkranz studied vancomycin, employing in his study ten cases. They included such diverse diseases as pneumonia, meningitis, parotitis, bacteremia, osteomyelitis, and arthritis. It was a valuable test study for vancomycin, for as Ehrenkranz said,

> Every patient in the study was considered to be critically ill. In 9 cases [of 10] the infection appeared life-threatening.[282]

Three of the ten, in fact, were so critically ill that they died despite all therapeutic efforts, but in each of those cases "serious underlying medical disease was present" (for example, a diabetic in a coma, and similar disabilities).[283]

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same, but occurred in a reorganized and improved format. In that second paper he provided a chart of the first (?) nine cases treated.

[282] N. J. Ehrenkranz, "Clinical Evaluation of Vancomycin in Treatment of Multi-Antibiotic Refractory Staphylococcal Infections," Antibiotics Annual, 1958-1959, p. 587-594. Quotation on p. 587.

[283] Ibid., p. 589.

Ehrenkranz, like others, had rather impure material. Even so the side effects were rather limited. His patients had large rashs on the body and face, hives, fever in one (treated by antihistamines), and phlebitis (though only in one patient on prolonged therapy). Such reactions were seen in four of his ten cases. Only one of his cases did Ehrenkranz consider a failure, though no post-mortem was done to demonstrate why.

All of the individuals had staphylococcal diseases, including the pneumonia victim who died. All ten had been treated unsuccessfully with two or more antibiotics prior to vancomycin therapy. Seven were total cures with vancomycin. Ehrenkranz in his conclusion remarked that "Vancomycin failures were not due to resistant bacteria."[284]

By 1955-1956 the Lilly researchers and the outside clinicians had come to consider vancomycin a life-saving antibiotic. In other words physicians often withheld the antibiotic until it was clear that no other agent was effective in a given case. The attitude that developed then has persisted right up to the present time in regard to vancomycin. For severe staphylococcal diseases it was held (in 1973) that

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[284] Ibid., p. 594.

> If the patient cannot be treated with the penicillins or the cephalosporins because of antibiotic resistance or adverse drug reactions, vancomycin is the antibiotic of choice.[285]

The work of Kirby and various of his colleagues during the second phase trials was as significant in setting the trend for future employment of vancomycin as was that of Geraci and of Ehrenkranz. Kirby began his studies with a small number of patients (15) as had Ehrenkranz and of those, "most were quite ill."[286] From Kirby's patients, 21 strains of Staphylococcus aureus were isolated. Nineteen showed no increased resistance during therapy. One increased in resistance a mere one-third-fold, the other less than two-fold, but no further increases in resistance were seen.[287]

Kirby's findings as to side-effects were interesting. He saw three classes of such effects: phlebitis, drug fever,[288] and renal irritation. He found that giving the antibiotic in dilute solutions

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[285] Wise, "Management," 301.

[286] W. M. M. Kirby and C. L. Divelbiss, "Vancomycin: Clinical and Laboratory Studies," Antibiotics Annual, 1956-1957, p. 107-117. Quotation on p. 108.

[287] Ibid., p. 109.

[288] Drug fever is a non-committal term employed when circumstantial evidence suggests that such elevated temperatures are due solely to the action of the drug and not to the disease processes themselves. Proof of the reality of drug fever remains very difficult and so must be considered cautiously as a legitimate class of side-effects. Kohlsteadt, TRI, p. 23 ff.

lessened the symptoms of the first class. By 1956 and beyond, it became common either to inject vancomycin directly into the vein very slowly, thus achieving increased dilution, or to give it by intravenous drip diluted in glucose or saline solutions. That remained the common practice from then on. Even before later improvements in the purity of the dehydrated preparation, slow administration helped to lessen phlebitis and pain upon injection. In the class of drug fevers Kirby noted that all such patients presenting that syndrome had "extremely complicated illnesses."[289] Thus he was very cautious on assigning that class as a real side-effect. The renal irritation class also remained equivocal for "some patients with renal damage tolerated therapy will without signs of nephrotoxicity."[290]

About two years later Kirby had added 32 more patients to his growing trials. In his initial 15 his results had been excellent. Thus he could say of some of the 15 that the response was "excellent," or was a "prompt cure," or even "dramatic" in the case of a long-term osteomyelitic patient.[291] In the case of the 32 additional patients, Kirby noted that

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[289] Kirby, "Vancomycin," p. 115.

[290] Ibid.

[291] Ibid., Table III, p. 112-113.

> Improvements in manufacture have since [the early study] led to a more purified preparation that is much better tolerated and produces relatively few side effects.[292]

The "improvements in manufacture" referred to the Dowex purification technique, which had eliminated most of the pyrogenic substances.

Kirby again tested Staphylococcus aureus strains in vitro and all 70 types were killed by vancomycin in the usual low concentration (i.e., usually less than 2.0 micrograms/ml. of broth). And in two groups of streptococci, comprising 83 strains, all were killed by 1.0 microgram/ml.

Again results from vancomycin in the treatment of the 32 patients were impressive. The majority of the cases were "debilitated [and presented a] therapeutic challenge."[293] Of the total only eight died, four during vancomycin therapy and four later due to underlying causes. One sector of the total was of special interest, however, Kirby noted that

> The clinical results were particularly striking in the 17 patients with staphylococcal septicemia, in view of the high mortality associated with this disease.[294]

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[292] W. M. M. Kirby, et al., "Present Status of Vancomycin Therapy of Staphylococcal and Streptococcal Infections," Antibiotics Annual, 1958-1959, p. 580-586. Quotation on p. 580.

[293] Ibid., p. 582.

[294] Ibid.

Of the 17, 13 were outright cures; the deaths being due to virtually untreatable conditions. For example, one patient had severe third degree burns over the entire body and another was a debilitated 88 year-old who died of heart failure. It is interesting to note, however, that in all of the four patients who died the staphylococcal infections were clearing well prior to death.

There had been "a striking lack of toxicity and side effects" associated with the Dowex-purified material.[295] Mild phlebitis occurred with only a few patients. One reaction was notable. After two weeks of vancomycin therapy one patient went totally deaf. What was important, though, was that he had been on parenteral neomycin therapy for over a month prior to institution of vancomycin. He had a demonstrable hearing loss during the neomycin therapy. Ototoxicity eventually became such a severe problem with neomycin that is it no longer used systemically. Irreversible deafness occurred in as little as seven days of treatment and had been observed as early as 1954.[296]

It was in December of 1957 that Griffith first alerted the PDC to the problem of decreased auditory sensitive with use of vancomycin.[297] From that time on a few cases came to the attention of the

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[295] Ibid., p. 584.

[296] Kucers, Use of Antibiotics, p. 156.

[297] PDC, Series 1570-C, p. 6. Copy in the author's files.

PDC. For example, in the following February one case was reported. It was in a series of six cases of severe endocarditis in which high doses of vancomycin were given. It was typical of aminoglycoside antibiotics (streptomycin and its relatives) to cause some eighth (auditory) nerve damage, and today remains a problem.[298] Although vancomycin is generally considered as of a monotypic class, it bears much resemblance to the aminoglycosides.[299] The fact that the endocarditis patient was given high doses of vancomycin helps to explain why decreased auditory function was observed. In cases of renal insufficiency the problem was heightened. Ototoxicity remains as one of the difficulties with vancomycin's use. However, much of the hesitation of employing it because earlier literature mentioned possible eighth nerve damage may be, in part, not well founded. So significant has this side-effect been in vancomycin's history that a section later in this chapter is devoted to ototoxicity.

Kirby concluded with a sentiment which has become incorporated into more recent practice, namely that vancomycin should not be considered totally a last-resort agent. He said,

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[298] Kucers, Use, p. 116 ff.

[299] Ibid.

> In a number of instances we have withheld vancomycin until too late, hoping to eradicate a moderately severe staphylococcal infection with other antibiotics. Our tendency at present is to use vancomycin more frequently and start it earlier.[300]

An earlier quotation indicated that today vancomycin is the agent of choice where the penicillins or cephalosporins are contraindicated either because of microbial resistance or host allergy. Vancomycin under those terms is not indicated, then, merely as a last-resort effort to be avoided until no choice is left. This newer (1973) attitude has its roots in the 1950's with the work of Kirby. Evidentally during the 1960's there had been some waning interest in vancomycin's use. For in 1970, one worker stated that

> Vancomycin, because of its uniquely potent antibacterial action, has continued to be a valuable agent in the treatment of staphylococcal infections in selected patients. Moreover because of the inexorable sequence of events in the host-parasite-drug relationship [i.e., resistance], vancomycin <u>may again resume its role</u> as a very useful antistaphylococcal agent.[301]

What the basis is for the statement "may again resume its role" is not entirely clear. It must be assumed, however, that the author (who published often on vancomycin) felt that the literature of the 1960's relegated vancomycin too much to the category of last-resort

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[300] Kirby, "Present Status," p. 585.

[301] H. D. Riley, "Vancomycin and Novobiocin," <u>Med. Clin. North Amer.</u>, 54 (1970): 1277-1289. Quotation on p. 1277. Emphasis added.

agents. An examination of the literature does not support that conclusion entirely. There does remain an important point here, however. From the beginning vancomycin has been referred to as a life-saving antibiotic and, as Kohlsteadt would agree, that attitude has remained viable. Kucers, in his handbook on the use of currently available antibiotics, remains conservative in his attitude. He calls vancomycin a reserve drug for the treatment of severe staphylococcal diseases.[302] His attitude antedates the 1973 concept cited twice earlier.

By mid-1959, Kirby had added an additional 33 cases to his, by then, rather large trial. Twenty were outright cures. Six were improving, but died of underlying causes. The remaining seven were conservatively called vancomycin failures. Most of those patients had overwhelming infections when therapy was begun and they expired very shortly thereafter.[303] He noted that in over 100 patients so far treated no staphylococcal resistance was ever encountered. He also found that phlebitis, occurring when vancomycin was infused by drip technique in glucose, was no more notable than with the glucose drip alone.[304]

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[302] Kucers, Use, p. 305.

[303] W. M. M. Kirby, et al., "Treatment of Staphylococcal Septicemia with Vancomycin," New England J. Med., 262 (1960): 49-55.

[304] Ibid., p. 53.

The period preceding vancomycin's release was taken up heavily by the work of the three investigators (exclusive of Griffith) just discussed. Other workers were employing the agent also, but usually in single cases.

At one point in February of 1955 the PDC decided to hold some antibiotic in reserve for both small and large scale emergencies (e.g., natural disasters) where it could be tested on many cases at once. Several such emergencies did arise about the period of commercial release. Mrs. G.S. of Tulsa wrote Lilly thanking them for discovering vancomycin. She said that her physician gave her one change in fifty of recovering. Evidentally she was the first person to be treated by vancomycin after its formal release.[305] Just a few days prior to November 24, 1958, a man at Mt. Sinai Hospital in New York was saved by the antibiotic. This came after a midnight delivery of an emergency supply by a Lilly representative.[306]

A third incident occurred in December of 1958. A disastrous fire occurred in the Our Lady of Angels school in Chicago. One hundred vials of vancomycin were sent gratis by Lilly. This was intended for treating any occurrence of resistant staphylococci

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[305]Letter from Mrs. G.S. to Eli Lillie [sic] Co., May 14, 1959. Original in the Lilly Archives. Copy in the author's files.

[306]Letter from Dr. J.B. to then President E. Beesley [of Lilly], November 25, 1958. Copy of the author's files.

anticipated with such burn victims.[307]

Other trial uses prior to commercial availability offered several important additions to the thinking of the PDC. The only case of an anaphylactoid reaction ever to appear in the literature was reported for a known rheumatic heart disease patient.[308] The 17-year old girl developed classic signs of anaphylaxis--dyspnea, urticaria, swelling of the eyes, barely palpable pulse, etc.--ten minutes after vancomycin administration. Rapid administration of epinephrine and oxygen revived her. Within 15 minutes virtually all symptoms were gone. She later developed an allergy to novobiocin, and was already known to be allergic to the sulfonamides. Individuals known to have reactions to one drug can often be expected to have reactions to others.[309]

Others who used vancomycin during the trial period were especially alert to possible side effects. In one study with seven patients, two had phlebitis, none demonstrated ototoxicity.[310] In comparing several antibiotics to one another in toxic reactions of the

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[307] The Chicago Times, Dec. 11, 1958, p. 6.

[308] H. J. Rothenberg, "Anaphylactoid Reaction to Vancomycin," J. Amer. Med. Assoc., 171 (1959): 1101-1102.

[309] Kohlsteadt, TRI, p. 23.

[310] W. L. Wilson, "The Use of Vancomycin in Staphylococcal Infections," Antibiotic Med. Clin. Therapy, 6 (1959): 167-172.

skin, vancomycin was found to be no more toxic than the nearly innocuous penicillin.[311]

In several studies vancomycin was used in pediatrics. In one all 25 patients were considered seriously ill and of those most had a "serious or generally fatal underlying disorder."[312] Nevertheless, most were cured promptly by vancomycin. One case in particular, considering the near-moribund status of the patient, was exceptional. A six-week old boy had extensive penumonia and empyema upon hospital admission. Erythromycin and chloramphenicol therapy earlier had failed. The bacterium was sensitive in vitro to kanamycin and there was, at first, clinical improvement when it was administered. Shortly, blood cultures again became positive. In the meantime the patient developed multiple lung abscesses and pulmonary collapse. Vancomycin therapy was begun and an immediate cure was effected.[313]

The medical literature for the trial period for vancomycin contains many other case histories. Virtually without exception all report similar findings. In the vast majority vancomycin succeeded

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[311] J. C. Lawrence, "The Comparative Toxicity of Antibiotics to Skin," Brit. J. Pharmacol., 14 (1959): 167-173.

[312] H. D. Riley and N. J. Ryan, "Treatment of Severe Staphylococcal Infections in Infancy and Childhood with Vancomycin," Antibiotics Annual, 1959-1960, p. 908-916. Quotation on p. 909.

[313] Ibid., p. 913-914.

where previously tried antibiotics failed. Actual vancomycin failures, exclusive of deaths due to underlying causes, and vancomycin's having been administered too late, are quite small. Reports of phlebitis primarily, and also a certain number of ototoxic cases, are seen. In most instances vancomycin was held in reserve and so was used as a last-resort effort.

The availability of vancomycin from about 1955 onward precipitated one not unexpected response from the medical community. The antibiotic was closely compared to the other then available agents. In a period when one antibiotic after another became useless against the resistant staphylococci, a certain amount of skepticism about the efficacy of another new agent was to be expected. Just how vancomycin fared in such comparative studies was important to the establishing of its place in the antibiotic armamentarium.

6.2. Vancomycin and Other Antibiotics--A Comparison

The rise of staphylococcal resistance to antibiotics was by no means limited to the pre-1955 period (as was discussed in Chapter 4), of course. Beyond that time the resistance continued to rise. Indeed, between 1949 and 1969 the proportion of penicillinase-producers outside hospitals alone rose to about 60%.[314] After 1954,

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[314] That was true in Britain, at least. See D. J. Goldie, et al., "Changes in the Drug Resistance of Staphylococcus aureus in a

such resistance was particularly notable for at about that time bacteriophage type 80 staphylococci rapidly increased both in Europe and America.[315]

The earliest comparisons made between vancomycin and other antibiotics came about in 1956. The work was done in London, England, and included several American- and European-made antibiotics. The antibiotics erythromycin, spiramycin, cephalosporin P_1, micrococcin, albomycin, and novobiocin were tested, as was vancomycin. Two other antibiotics which were as yet not formally named were also included. Against Staphylococcus aureus vancomycin, after two hours contact in vitro, "gave the lowest survivor counts of all" the antibiotics tested.[316] The order of the antibiotics listed just above represented the order wherein the most to least resistance was demonstrated by the bacterium. Induced resistance to vancomycin increased 16-fold with some strains, but that was the highest induced resistance ever demonstrated in vancomycin's history.[317]

Non-Hospital Population During a 20-Year Period," J. Clin. Path., 24 (1971): 44-47.

[315] Ibid., p. 45. This bacteriophage type was also implicated repeatedly by Kirby and others who dealt with vancomycin.

[316] L. P. Garrod and P. M. Waterworth, "Behaviour In Vitro of Some New Antistaphylococcal Antibiotics," Brit. Med. J., 2 (1956): 61-65. Quotation on p. 63.

[317] Ibid., p. 64. This 16-fold resistance was referred to in the previous chapter.

In 1957 in Minneapolis several workers compared a dozen antistaphylococcal antibiotics. For the most part, they made the comparison by using combinations and permutations of two agents at a time. The interpretation of results, insofar as the efficacy of any given antibiotic alone was concerned, was confounded by the technique. They, nevertheless, reported that vancomycin with neomycin or streptomycin was the most bactericidal of the 12 studied.[318] The use of combinations of antibiotics seems predicated upon the fear that resistance to any single agent was sure to develop. Therefore, they immediately tested the new antibiotic in combinational form.

At the time, novobiocin was one of the most popular antistaphylococcal agents available. More recently it has been much less used against the staphylococci.[319] During the late 1950's, however, it was considered a main line of defense. In comparison to vancomycin in 1957, it was shown to cause greater induced resistance.[320] Novobiocin has been recognized to bind serum proteins, causing irreversible loss of activity. Of all antibiotics, novobiocin is

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[318] H. J. Elliott, et al., "Studies on Synergism with Twelve Antibiotics Against Thirty Hospital Strains of Staphylococcus aureus," J. Lab. Clin. Med., 50 (1957): 242-249.

[319] Kucers, Use, p. 233.

[320] L. A. Rantz, et al., "The Effects of Vancomycin, Oleandomycin, and Novobiocin on Staphylococci in Vitro," Antibiotics and Chemotherapy, 7 (1957): 399-409.

among the most highly bound.[321] Other researchers tried novobiocin and vancomycin in combination in 1957 too, and found no synergism to occur. Moreover, it was "shocking to discover" the rate at which resistance built-up in the presence of novobiocin.[322] They recommended it be used only rarely. Nevertheless, from 1955 to 1958, there were approximately 28 publications reporting on more than 500 patients treated with novobiocin with a cure-rate of 94%. But severe septicemias were virtually never cured.[323] It is in just such cases that vancomycin has done exceptionally well.

Oleandomycin was available at this time and was used in combating the staphylococci. A macrolide antibiotic, it was not much different from erythromycin, and must less active. They were shown to be almost exactly alike in developing cross-resistance, which occurred in as little as 48 hours after initiation of treatment. Oleandomycin has not survived the test of time, and although still

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[321] Kucers, Use, p. 231.

[322] E. Jawetz, et al., "The Participation of Novobiocin and Vancomycin in Combined Antibiotic Action Against Staphylococci," Antibiotic Med. Clin. Therapy, 4 (1947): 40-44. Quotation on p. 42.

[323] M. Finland (moderator), et al., "The Current Status of Erythromycin, Kanamycin, Novobiocin, Oleandomycin, Ristocetin, and Vancomycin, With Particular Reference to Their Use in Staphylococcal Disease," Antibiotics Annual, 1958-1959, p. 1050-1072. Citation on p. 1051-1052.

available "it is doubtful it has any place in current therapeutics."[324]

At least two other antibiotics were investigated intensively and compared to vancomycin in the 1950's. They were kanamycin and ristocetin. Antibiotics available in the 1950's, such as erythromycin and the penicillins, had not lost popularity when resistance was not a consideration.

By 1958 kanamycin had been available in the U.S. for approximately ten months. It had been shown to have a very broad spectrum but was very toxic to the host, and resistance was a problem. Within a few years of its introduction, a 30% rise in resistance was observed.[325] One example of kanamycin-resistance (and exemplary of that of many other antibiotics) was particularly striking. S.S., a 62-year old man with a genital infection, was treated unsuccessfully with chloramphenicol. Two days later penicillin and streptomycin were added, but a day after that a fulminating staphylococcal enterocolitis developed. Kanamycin and erythromycin were used to no avail, for within four days resistance had been induced by both of them. The organism was resistant to penicillin, streptomycin, tetracycline, novobiocin, nitrofurantoin, chloramphenicol, bacitracin,

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[324] Kucers, Use, p. 226.

[325] S.M. Finegold and D.W. Gaylor, "Enterocolitis Due to Phage Type 54 Staphylococci Resistant to Kanamycin, Neomycin, Paromomycin, and Chloramphenicol," New England J. Med., 263 (1960): 1110-1116.

paromomycin, and neomycin. Treatment by vancomycin was begun and three days later the man was asymptomatic and remained so.[326]

The one antibiotic of the period that was most comparable to vancomycin was ristocetin. During an antibiotics symposium held in 1958, at least a half-dozen papers were read wherein ristocetin was shown to have cured resistant staphylococcal infections.[327] Ristocetin can be compared to vancomycin in many ways. Like vancomycin it had two major fractions (A and B), the chemical structure of which is still being investigated. It was highly bactericidal, though less so than vancomycin. For instance, where vancomycin killed 12 of 13 strains tested, ristocetin killed only one.[328] It is an antibiotic of high molecular weight like vancomycin. Resistance did develop to it, but slowly. The side-effects, however, presented a severe problem. It is nephrotoxic, and especially damaging to the hematopoietic system. So severe were its side effects that by 1968 it had been withdrawn from the American market.[329]

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[326] Ibid., p. 1112 and p. 1115.

[327] Cited in Finland, "Current Status," p. 1957.

[328] Cited in H.D. Riley, "Vancomycin and Ristocetin," Ped. Clin. N. Amer., 8 (1661): 1073-90. See p. 1081.

[329] E. Jawetz, "Polymixins, Colistin, Bacitracin, Ristocetin, and Vancomycin," Ped. Clin. N. Amer., 15 (1968): 85-94. See p. 92.

During the 1960's, several other antistaphylococcal agents came into general use. The older antibiotic, bacitracin, available since 1946, was used considerably for severe systemic staphylococcal infections up to about 1960. Because of its extreme toxic effects it was almost totally superceded by vancomycin.[330] It has, however, continued to be useful for topical applications.

At about 1960 methicillin, a semi-synthetic penicillin, became available. It has been used intensively in Europe and like its parent penicillin, can be resisted by many staphylococcal strains. The problem became acute in Europe during the late 1960's. In Denmark, 10% of all bacteremias caused by Staphylococcus aureus were methicillin-resistant; in Zurich, Switzerland, the resistance climbed from 9.7% in 1965 to 16.1% in 1967.[331] However, all 66 strains tested in Zurich in 1968 were vancomycin-sensitive. By 1972 from 30% to 55% of all Staphylococcus aureus infections in Zurich hospitals were caused by methicillin-resistant forms.[332] Resistance to other antibiotics was high there, as well. Chloramphenicol resistance went

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[330] Ibid., p. 91.

[331] E. J. Benner and F. H. Kayser, "Growing Clinical Significance of Methicillin-Resistant Staphylococcus aureus," Lancet, 2 (1968): 741-744.

[332] F. H. Kayser and T. M. Mak, "Methicillin-Resistant Staphylococci," Amer. J. Med. Sci., 264 (1972): 197-205.

from 17% (in hospital) in 1965 to 36% in 1970-71, and in outpatients it increased from 0% to 9% during the same period.[333]

Similar reports come from as diverse areas as Portland, Oregon and Paris, France. Of 22 strains isolated by the Portland workers from such places as England, Columbia (South Carolina), Houston, and New York City, all were methicillin-resistant. None were vancomycin-resistant.[334] In Paris, in 1973, all of the 31 strains tested were methicillin-resistant. In addition, 20 of those strains were resistant to a cephalosporin alone, or to a cephalosporin-aminoglycoside combination. No strain was vancomycin-resistant.[335]

The problems of staphylococcal resistance in the 1940's and earlier 1950's remained a problem after vancomycin came into use. The majority of strains of Staphylococcus aureus treated are sensitive to one or more members of the penicillin and/or cephalosporin antibiotic classes. Only a certain percentage of staphylococcal strains, are resistant to essentially all antibiotics. It has been for these resistant strains that vancomycin has been required. Among such

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[333] Ibid., Table I, p. 198.

[334] E. J. Benner and V. Morthland, "Methicillin-Resistant Staphylococcus aureus," New England J. Med., 277 (1968): 678-680.

[335] J.F. Acar, et al., "Methicillin-Resistant Staphylococcemia: Bacteriological Failure of Treatment with Cephalosporins," Antimicrobial Agents and Chemotherapy, 1970: 280-285 (volume numbers not in use in this journal prior to 1971).

difficult cases for treatment, one group stands out as especially notable. Bacterial endocarditis, both staphylococcal and streptococcal, has long been a difficult disease to treat.

6.3. Bacterial Endocarditis

> Infective endocarditis was long regarded as one of the great diseases by such eminent clinicians as Osler and Thayer because of the challenge of diagnosis. Despite major technical advances in many areas of cardiology, the challenge now [1967] is as baffling as it was, and in many instances, the presence of endocarditis is not detected until exceedingly late in the course of the illness, if at all. When unexpectedly exposed at post-mortem examination, its presence remains one of the major therapeutic tragedies.[336]

When it is considered that 50% of acute bacterial endocarditis is caused by Staphylococcus aureus, most of which are resistant, the full impact of the sentiments expressed above becomes clear.[337] Furthermore the prognosis is for well above 50% mortality in staphylococcal endocarditis.[338] It is not surprising, then, that any new antistaphylococcal agents would be immediately applied to cases of endocarditis. That was true for vancomycin.

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[336] P. A. Tumulty, "Management of Bacterial Endocarditis," Geriatrics, 22 (1967): 122-129. Quotation on p. 128. The use of the term infective was preferred by Tumulty since fungi as well as bacteria may be indicated in cases of endocarditis. (Vancomycin is ineffective against fungi.)

[337] Ibid., p. 123.

[338] Ibid., p. 137.

The first recorded individual with staphylococcal endocarditis treated with vancomycin was a patient of Geraci's in early 1956. A 71-year old man had undergone amputation of both legs. He was known to have a staphylococcal infection, was acutely ill, being toxic, tremulous, stuporous and almost comatose. Several of the classic signs of endocarditis were present, i.e., retinal hemorrhages, heart murmur, and finger lesions. Treatment with vancomycin was commenced with the first report of positive blood cultures. Geraci reported that "the results of the therapy were impressive."[339] Within a few hours the tremulousness and toxicity disappeared and the temperature began to drop. In three days a lasting cure had been effected.

By 1960, Geraci had treated 22 patients with staphylococcal endocarditis, over three and a half years. Of these, 18 were treated with vancomycin. The first six had been discussed in the literature by April of 1958, and, by June of 1960, an additional 12 had been treated. Geraci's first patient has been discussed above. In the remaining five of that first group of six, the staphylococci varied in response to eight different antibiotics. But unlike the over 50% resistance to all antibiotics (excepting vancomycin) seen in 1967,

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[339] Geraci, "Some Laboratory Experiences," 579.

a decade earlier Geraci found resistance to be 30% to 50%.[340] Though in vitro sensitivities were encouraging for various antibiotics, cures were not forthcoming with most of his patients without recourse to vancomycin. In all patients treated by Geraci the incidents of side effects due to vancomycin were small. In one case a perceptible hearing loss was observed. All patients suffered varying degrees of phlebitis.[341]

Acute staphylococcal endocarditis, caused by broadly resistant organisms, was a 100% fatal disease prior to the advent of vancomycin.[342] The picture was altered sharply after the appearance of vancomycin, as has been outlined above. Though 50% of staphylococcal endocarditis is caused by Staphylococcus aureus, most of the remaining 50% is caused by Streptococcus viridans.[343] Vancomycin in use against the streptococci has also been successful.

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[340] J.E. Geraci, et al., "Antibiotic Therapy of Bacterial Endocarditis. VII. Vancomycin for Acute Micrococcal Endocarditis," Proc. Staff Meet. Mayo Clin., 33 (1958): 172-181.

[341] J.E. Geraci and F.R. Heilman, "Vancomycin in the Treatment of Staphylococcal Endocarditis," Proc. Staff Meet. Mayo Clin., 35 (1960): 316-326.

[342] W.M.M. Kirby, "Vancomycin," in Antibiotic Therapy for Staphylococcal Diseases, ed. by H. Welch and M. Finland (New York: Medical Encyclopedia Inc., 1959), p. 123-137. See p. 133 herein and Kohlsteadt, TRI, p. 16.

[343] Tumulty, "Management," p. 123.

Streptococci have never shown resistance of the magnitude of that in the staphylococci as was mentioned in Chapter 4. Therefore, in most instances, penicillin or penicillin and streptomycin would effect cures for these endocarditis infections. Other less notable causal agents of endocarditis, penumococci, meningococci, gonococci, and Streptococcus pyogenes, are usually penicillin-sensitive.[344] For that reason streptococcal infections seldom require treatment with vancomycin.

As has been discussed for a patient allergic to penicillin, vancomycin is a viable alternative in any type of endocarditis infection. Kirby reported on such a case in 1958. A patient with Streptococcus viridans endocarditis was given penicillin and developed a severe drug reaction. On being given vancomycin an immediate improvement was seen. Blood cultures became negative overnight and a lasting cure was soon effected.[345] This was the only case recorded in the literature prior to 1968.

The use of vancomycin in such cases has become increasingly more common in very recent years. In 1968, six patients were

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[344] J.E. Geraci, "The Antibiotic Therapy of Bacterial Endocarditus: Therapeutic Data on 172 Patients Seen From 1951 Through 1957: Additional Observations on Short-Term Therapy (Two Weeks) for Penicillin-Sensitive Streptococcal Endocarditis," Med. Clin. North Amer., 1958, p. 1101-1140.

[345] Kirby, "Present Status," p. 583.

treated with vancomycin for either Streptococcus viridans (two individuals) or enterococcal endocarditis (four individuals).[346] Vancomycin was used either because of penicillin allergy or because of an organism's penicillin-resistance. The disease is usually 100% fatal even if treated by bacteriostatic antibiotics to which the causal agent is sensitive. A bactericidal agent is needed. Only a few of all known antibiotics are clearly bactericidal in low dosages.[347] Of the six patients, vancomycin cured them all.

In 1969, clinicians immediately began to recommend vancomycin as the drug of choice where warranted, such as the cases discussed above.[348] In one year the idea was firmly planted and expanded upon. Investigators discovered that a synergism existed between vancomycin and streptomycin. This combination was especially efficacious in stubborn cases of enterococcal endocarditis. Testing of 20 strains of enterococci, in vitro showed streptomycin alone to be totally ineffective. Vancomycin was bacteriostatic for all strains and bactericidal in low concentration for many. However, the

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[346] C.K. Friedberg, et al., "Vancomycin Therapy for Enterococcal and Streptococcus viridans Endocarditis," Arch. Int. Med., 122 (1968): 134-140.

[347] Ibid., p. 138.

[348] G.O. Westenfelder and P.Y. Paterson, "Life-Threatening Infection: Choice of Alternate Drugs When Penicillin Cannot Be Given," J. Amer. Med. Assoc., 210 (1969): 845-848.

combination of the two agents gave greatly improved results over vancomycin used alone.[349]

Both agents alone have established reputations of producing toxic side-effects, streptomycin being by far the more toxic. Thus it was not surprising that there was hesitation in using the two antibiotics in combination in man. The first case treated was reported in 1973. A 61-year old woman presented classic signs of microbial endocarditis and was acutely ill upon admission to the hospital. Penicillin G therapy was begun. Seven days later her symptoms were more severe. Some hearing loss had already occurred so streptomycin, which is quite ototoxic, was withheld. Ampicillin (a semisynthetic penicillin) replaced the penicillin G therapy. Three days later, her condition had further deteriorated. Penicillin G and streptomycin were then instituted, followed by further deterioration. Finally vancomycin-streptomycin therapy was initiated. Within a few days all symptoms had vanished and no ototoxicity was detectable. A lasting cure had been effected.[350]

With the advent of vancomycin, severe Staphylococcus septicemias, including endocarditis, present a lessened threat.

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[349] G. L. Mandell, et al., "Synergism of Vancomycin and Streptomycin for Enterococci," Amer. J. Med. Sci., 259 (1970): 346-349.

[350] G. O. Westenfelder, et al., "Vancomycin-Streptomycin Synergism in Enterococcal Endocarditis," J. Amer. Med. Assoc., 223 (1973): 37-40.

Certain side-effects have been repeatedly observed when vancomycin has been employed. Phlebitis has never been totally eliminated, but does not present a serious impediment to therapy. The only well-documented side-effect that has given the medical community reason for some concern has been ototoxicity.

6.4. The Question of Ototoxicity

The aminoglycoside antibiotics (e.g., streptomycin, kanamycin, gentamycin, neomycin, framycetin, and paromomycin) and the somewhat similar vancomycin are indicted most often in ototoxic phenomena.[351] Generally they destroy cochlear hairs, causing hearing loss, or destroy the neuroepithelium of the cristae of the semicircular canals, causing ataxia and balance problems. Vancomycin has never been indicted in the latter difficulty, but only in connection with hearing loss. Most of the highly ototoxic aminoglycosides are poorly absorbed by the gastrointestinal tract. They are excreted primarily through the kidney route. It is in cases of impaired renal function that ototoxicity becomes a threat. Many drugs are excreted via the kidneys and if kidney function is impaired, blood levels of such drugs rise. This rise can reach such a point that damage can be caused, especially in the region of the ear.

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[351] J. W. Walike and J. M. Snyder, "Recognizing and Avoiding Ototoxicity," Postgraduate Med., 52 (1972): 141-145.

Vancomycin was early demonstrated to be cleared from the body by the kidney. Griffith did quantitative studies on concentrations of vancomycin in the urine. A 100 milligram dose of the antibiotic was given and blood and urine concentrations were monitored. At six hours post-administration the blood level was 0.8 micrograms per milliliter, the urine level was 102.4 micrograms.[352] This indicated rapid removal by the kidney. During the earliest years, the average or usual dose[353] was agreed to be approximately two grams per day for optimal bactericidal activity. Body weight was evidently not considered in setting the dosage (but see below). The blood level should be one to four micrograms per milliliter for best performance. Such levels are accepted for most applications of vancomycin today.[354]

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[352] Griffith, "Vancomycin III." p. 621.

[353] Kohlsteadt considers the use of the term usual dose to be tantamount to nonsense. Insufficient doses do not get cures and/or may lead to increased resistance to the antibiotic (excepting vancomycin) being administered. The term usual dose is "from the standpoint of [both] the physician and pharmacist . . . absolutely meaningless" (TRI, p. 12). If a disease process requires a higher than usual dose, then the physician must give it or look for a replacement agent. There are, of course, legal implications. For example, if a clinician gives higher than the usual dose and theretofore expectable side-effects do, in fact, occur in the patient the physician may be liable. If in the first place the term dosage range was used (as Kohlsteadt has suggested: TRI, p. 12), such difficulties may well have been prevented.

[354] Kucers, Use, p. 304.

The matter of serum levels and ototoxicity in regard to vancomycin presents an intriguing historical problem. Reluctance of physicians to use vancomycin immediately in treatment, as has occurred often throughout its history, may not be entirely well-founded; that is, if fears of possible ototoxicity are alone the deciding factor. An examination of attitudes and data on this topic over time presents a fascinating picture.

Renal damage per se had never been conclusively associated with vancomycin, although it has been associated with bacitracin, the polymyxins, neomycin, and kanamycin.[355] If renal insufficiency was present, ototoxicity would be expected with vancomycin. That has been noted occasionally above. Nevertheless, with the proper control, vancomycin can be successfully employed in cases of established renal insufficiency. Such a case occurred rather early (1960) in vancomycin's history. Two clinicians in Ireland showed that treatment was "effective and relatively simple."[356] In a ten-year old boy with acute pyelonephritis, the agent, Staphylococcus aureus, was resistant to all antibiotics, except vancomycin and three others. Two

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[355] C. M. Kunin and M. Finland, "Restrictions Imposed on Antibiotic Therapy by Renal Failure," Arch. Int. Med., 104 (1959): 1030-1050.

[356] I. R. Wallace and N. A. J. Carson, "Staphylococcal Septicemia Treated With Vancomycin in the Presence of Chronic Renal Failure," Lancet, 1 (1960); 519-520.

of those (erythromycin and chloramphenicol), being only bacteriostatic, were found ineffective after six days of treatment. Vancomycin was substituted. The physicians realized that

> Because of the boys poor renal function, control of the vancomycin dosage by serum assay was essential to avoid dangerously high serum levels.[357]

The researchers thus developed a simple, daily microbial assay that indicated a bactericidal concentration was being maintained at the dosage level being employed. Vancomycin was given with a slow glucose infusion over four hours each day. Initially, the clinicians thought they were underdosing the boy and so increased the dosage on the third day. Results indicated that the initial dosage was sufficient. Indeed, the increased dosage was potentially ototoxic.[358] No further vancomycin was needed for three days as the level remained bactericidal. Over ten days, less than one gram of vancomycin was needed to eradicate the stubborn infection. No ototoxicity was demonstrable.

By 1966, a specific dosage schedule had evolved, that would maintain effective serum levels. Six individuals on regular hemodialysis were studied. Since vancomycin is not lost across the dialysis membrane, levels could be maintained for long periods with initial

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[357] Ibid., p. 519.

[358] A potentially ototoxic level of 70-100 micrograms per milliliter of serum was established by Geraci in 1958.

small doses. The investigators found that the serum level of the minimal inhibitory concentration against their strain of Staphylococcus aureus was reached in 24 hours in normal patients. However, it took 21 days to obtain that level in oliguric individuals.[359] No ototoxicity resulted in any of the cases.

It was not until 1971 that the potential threat of ototoxocity using vancomycin could be clearly diminished. Whether renal insufficiency exists due to damage to the kidney, or to decreased fluid intake, ototoxicity can result.[360] Proof was wanting that extensive use of vancomycin over much time and with many individuals, was necessarily an ototoxic threat. True, if levels rise in the serum it can be a real threat, but if levels are monitored in any individual with or without renal damage there is no reason that vancomycin need be toxic.

A study done in 1971 must be regarded as a milestone in vancomycin's history. The large set of data obtained indicate just how safe vancomycin can be. Twenty-five patients were on regular hemodialysis for a total of 20 patient-years. The occurrence of occasional infections of Staphylococcus aureus would be expected at

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[359] D. D. Lindholm and J.S. Murray, "Persistence of Vancomycin in the Blood During Renal Failure and Its Treatment by Hemodialysis," New England J. Med., 274 (1966): 1047-1051.

[360] Kohlsteadt, TRI, p. 15.

the site of the hemodialyzer-vein communication (i.e., shunt). Such infections did not occur, for those patients were on continuous prophylactic treatment with vancomycin. Six of the 25 had bactericidal serum levels for 600 consecutive days and one for 730 days. Not one instance of any side-effect, ototoxic, phlebitic, or otherwise was observed in those 20 patient-years.[361] Indeed, the researchers found that

> In contrast to the aminoglycosides, the ototoxicity of vancomycin does not appear to be a function of total dose or duration of therapy. As much as 54 grams have been administered over a two year period to patients in the present study without deterioration of auditory acuity as measured by audiometry.[362]

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[361] A. J. Morris and R. T. Bilinsky, "Prevention of Staphylococcal Shunt Infections by Continuous Vancomycin Prophylaxis," Amer. J. Med. Sci., 262 (1971): 87-92.

[362] Ibid., p. 91.

7. CONCLUSIONS

7.1. Questions Examined and Suggested by the Dissertation

In the Introduction to the dissertation several questions were posed. These included the following:

1. Was there a trend in the progress of the history of chemotherapeutic medicine related to antibiotics which had both a definable beginning and a definable end?
2. Are the characteristics of the pattern of discovery and development such that one could actually define the period of 1940-1960 in antibiotic history?
3. Can the characteristics which seem so similar in the growth of a wide variety of antibiotics be used as elements to define a historical period?
4. Is any such definition evolved peculiar to a certain time-span and no other?

For convenience these four questions are condensed into one more manageable general question: Was there a discovery era in the history of antibiotics, and can it be defined.

These same four questions were set as integral to the thesis of this dissertation. A closer examination of each question will support the proposition that in fact a definable era did exist. As answers are forthcoming regarding these four questions, more will be generated

concerning the discovery era.

In answer to the first question--was there a trend in the history of chemotherapeutic medicine which had a definable beginning and end--several points are raised.

Prior to the advent of penicillin, various agents were discovered which were antibiotics in the sense of Waksman. In the two volume work Antibiotics (cited earlier), Flory and his colleagues devoted a long and well-documented chapter on the history of antibiotics of the pre-penicillin era. They limited themselves to the true antibiotics (as has been done in the present discussion) and avoided such agents as mercurials and other therapeutic agents with known antimicrobial effects. Their findings indicate very strongly the lack of any research trend in antimicrobial medicine prior to that inaugurated at the discovery of penicillin. Incidental discovery of selective or wide-spectrum kills of infectious microorganisms by specific chemical compounds never resulted in a period which can be clearly circumscribed as an era.

Neither the findings of Pasteur of antibiotic action in the 1870's or the findings of Ehrlich in chemotherapeusis at the turn of the century ever led to a viable new period in chemotherapeutic medicine. This was true of all other substances investigated during this century, even including the sulfonamides. As useful as these agents have been, a new era in chemotherapeutics was not inaugurated by their

discovery in the mid-1930's. Perhaps this is so because they have consistently remained limited in application due to their inherent toxicity. Further, a massive burgeoning in medical-industrial technology was not initiated by them as was true with the antibiotics. Even by the early years of the penicillin period (late World War II), the sulfonamides had not gained the immense popularity seen with penicillin soon after its availability (see Chapter 2). One could not argue that the true antibiotics displaced (by their appearance) a possible new era of sulfonamide medicine. The sulfonamides, due to their toxicity (especially in comparison to the antibiotics), would not likely have ever become so integral to therapeutic medicine. Over the past four decades since their introduction, the sulfonamides remain limited in application.

Examination of the literature concerning the treatment of bacterial diseases prior to the era in question indicates clearly that such maladies remained essentially untreatable. Mercurials and arsenicals were doing much to combat trypanosomiases and spirochaetoses, but were ineffective against eubacterial diseases. The inauguration of the discovery era, then, set a trend in the progress of chemotherapeutic history. This was true because widespread, and heretofore untreatable bacterial diseases were finally curable in the vast majority of instances.

Several questions are raised by the fact that the discovery era could not have begun before 1940. The most obvious question is why the era did not begin about 1928 when Fleming discovered penicillin? There are several possible answers to this. In the first place Fleming's discovery was not nearly so spectacular as it later was made out to be. One is wont to speculate why he shared the Noble Prize with Florey (who was included only late in consideration for the award). This is all the more interesting when one considers that Tyndal had observed the same phenomenon a half-century earlier. Florey's biographer made it clear by citing conversations between Florey and Fleming that even in 1939-1940 the latter did not realize the singular significance of penicillin. Fleming recognized his limitations in the knowledge of chemistry, but did not study that subject further so that he could devote himself to penicillin research. Florey, on the other hand, steeped himself in chemical studies when he realized his own shortcomings in that subject. This he did for the very reason that he wished to study chemotherapy in greater depth.

A second possible answer is that the state of biomedical technology did not permit the possibility of opening the antibiotic era in 1928 as it did in 1940. Conover's statement that "by 1940" the state of the art allowed certain things to occur. Did Conover suggest that one or more crucial technologies were not extant before 1940? He did not elaborate upon this and the point is worthy of further research to

make it even more clear why 1940 was such a germinal year.

A second question posed when one dates the ending of the era as 1960 is as follows. Was the fact that all major antibiotic chemical families were known by 1959 the sole reason the era closed? Or was the fact that semisynthetic agents just becoming useful then were of equal import in closing the era? It may well be that the close of the era was due to a variety of factors. This is certainly open to further research.

The second question concerning the characteristics of the era pattern requires discussion. The growth (discovery and especially development) of several antibiotics bespeaks of the existence of a pattern. Penicillin was the precedent and in retrospect it can be seen just how true this was. The development of virtually all other antibiotics followed the pattern set by the evolution of penicillin. Hare and Bickel, in their respective histories of penicillin, document this pattern. An examination of the historical development (via the technical literature) illustrates how development of other discovery era agents parallels the pattern set by penicillin.

Of course, definite deviations from pattern fit must be anticipated in each post-penicillin agent. Each is a different chemical compound and must be handled according to its own peculiarities. Vancomycin, for example, is not entirely known chemically. Penicillin has been well-defined chemically for a long time. The

producing organisms is another consideration also. Penicillin, as precedent, in several ways differs somewhat from most other discovery era antibiotics. It is produced by a true fungus, most other agents by actinomycetes. Waksman found mutagenesis efforts, so effective with Penicillium very difficult with Streptomyces.

Deviations from the pattern, however, are inconsequential when compared to the similarities shown by the history of various agents. Vancomycin, in its fit to the overall pattern, functions well as a microcosm of the greater universe of antibiotics discovered during 1940-1960.

Probably the most tantalizing unanswered question is, how closely do all discovery era antibiotics fit the era pattern? As we have explored vancomycin's history deeply, it would be desirable to do the same with each of the remaining successful antibiotics of that era. If it would be possible to have access to corporate records for the developmental phases of aureomycin, streptomycin, terramycin and many others, would it be found that the era pattern is even more predictable than suggested here? Certainly the technical literature concerning each agent suggests this. In order to achieve a greater understanding of the factors operative in antibiotic history during 1940-1960 then a thorough history of each antibiotic is desirable. In this way it would be possible to achieve a greater accuracy in defining and understanding this vital historical period.

The third question cited above asks if era pattern characteristics can be used as elements of definition. Given what has been discovered during the course of this research, the answer is yes.

Conover's statement on the state of the art of antibiotic technology in 1940 has led this author to examine the entire discovery era in that light. But to construe Conover's assessments as a definition would be simplistic. Many factors besides technology must be considered in presenting a definition of a historical period. The mechanisms and results of human decision-making and the machinations of individual researchers are essential elements in defining an era. It would be difficult to include factors such as willingness to bear great financial indebtedness in developing an antibiotic in a definition without examining the corporate decision-making process first-hand. Conover did not propose a definition. He did, however, provide one framework by which to examine further evidence and to eventually devise one. The elements given by him plus certain others discovered during the study of the development of the history of vancomycin and other agents help provide a definition.

As remarked above, a most desirable avenue would be to have access to corporate research and development records for each major antibiotic's history. The points raised by this question of definition elements remain possibly the most difficult of assessment. Do corporate decisions for all antibiotic developmental histories follow a

somewhat predictable pattern, or do they differ radically. Certainly the published technical literature suggests in a way that they do. For example, to abandon soybean meal as a nitrogen source in favor of some other source for both vancomycin and aureomycin (and others?), is a reflection of decision-making. Is it based on knowledge of a pattern formed during the early 1940's? Was the decision made at the corporate management level or the research laboratory level? What role does the published technical literature have on those individuals making decisions? How much inter-corporate discussion goes on? The only answer to these and similar questions will come when it is possible for the historian of antibiotics to examine corporate records as has been done for vancomycin's history.

A fourth question was posed. That is--Is the definition peculiar to a certain period? The answer is yes, in most respects. Of course, antibiotic discovery and development did not cease in 1960. What did cease was the discovery of any further new chemical families of antibiotics. Evidence for this has already been given in the introduction.

What remains to be answered is this. If the era ceased, but antibiotic medicine is still a major area of medical practice, what superceded the discovery era? It is always difficult to deal with recent history. In this case it is especially true because current technical literature does not hint at the close of an era which may be

supposed to have begun in about 1960. The genesis of the era which began then can be defined when the close of the discovery era is more intensively studied. It does not seem unreasonable to assume that when one era closes another opens. Whether it is reasonable to attempt a definition for any era beginning about 1960 does not seem as wise. It must first be shown that such an era can be fully circumscribed. Evidence in the modern technical literature does not suggest that that is possible.

7.2. The Antibiotic Discovery Era--A Proposed Definition

All published material examined concerning the 1940-1960 period in antibiotic medicine suggests that certain distinctions can be made about it. When these distinctions are taken together they provide a definition of the era.

Returning to Conover's statement, it will be seen that he gave an approximate definition for the antibiotic discovery era. After careful examination of the histories of several well-established antibiotics and the intensive look at one in particular, a full definition of the discovery era is given.

The discovery era in antibiotic medicine can be characterized as follows:

By the initiating period of the era (1940) certain facets of technological and industrial management development allowed for

a. Facile collection of a wide variety of microbial types from nature, especially from soils, and

b. Isolation and characterization, of not only morphologically but biochemically (physiologically) disparate groups of microorganisms, and

c. Demonstration of antibiotic potential, most commonly by team approach, within a given industrial firm capable of all aspects of production of chemotherapeutic agents, and

d. Employment of several significant techniques including massive sampling programs and sophisticated biochemical testing capable of selecting for desirable variations in active fractions of naturally-occurring compounds, and

e. Willingness to expend very large sums of money on research and development, and

f. The capacity (through law) to employ living organisms in test situations, even in apparent incurable human ailments, and

g. Use of widely varying microbiological methods (mutagenesis, strain improvement, phage manipulation) to attain high-producing strains of microorganisms, and

h. The development of industrial-scale production of naturally occurring agents by techniques previously unknown or untried, such as submerged fermentation, and in recovery the precipitation and ion exchange of the product.

By the close of the era the following conditions ceased to be of primary import:

a. Massive sampling programs, and

b. Need for extensive strain selection where this is intended to avoid production of unwanted fractions, and

c. Dependence upon only naturally-occurring compounds as the sole source of antibiotics.

The beginning of the next era in the history of antibiotic medicine was characterized by

a. Employment of all aspects of the discovery era excepting the three noted immediately above, and

b. Initiation of production of semi-synthetic antibiotics produced by molecular manipulation of members of known chemical families of antibiotics.

This later era began about 1960 and may still be in effect.

BIBLIOGRAPHY

The Bibliography has been divided into two sections: Critical and General. The Critical section includes annotated milestone papers (e.g. announcement of penicillin's discovery), popular histories of antibiotics, and scholarly histories of antibiotics (there are very few of these).

The General Bibliography includes all materials cited in the footnotes and in the Critical Bibliography.

CRITICAL BIBLIOGRAPHY

Abraham, E.P. and E. Chain. "An Enzyme from Bacteria Able to Destroy Penicillin." Nature, 146 (1940): 837.

This short letter to the editor is the first indication in print of known activity in bacteria against a given chemotherapeutic agent. Although Ehrlich was aware of acquired immunity on the part of trypanosomes against his Salvorsan he did not clarify any mechanism for this action. Later (i.e. after 1940) bacterial resistance to antibacterial therapy increased in importance, though the mechanism was not always enzymatic.

Baldry, E.I. The Battle Against Bacteria: A History of the Development of Antibacterial Drugs, for the General Reader. The University Press, 1965.

Among all the classes of books on this topic, Baldry's work is one of the best. Its title is certainly more informative than most and "the general reader" can tell it is meant for him. It is without citations and bibliography, but in that it is short (99 pages) and very well written it is still to be recommended. The author stays very much within the limits suggested by the title. There is no pretense of erudition nor masking of the simpler aspects of this fascinating history by overuse of medical vernacular (Baldry is a physician). In the sections on penicillin and streptomycin the accuracy is clearly apparent. For instance, in the section on streptomycin there can be no doubt that Baldry read Waksman's autobiography (My Life With the Microbes) and condensed its essentials into a few readable pages. There are a few plates, and they are well reproduced. Doehle's 1889 photograph of antibiosis on a Petri plate (the first such photo ever taken) and a portrait of Ernst Chain are especially attractive. The book is well worth the investment of the small mount of time necessary to read it.

Bickel, Lennard. Rise Up to Life: A Biography of Howard Walter Florey Who Gave Penicillin to the World. London: Angus and Robertson, 1972.

Without doubt this very new book, which is certainly a biography of Florey, is much more than that; it is the single most comprehensive study of the history of penicillin. Without malice, the author throughout the text dethrones Fleming and gives the reader a much more believable hero for giving penicillin to the world. In exciting narrative Bickel leads one through each and maybe all the fine, intricate and very complex details of the story of penicillin. It goes from Florey's

involvement in 1938 to penicillin's availability to civilians nearly a decade later. The role of Mary Ethel Florey is clearer here than in any other source, as well as clarification of the impact the war had upon the whole project. Indeed this latter point gives more structure to the story than has heretofore been realized in other penicillin histories. The sole outstanding drawback is the author's reluctance on citing all sources. This is a severe handicap. Yet no other history of penicillin can even come near to being so complete.

Böttcher, Helmuth. Wonder Drugs: A History of Antibiotics. Translated by Einhart Kawerau. Philadelphia: Lippincott, 1964.

If one is to keep pure the definition of the term antibiotic as defined by Waksman (see Chapter Two in this dissertation) then this book is not a history of antibiotics. Only one-fifth of the book's pagination is devoted to the true antibiotics and within the story of the discovery of penicillin major errors are to be found (compare to Hares' more accurate history). The work should be entitled as a history of folk medicine and chemotherapy. Beginning with Isis' treatment (by micturation) of Horus' supposed malaria (inflicted by Anopheles flies [sic]), the author leads the reader through the treatment of diseases in the ancient world. The Babylonians, Jews, Incas, east Indians and others are discussed. The style is often somewhat poetic, but the work is totally without citations or bibliography. If the inaccuracies seen in the penicillin story are at all indicative of the book as a whole the reader must be cautioned in drawing from it anything which he feels is surely factual.

Chain, E., et al., "Penicillin as a Chemotherapeutic Agent." Lancet, 239 (1940): 226-228.

This short paper is the first announcement on the great possibilities of penicillin emanating from Florey's "Oxford team." Chain's name appears first as Florey always chose to have the authors appear in alphabetical order. It is a typical paper describing a subject of science to readers of science. Several historical paragraphs begin it, a table showing therapeutic effects on mice is included with a discussion on animal trials, and it is ended with a conclusion attesting to its efficacy in animals. No hint, hope, or suggestion is made regarding human treatment. It is cool and scientific and gains increased significance through the eyes of the historian more than, we may presume, those of the then contemporaries.

Duthie, Edward S. Molecules Against Microbes. London: Sigma, 1946.

This member of the Sigma Introduction to Science series is just that, an introduction. It is usually a simple, sometimes complex description of the state of the art of chemotherapy in 1946. It whisks its readers from C-stands-for-carbon to the intricacies of condensed aromatics within a mere eight pages. The historical statements regarding the history of bacteriology are very uncritical. Fifty-three of its 156 pages are devoted to protozoan afflictions (principally tropical). The final chapters on future prospects has a good deal of history in it that one would expect in a first chapter. At the end of the text there occurs a list of Landmarks in Chemotherapy which is the most useful part of the book for a modern reader in chemotherapy. The book probably served well in 1946, but now must be ranked low on the reading list for the historian of chemotherapy.

Epstein, Samuel, and Beryl Williams. Miracles from Microbes: The Road to Streptomycin. New Brunswick: Rugers Univ. Press, 1946.

Unlike its subtitle might suggest, this book gives only a sixth of its pagination to streptomycin. Of its five chapters the first two are especially useful mentioning many early, seldom remembered attempts in antibiosis. Unfortunately there are no citations, no index, and no bibliography. Nevertheless, the veritable goldmine of usable leads in Chapters 1 and 2 make the book worth reading. The devotion of a full chapter (Chapter 3) to tyrothricin seems ill-founded, but it does cover the subject fairly well. There is the usual chapter on penicillin and the book remains a prime source for its semi-popular discussion of streptomycin.

Fleming, Alexander. "On the Antibacterial Action of Cultures of Penicillium, With Special Reference to Their Use in the Isolation of B. influenzae." Brit. J. Exper. Path., 10 (1929): 224-236.

This is the original paper on penicillin. The title indicates the primary thrust of the article which includes a small discussion on the biochemical investigations carried out by Craddock and Ridley, assistants to Fleming. The paper is notable for what it leaves out. An excellent discussion, indeed a thorough post-mortem on this classic paper, is included in Ronald Hare's The Birth of Penicillin. He analyzes the content through the eyes of a man present in Fleming's laboratory in 1928-1929.

Fleming, Alexander. Chemotherapy: Yesterday, Today, and Tomorrow, The Linacre Lecture (May 6, 1946). Cambridge: The University Press, 1946.

This short (39 pages) discussion deals lightly with the subject of chemotherapy in the mid-1940's. It does not discuss early history per se, but does outline the direction chemotherapy had taken before about 1939. Several antiseptics receive such consideration that it becomes apparent that Fleming feels they represent the main thrust of chemotherapy prior to penicillin. The sulfas and gramicidin get several pages each. Interestingly, in regards the German "flit-gun" approach, Fleming feels that "no one can expect the result to be revolutionary." But by the time of his lecture just such a massive approach had given the world streptomycin and later this approach would be repeated many times with many triumphs.

Florey, H.W., et al. Antibiotics: A Survey of Penicillin, Streptomycin and Other Antimicrobial Substances from Fungi, Actinomycetes, Bacteria and Plants. 2 volumes. London: Oxford, 1949.

In Chapter One of the first of this massive two-volume set, Florey and his co-workers have provided an extensive (73 page) historical background to the antibiotic era. Beginning with ancient folk medicine (Mayans and others) they trace the use of antibiotics up to the 1940's. Although the term antibiotic is not used in the strictest Waksman sense, Florey had avoided general chemotherapy (heavy metals as drugs, etc.), thus Ehrlich and Domagk, and others are not included. The survey (as it is not truly a history) appears to be one of the most exhaustive anywhere in print. In order to appreciate antibiotic discoveries and research prior to the 1940's this survey is the best single source. Unlike so many semipopular works, it is very thorough and very well-documented.

Florey, H.W. and E.P. Abraham. "The Work on Penicillin at Oxford." J. Hist. Med., 6 (1951): 302-316.

This generalized account is, in part, extracted from the authors' magnum opus entitled Antibiotics. It comprises a portion of Chapter 15. It is a general, though condensed, account giving major names, dates, and events at Oxford from 1939-1943 and mention is made of other associations (at Peoria, Illinois, North Africa, etc.). Florey's book (above) and his biography (by Bickel) are much more complete.

Goldsmith, Margaret. The Road to Penicillin: A History of Chemotherapy. London: Drummond, 1946.

Although this book is not in the semi-popular style of, say, Yellow Magic by Ratcliff, it is neither a scholarly work. It provides a bibliography, but is woefully short on textual citations. It is broad including such figures as Leeuwenhoek and Perkin, to whom complete chapters are respectively given. This is possibly less justified than giving a full chapter to Lister. Happily, Florey has a complete chapter unto himself, as does Fleming. The chronology begins with Hippocrates and ends with Florey giving a suitable weight to the various milestones inbetween (with the exceptions noted above). As a short work it ranks as one of the best in its subject area.

Hare, Ronald. The Birth of Penicillin: And the Disarming of Microbes. London: Allen and Unwin, 1970.

This book, along with Bickel's biography on Howard Florey, represents the best and most recent statement on penicillin's history. The present text is, unlike Bickel, autobiographical, and stresses not the industrial history, but as the title says, the birth of penicillin. Hare worked under Fleming at the time of the latter's momentous and very fortuitous discovery. Fortuitous because Hare takes a great portion of the text to go into the very complex details of why Fleming's discoveries hung so much on pure luck. It is a detective story to thrill even the most hardened laboratory bacteriologist, for whom this portion of the book holds special interest. Other chapters are more autobiographical and are not always dealing with antibiotics, as Hare's career was well varied within microbiology. An excellent discussion of the University of Toronto's Connought Laboratories is included plus a useful history of twentieth century attempts and accomplishments in antitoxins, vaccines, etc. Unlike other autobiographies (e.g. compare to that of Selman Waksman) it is virtually never self-laudatory and rings more like the way things were and not like they were imagined to be. It is clear, easily read and most importantly gives a more believable perspective on Fleming, demythologizing him, but without deprecation. For one searching the history of antibiosis it cannot be left unread.

Havinga, E., et al. Modern Development of Chemotherapy. New York: Elsevier, 1946.

The book is highly technical and treats primarily of the chemistry of the sulfonamides. It was published, as the Preface authors state, to show that the Dutch were not idle in their scientific research during the war years. To the history of antibiosis the book

will provide primarily technical data on the behavior of the drugs ex and in vivo from the pharmacological, physico-chemical and bacteriological standpoints. Historical discussion is virtually non-existent. Expansine, an obscure antibiotic, is given considerable space, whereas this is not the case in other books of this period. The literature lists at the end of each chapter contain not only a broad spectrum of various European and American authors, but are goldmines of the Scandanavian sources. The book's usefulness to the historian of chemotherapy will be limited to the materials noted in the first sentence above.

Hepler, Opal E. and Arlene Snow. "Penicillin: A Review." Quart. Bull. Northwestern Univ., 17 (1943): 218-228.

As a review this article does not pretend to be a critical history. Nor should it be given its early date. It is very important in that it gathers together the already rapidly growing literature on penicillin. It lists 65 citations virtually all of which date in the early 1940's. It appears to be the most complete listing of early penicillin works. The text is divided into sections which emphasize current knowledge regarding production, assay, action, toxicity, etc.

Marti-Ibanez, Felix. Men, Molds, and History. New York: M.D. Publications, 1958.

This book is a collection "of articles and addresses on philosophical and historical aspects of antibiotics," as the author states in his dedication. As regards his history, it is weak on several accounts. It is not at all well-documented, nor is a suitable bibliography given. Many statements must be taken on faith. Given Marti's glorioso style, however, one looks on more with a jaundiced eye than with open arms. As for the "philosophical aspects," this is in prime what the book is. Much future speculation, much applause of the past, and much trepidation about the present (1953-1958) fill the most of the text. In a memoriam chapter to Fleming the savant is once more lauded. Florey (as is common with most works) is buried elsewhere in the polysyllabic text. The usefulness to the modern reader in antibiotic history is one principally as a source of the tenor of the times (the mid-1950's).

Maurois, Andre. The Life of Alexander Fleming. Translated by Gerard Hopkins. New York: Dutton, 1959.

This is considered the definitive work on Fleming, yet it contains certain errors of interpretation. The role of Almroth Wright, as portrayed by Maurois, is found wanting according to Ronald Hare (author

of The Birth of Penicillin) who, unlike Maurois, was a first-hand witness in 1928 and who worked under Wright. Since Maurois is primarily a novelist his style is very pleasant and thus the text is highly readable. When read in conjunction with Hare and Bickel's biography of Florey one can feel he has a fair grasp of the penicillin period. This book is, of course, a must for anyone interested in the subject contents, and further as an example of a well-done biographical treatment.

Ratcliff, J. D. Yellow Magic: The Story of Penicillin. New York: Random House, 1945.

The foreword of this book is by Chester Keefer, M.D., who had the unenviable job of allocating the small lots of the first penicillin produced. The introduction is by Morris Fishbein, M.D.

The book gives the whole penicillin story to 1945 and is very patriotically oriented. Fleming is well presented, and the sad lapse of time from his discovery to the understanding of its significance is equally well discussed. Although the sulfa drugs are discussed, Rene Dubos is given much more space than is Gerhardt Domagk. Domagk was German and this book is dated 1945. The American industrial contribution takes up fully two-thirds of the pagination. The work is semi-popular, but assumes some medical knowledge on the reader's part.

Sokoloff, Boris. The Story of Penicillin. Chicago: Ziff-Davis, 1945.

One wonders at the aims or ethics of the publisher, author or both when an expanded form of an already published book becomes available. By noting the discussion on the author's The Miracle Drugs (1949) it becomes apparent that this book (i.e. The Story of Penicillin) can simply be discarded--all the same information, in virtually the same wording, appears again in the 1949 book. No indication is given of this borrowing in the latter publication, however.

Sokoloff, Boris. The Miracle Drugs. Chicago: Ziff-Davis, 1949.

Unlike many other popular (this book), or semi-popular works of the 1940's, The Miracle Drugs deals little with history, but much more with the contemporary status of antibiotics. The first three chapters are completely historical. Curiously Chapter 3 ("When Dog Eats Dog") appeared in Sokoloff's 1945 book (same publisher), The Story of Penicillin, in exactly the same wording (paragraphs are structured differently). No hint or reference is given in this publication of this reprinted chapter. This same thing is done with three other chapters borrowed from the earlier book (but with minor word

changes). The last two chapters in the 1945 book can be found in rearranged and expanded form in the present volume. These are in part, the remaining 13 chapters of this current book.

There are no literature citations throughout, but an outstanding bibliography of 25 pages can be found. The book is useful primarily for providing a sense of how the scientist and physician (Sokoloff was both) looked upon the burgeoning new field of antibiosis. His style is reminiscent of Paul DeKruif in Microbe Hunters, Life Among the Doctors, and others--a very pleasant style. The bibliography remains a high point of the value of this publication today.

Taylor, Frank S. The Conquest of Bacteria: From Salvarsan to Sulphapyridine. New York: Philosophical Library--Alliance Book Corp., 1942.

This book, written in 1942, just missed the possibility of putting in the most exciting chapter of all--that on penicillin. The production of penicillin was allocated a AAA rating by a war production board about the time this book came into print. That rating meant that research and production of penicillin was to be considered as important as getting out a new B-29 or battle-ship. When the author wrote his introduction, however, things were considerably different. The following quotation deserves space here; if only for its prose value it is of decided appeal.

> The world is again at war and is spending perhaps some twenty millions a day on weapons of death and defenses against them. Yet we will not spend five thousand dollars a day in the hope of winning a permanent victory over the grim wolf who was with us when Egypt warred with Babylon, who each year carries off tens of thousands of men, and who, if we help not ourselves, will still be slaying us when the present war is but a dusty item in the historian's count of crime and its reward.

Sigerist, in his Foreword, calls the book popular, and it is just that. Some education, but little previous knowledge of science or medicine, is called for on the part of the reader. It considers in its 175 pages the history of bacterial diseases and early chemotherapeutic attempts. Major chapters consider sulfonamides, prontosil, and sulphapyridine. These are the most valuable sections. Considering Domagk's German heritage he is given a very good treatment. Considering just how much was known of penicillin at this time it finds absolutely no mention in the book. The book is pleasant, but not of critical value to the historian of antibiosis. It is full of typographical errors and is often extremely repetitious.

Waksman, Selman A. My Life with the Microbes. New York: Simon and Schuster, 1954.

The book is autobiographical and is immensely personal. It is singularly unenlightening insofar as Waksman's work with the actinomycetes is concerned, at least for the scientifically-trained reader. It is written on a very simple level and should not be a challenge even to a poorly-educated reader. It is repetitive and is in general a travelog of Waksman's many European peregrinations. Waksman's cultural heritage shows through clearly in his choice of syntax and expression making this the most entertaining angle. In short, the book is much longer than needs be and is understandably biased. The reader concerned with economy of time would not suffer in passing it over.

Welch, Henry and Marti-Ibanez, Felix. The Antibiotic Saga. New York: Medical Encyclopedia, 1960.

Although this book is written by two men who have been active in the laboratory and with pen throughout the antibiotic era, it is again popular and devoid of any citations or bibliography. The writing style is evidently a blend of the two authors as Welch's very exact scientific style and Marti-Ibanez' high erudition are both lacking. It is clearly popular (e.g. in reference to Mycobacterium tuberculosis: "This studdy, pencil-like creature has a thin but waxy coat, a natural armour, to protect him like a magic shield from the impact of the magic bullets the physician so hopefully carries in his little black bag."). It is pleasant reading, but will not expand the knowledge of one who has read dozens of popular and semi-popular accounts of the history of antibiosis.

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