Association of Toxic Capsule and Cell Wall Mucopeptide with Virulence in *Gaffkya tetragena*¹

RICHARD P. MAGEAU² AND BOB S. ROBERSON

Department of Microbiology, University of Maryland, College Park, Maryland 20742

Received for publication 20 August 1968

Nine strains of organisms morphologically and physiologically identified as *Gaffkya tetragena* were obtained from various sources to study their pathogenicity. Initial virulence analysis of all strains by mouse intraperitoneal injection of viable cells revealed that only three strains, recently isolated from and associated with respiratory infections in hospitalized patients, caused death of mice within 48 hr. The LD₅₀ for these virulent, encapsulated strains was 1×10^7 to 6×10^7 viable organisms. To associate virulence with a toxic component, the following fractions were purified from all strains: capsular material, cell walls, mucopeptide preparations from cell walls and whole cells, grouplike material, cytoplasmic material, and culture filtrate with and without added reducing agent. Rabbit and mouse dermal toxicity testing of these fractions revealed that the capsular material, cell walls, and mucopeptide preparations of the virulent strains were toxic. None of the nonvirulent strains contained toxic components, with the exception of one strain which yielded capsular material equal in toxicity to that of the virulent strains. The capsular material induced a soft pustular lesion persisting for approximately 22 days. Cell walls and mucopeptide preparations produced a hard nodular lesion, identical to that produced by autoclaved whole cells, that persisted for 25 to 30 days. One strain may represent a virulence intermediate between the virulent and nonvirulent strains, since it contains toxic capsular material but nontoxic cell wall mucopeptide. The results indicate that the virulence of this organism is associated with toxic capsular material and cell wall mucopeptide.

The status of the organism *Gaffkya tetragena* (*Micrococcus tetragenus*) among pathogens is uncertain at this time. Several reports (3, 14, 22, 24, 25, 27, 29) illustrate the ability of this organism to cause a variety of pathological conditions in man. Other reports (2, 15, 23, 27, 28) indicate variability in the pathogenicity among some strains of this organism for man and laboratory animals.

Although sufficient information is known about the organism to make identification possible and practicable, Mock and Wynne (11) and Reimann (14) have indicated that many more progressive infections are caused by *G. tetragena*, either as a primary or secondary invader, than are reported or detected. The leading cause of this omission may be due to the close resemblance of *Gaffkya*

infection to that caused by *Staphylococcus aureus* or other gram-positive cocci.

The purpose of this study was to determine toxic factor(s) associated with virulence in strains of *G. tetragena* by subjecting purified cellular components to sensitive *in vivo* tests. It also was desirable to clarify the position of this organism as a pathogen and to describe a possible mechanism by which it may cause disease.

MATERIALS AND METHODS

Bacterial cultures and growth conditions. The source and designation of nine strains of *G. tetragena* employed in this study are given in Table 1. Strains GT-1 to 6 are from various culture collections, whereas strains GT-7, 8, and 11 represent fresh clinical isolates of the predominant microbial flora of three hospitalized patients with respiratory infections.

All organisms were grown at 37 C for 24 to 48 hr in either Trypticase Soy Agar or Trypticase Soy Broth (BBL). Large quantities of cells were obtained by growing the organisms in 100-ml portions of Trypticase Soy Broth, dispensing in separate flasks, and incubating on a rotary shaker. Cells were harvested by centrifugation at $5,000 \times g$ for 15 min, washed with

¹ This report represents part of a dissertation submitted by the senior author to the Graduate School of the University of Maryland in partial fulfillment of the requirements for the Ph.D. degree. Presented in part at the 68th Annual Meeting of the American Society for Microbiology, Detroit, Mich., May 1968.

² Present address: Department of Biology, Kansas State College of Pittsburg, Pittsburg, Kan. 66762.

sterile distilled water, frozen, and stored at -10°C until needed. An enriched medium was employed by supplementing Trypticase Soy Agar with 5% human type O blood.

Morphological procedures. To assure the use of organisms typical of *G. tetragena*, all strains were subjected to negative staining by phosphotungstic acid and examination in an RCA EMU 3F electron microscope at a magnification of 14,000.

Capsules were determined by the use of Duguid (5) and Maneval (10) capsule stains.

Serial mouse passage. Virulent strains of *G. tetragena* were subjected to serial mouse passage to aid in the determination of component(s) associated with virulence. Growth from a 48-hr blood-agar plate was suspended in 10 ml of sterile 0.85% saline and dispersed. This suspension (1 ml) was injected intraperitoneally into several 5- to 6-week-old Swiss Webster white mice from an inbred colony. Organisms were recovered by aseptically opening the peritoneal cavity immediately after the mice had died, and streaking some of the exudate on a blood-agar plate. A pure culture of the passed organism was obtained after suitable incubation, and the process was repeated for each subsequent passage.

Determination of LD_{50} . LD_{50} determinations were performed by the intraperitoneal injection of 1 ml of twofold, serially diluted, saline suspensions of viable cells of each strain into several 5- to 6-week-old white mice. Deaths were recorded for 1 week. Standard plate counts of each undiluted cell suspension were performed at the time of injection in duplicate in Trypticase Soy Agar. From the resulting deaths and the original cell counts, the LD_{50} for each strain was calculated by the method of Reed and Muench (13).

Toxicity testing of cellular fractions and determination of lesion index. All purified cellular fractions were tested for toxicity by the intradermal injection of a known quantity of material in an 0.1-ml volume of sterile distilled water in the shaved flanks of 2.5- to 3.5-kg New Zealand albino rabbits, as described by Schwab and Cromartie (19), Abdulla and Schwab (1), and Schuster, Hayashi, and Bahn (18). Quantitation of material prior to injection was achieved by dry weight determinations of the material dried to a constant weight at 90°C .

The response of cellular fractions was recorded as a lesion index, which was determined by measuring the major and minor axis of the lesion in millimeters, taking the square root of the product of the two and adding one point each for erythema, hardness, thickness, and pus formation. These lesion indexes were determined daily for the first week of the test, then every other day for an additional 3 weeks.

Preparation of capsular material. Capsular material was obtained by the sodium acetate-ethyl alcohol extraction procedure, described by Kabat (8), from a cell-free aqueous extract obtained by blending 5 g (dry weight) of whole cells in 100 ml of sterile distilled water for 10 min at high speed in a Waring Blendor. Deproteinization was accomplished by the phenol-water extraction method of Westphal, Lüderitz, and Bister (26). The capsular material was reprecipitated by a second sodium acetate-ethyl alcohol extraction

and resuspended in sterile distilled water. Residual phenol was removed by extraction with anhydrous ethyl ether. Residual ether was removed by evaporation. The material was subjected to a spectrographic scan from 400 to 200 nm on a Beckman DB recording spectrophotometer; it was assayed for protein by the method of Lowry et al. (9) with crystalline bovine albumin as a standard, and by the method of Ikawa and Niemann (7) for total carbohydrate. Dry weights and sterility checks were performed, as on all other preparations, prior to toxicity testing.

Cell wall preparations. Modifications of the procedure of Roberson and Schwab (16) were used to prepare pure cell walls from all strains. Cells were ruptured with Ballotini glass beads in a 10-kc Raytheon sonic oscillator, followed by glycerol density gradient centrifugation. Crude cell walls were washed free of glycerol and were treated successively with 100 μg of ribonuclease per ml and 200 μg of trypsin per ml at 37°C for 3 hr.

Mucopeptide preparations. Preparations which were considered to be essentially mucopeptide were made from isolated cell walls by the formamide extraction method of Fuller (6), and from whole cells by the trichloroacetic acid extraction procedure of Park and Hancock (12).

Preparation of grouplike substance. The supernatant fluid from the formamide mucopeptide extraction procedure was used to prepare grouplike material by the method of Fuller (6); this method is commonly employed for streptococci.

Cytoplasmic material. A fraction consisting of cytoplasmic material was obtained by sonic treatment of whole cells with glass beads so that 99.9% rupture of cells was achieved; collection of the supernatant fluid from centrifugation at $81,500 \times g$ for 1 hr was carried out in a no. 40 rotor of a Spinco Model L ultracentrifuge.

Culture filtrate. A cell-free culture filtrate was obtained from all strains by centrifuging a 72-hr Trypticase Soy Broth culture at $15,000 \times g$ for 30 min in a refrigerated Sorvall centrifuge. The supernatant fluid was filtered through a 0.45- μm membrane filter (Millipore Corp., Bedford, Mass.). Toxicity tests were performed on these culture filtrates, and the filtrates were reduced by the addition (100 $\mu\text{g}/\text{ml}$) of cysteine to portions of the fluid prior to use.

Antisera. Antiserum to each of the organisms was prepared in rabbits by repeated intravenous injections of washed, Formalin-treated whole cells.

RESULTS

Initial characterization studies. All strains in this study were initially subjected to limited morphological and physiological testing to insure the use of strains typical of *G. tetragena*. The tetrad morphology exhibited by all strains in this study is typical for this species. Physiological tests, usually employed for members of the *Micrococcaceae*, were performed by standard methods on all strains in this study. All strains were uniformly negative for coagulase, hemolysis, hydrogen sulfide, indole and acetoin production, urea

hydrolysis, growth in 10% sodium chloride, manitol fermentation, oxidase, gelatin liquefaction, and citrate and ammonium phosphate utilization. It is believed that these morphological and physiological characteristics agree well with those given for *G. tetragena* in *Bergey's Manual of Determinative Bacteriology* (2), and that the strains in this study are considered typical of this species.

LD₅₀ determinations. Initial examination of the virulence of all strains by intraperitoneal injection in white mice revealed that only strains GT-7, 8, and 11 caused death within 24 to 48 hr. These three strains were serially passed 9 to 12 times in mice for virulence enhancement to aid in the determination of toxic component(s) possibly associated with virulence. Table 1 gives the level of virulence of all strains as expressed by LD₅₀ values after final mouse passage. Strains GT-7, 8, and 11, having an LD₅₀ in the range of 1×10^7 to 6×10^7 viable organisms, represent virulent strains causing death of mice within 48 hr. Symptoms of illness became apparent within 5 to 6 hr after injection of these strains in mice. Examination of the peritoneal cavity after death revealed a large gelatinous mass of organisms and phagocytic cells, but no gross pathological changes were observed in any of the internal organs. It was established that the virulence of the virulent strains was slightly enhanced by serial mouse passage, since the LD₅₀ determination on GT-7 prior to and after passage revealed a 1.7-log decrease in the LD₅₀ value. Strains GT-1 to 6 failed to cause death of mice at the highest concentration of organisms attainable or after repeated attempts at mouse passage.

Toxicity testing of cellular fractions. Toxicity testing was initiated by examining the response to whole cells of each strain injected in rabbit dermis. The response to 100 μ g (dry weight) of autoclaved whole cells is shown in Fig. 1. Three levels of

FIG. 1. Response of rabbit dermis to an 0.1-ml intradermal injection of 100 μ g (dry weight) of autoclaved whole cells. Symbols: ■, response to GT-2 which closely resembles GT-3, 4, and 5; ▲, response to GT-1 which closely resembles GT-6; ●, response to GT-7 which closely resembles GT-8 and 11.

virulence response are evident. Strains GT-2, 3, 4, and 5 produced a primary inflammatory response to the cellular material without the production of a lesion. Strains GT-1 and 6 showed an intermediate response by the production of small lesions which regressed quickly. The virulent strains produced the highest response with the production of large, hard nodular lesions, which gradually regressed within 30 days beyond that shown in Fig. 1. Partially shaved mice tested in the same manner showed identical responses, with the exception that the lesions produced were smaller and subsided more rapidly than those in rabbit dermis. Toxicity obtained from autoclaved whole cells can thus be attributable to stable cellular components.

Capsule stains revealed that strains GT-7, 8, and 11 contained large capsules. To assess the

TABLE 1. Designation, source, and LD₅₀ values for strains of *Gaffkya tetragena*

Designation	Source	LD ₅₀ ^a
GT-1	ATCC no. 10875	1.56×10^9 , no deaths
GT-2	Univ of Maryland collection, NC 22-1-A	1.23×10^9 , no deaths
GT-3	Univ of Maryland collection, 10-45	2.38×10^9 , no deaths
GT-4	Univ. of Maryland collection, 11-53	2.73×10^9 , no deaths
GT-5	Univ. of Maryland collection, 1-49	2.28×10^9 , no deaths
GT-6	Midwestern Culture Service, "Potentially pathogenic"	6.20×10^9 , no deaths
GT-7	Hospital isolate from sputum	1.37×10^7
GT-8	Hospital isolate from sputum	4.00×10^7
GT-11	Hospital isolate from sputum	6.20×10^7

^a The LD₅₀ value represents the number of viable organisms required to kill 50% of the mice following intraperitoneal injection, as estimated by the formula of Reed and Muench (13).

relative toxicity of this material in virulence, all strains were subjected to extraction procedures to isolate capsular material. Capsular material was isolated not only from GT-7, 8, and 11, as expected, but also from GT-2, a nonvirulent strain. Tests for protein by the method of Lowry et al. (9) were negative; no protein or nucleic acid was indicated by absorption spectroscopy, the curves showing only terminal absorption in the far ultraviolet. Estimation of total carbohydrate (7) as a percentage of dry weight established that the isolated capsular material is largely if not entirely polysaccharide. Double diffusion in agar of each preparation against its homologous whole cell antiserum gave a single band for two of the preparations, the other two showing a trace band in addition to the major band. The response to capsular material from GT-8 is shown in Fig. 2. Similar results were obtained with capsular material from strains GT-7, 11, and 2 using as little as 10 μ g of material. The lesion produced by this material was of a soft, pustular nature that was less severe and of a shorter duration when compared to the hard, nodular lesions produced by whole cells. The lesion produced by capsular material remained pustular for 10 to 15 days, and then gradually subsided to scar formation within 22 days. Since toxic capsular material was found in the nonvirulent GT-2 strain, it is apparent that additional factors are essential to virulence.

Since several studies (17, 18, 20) have shown the involvement of the cell wall of other gram-positive cocci in toxicity, cell walls of all strains in this study were tested. Figure 3 shows the response of rabbit dermis to 100 μ g (dry weight) of purified cell walls of all strains. The cell walls of the virulent strains are toxic, whereas those of the

FIG. 2. Response of rabbit dermis to an 0.1-ml intradermal injection of 50 μ g (dry weight) of purified GT-8 capsular material.

FIG. 3. Response of rabbit dermis to an 0.1-ml intradermal injection of 100 μ g (dry weight) of purified cell walls. Symbols: ▲, response to GT-1 which closely resembles GT-2, 3, 4, 5, and 6; ●, response to GT-7 which closely resembles GT-8 and 11.

nonvirulent strains are not. The response produced by these toxic cell walls was the typical hard, nodular lesion previously described for whole cells. The response to strains GT-1 and 6 are no longer significant in this dermal toxicity testing and are placed in the nonvirulent group. The reason for the intermediate virulence response to GT-1 and 6 in the whole cell study is not known. The cell wall toxicity results are in excellent agreement with those obtained for the LD₅₀ determination in relation to the virulence level of the various strains. The large differential response between the two groups of strains at day 7 should also be noted (Fig. 3).

Figure 4 shows, at day 7, the lesions from which the data were taken to plot the curves shown in Fig. 3. The slight responses of cell walls from nonvirulent strains GT-1 to 5 are shown in Fig. 4A; the large nodular lesions produced by cell walls of virulent strains GT-7, 8, and 11 are shown in Fig. 4B. The response to GT-6, not seen in Fig. 4A, was not different from those shown.

To study the possibility that a more basic unit of the cell wall, namely, the cell wall mucopeptide, might be involved in toxicity, two mucopeptide preparations were made from all strains and subjected to dermal toxicity testing. Figure 5 shows the results obtained from 100 μ g (dry weight) of formamide-prepared mucopeptide from pure cell walls. The mucopeptide from the virulent strains is toxic, whereas that of the nonvirulent strains is nontoxic. The response produced to the mucopeptide is identical to the hard, nodular lesion produced to whole cells and cell walls, although

FIG. 4. The 7-day response of rabbit dermis to an 0.1-ml intradermal injection of 100 μ g (dry weight) of purified cell walls. (A) Strains GT-1, 2, 3, 4 and 5 represented by their respective numbers. (B) Strains GT-7, 8, and 11 represented by their respective numbers.

slightly less in severity and duration. Trichloroacetic acid-prepared mucopeptide from whole cells tested in the same manner yielded results analogous to those of the formamide mucopeptide. A slightly higher response to the trichloroacetic acid-extracted mucopeptide of the nonvirulent strains was noted. Compared by immunoelectrophoresis, each preparation showed a single precipitin band with homologous whole cell antiserum. The position of the bands for the virulent strains was slightly more toward the anode than was that from the nonvirulent strains.

Toxicity tests with the capsular material, cell walls, and mucopeptide preparations in mouse dermis gave identical differential responses from the various strains compared to rabbit dermis response; however, in all cases, the response in mice was greatly diminished and persisted for a shorter period of time.

Each of the fractions was tested for its ability to cause death in mice, as determined by the intraperitoneal injection of 1 ml of each fraction in the most concentrated stock solutions available. For purified cell walls and mucopeptide preparations, 2 to 4 mg/ml was injected. Capsular material was injected in the range of 100 to 500 μ g/ml. During observation of the inoculated mice for 1

FIG. 5. Response of rabbit dermis to an 0.1-ml intradermal injection of 100 μ g (dry weight) of formamide-extracted mucopeptide. Symbols: ▲, response to GT-1 which closely resembles GT-2, 3, 4, 5, and 6; ●, response to GT-7 which closely resembles GT-8 and 11.

week, no deaths were produced by any of these fractions. Mice were protected with homologous whole cell antiserum against a lethal challenge of viable cells. The dermal toxicity of capsular material was neutralized by this antiserum, but the dermal lesion induced by the mucopeptide was not altered.

The remaining fractions subjected to toxicity testing were the cytoplasmic material, culture filtrate with and without added reducing agent, and the grouplike material from the formamide supernatant fluid. When these fractions from all strains were tested in rabbit and mouse dermis and intraperitoneally in mice, no toxicity for any fraction was demonstrated. The absence of toxicity from culture filtrates and cytoplasmic fractions of the virulent strains rules out the possibility of extra- or intracellular toxins associated with dermal toxicity under the conditions tested. The exact nature of the grouplike material isolated from the formamide supernatant fluid is unknown, but it appears that there is no group material in *Gaffkya* as is commonly thought of in relation to the streptococci. Failure of each of these extracts to react with specific, whole cell antisera supports this idea.

DISCUSSION

This study has demonstrated the presence of toxic capsular material and cell wall mucopeptide in pathogenic strains of *G. tetragena*. The direct correlation between virulence and possession of both toxic components demonstrated in this study

gives added significance to the failure of repeated attempts to establish an LD₅₀ for strains not possessing these two toxic components.

All strains lethal for mice were encapsulated, a state not remarkable in view of the known relation of capsules to virulence with *Diplococcus pneumoniae* serving as the best known example. This situation has been recognized in other organisms and recently reported by Bulmer and Sans (4) with encapsulated strains of *Cryptococcus neoformans*. Demonstration of dermal toxicity for capsular material was unexpected, and no reports of a similar capsular polysaccharide toxicity were found in the literature. The possibility exists that this capsular material may serve a dual role in the establishment of *G. tetragena* infection by allowing the organism to resist phagocytosis and by exerting a direct toxic effect itself upon the host.

Since strain GT-2 contained toxic capsular material but was nonvirulent, the importance of toxic cell wall mucopeptide in virulence is evident. The hard, nodular lesions produced in rabbit dermis to whole cells, cell walls, and cell wall mucopeptide of the virulent strains are very similar to those observed by Abdulla and Schwab (1) and Schuster et al. (18) under similar test conditions for streptococci and other gram-positive cocci. The extended duration of whole cell lesions compared to cell wall and mucopeptide lesions produced by the same amount of injected material in rabbit dermis may be related to particle size injected and rate of clearance of the material from the site of injection. Roberson, Schwab, and Cromartie (17) have shown that the size of lesion produced is related to particle size of cell wall complex injected; however, Schwab and Ohanian (21) have shown that the rate of elimination of injected streptococcal cell walls and mucopeptides is related to both properties of particle size and species from which these were derived. The prolonged response to whole cells may be due to a longer exposure to the toxic action because a longer period may be necessary for clearance from the injected site compared to that of the smaller cell wall and mucopeptide complexes.

It is unlikely that any other factor is responsible for the reaction in rabbit dermis other than the capsular material, cell walls, or mucopeptide preparations themselves. Hypersensitivity seems a highly unlikely mechanism of toxicity due to the regularity and predictability of the response which quickly follows injection of these materials into either normal rabbits or mice. This is also supported by the fact that while capsular material was neutralized in protection studies with the use of whole cell antisera, cell walls and mucopeptide preparations still induced lesions without modification in time of appearance, severity, or duration.

Preliminary chemical and immunological studies indicate that a complete, detailed chemical analysis of the capsule, cell walls, and cell wall mucopeptides of the strains in this study would aid greatly in defining toxicity in terms of exact chemical or structural features. Whether the mechanism of pathogenesis of *G. tetragena* in man is that of an opportunistic invader exerting its combined toxic effects under the protection of a capsule will require the analysis of additional isolates from hospital patients. A similar study performed on a wide variety of staphylococci and micrococci strains also would help to determine the exact taxonomic position and interrelationship of *G. tetragena* within the *Micrococcaceae*.

ACKNOWLEDGMENTS

We thank Zigfridas Vaituzis for the fine photography and electron microscopy performed during this investigation on whole cells and cell wall preparations.

This investigation was supported by a Public Health Service Predoctoral Fellowship 1-F1-GM-35,965-01 from the National Institute of General Medical Sciences and by Public Health Service grant AI 07863-01 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Abdulla, E. M., and J. H. Schwab. 1966. Biological properties of streptococcal cell-wall particles. III. Dermonecrotic reaction to cell-wall mucopeptides. *J. Bacteriol.* 91:374-383.
2. Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. *Bergey's manual of determinative bacteriology*, 7th ed. The Williams & Wilkins Company, Baltimore.
3. Brisou, J., J. Morichau-Beauchant, J. Gimenez, and A. Lenriot. 1952. Flore bacterienne des infections urinaires. *Bull. Soc. Pathol. Exotique* 45:439-442.
4. Bulmer, G. S., and M. D. Sans. 1968. *Cryptococcus neoformans*. III. Inhibition of phagocytosis. *J. Bacteriol.* 95:5-8.
5. Duguid, J. P. 1951. The demonstration of bacterial capsules and slime. *J. Pathol. Bacteriol.* 63:673-685.
6. Fuller, A. T. 1938. The formamide extraction of polysaccharide from haemolytic streptococci. *Brit. J. Exptl. Pathol.* 19:130-139.
7. Ikawa, M., and C. Niemann. 1949. A spectrophotometric study of the behavior of carbohydrates in 79% sulfuric acid. *J. Biol. Chem.* 180:923-931.
8. Kabat, E. A., and M. M. Mayer. 1961. *Experimental immunochemistry*, 2nd ed., p. 840-850. Charles C Thomas, Publisher, Springfield, Ill.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
10. Maneval, W. E. 1934. Rapid staining methods. *Science* 80:292-294.
11. Mock, F. S., and E. S. Wynne. 1950. The sensitivity of *Gaffkya tetragena* strains to penicillin and streptomycin. *J. Infect. Diseases* 87:10-12.
12. Park, J. T., and R. Hancock. 1960. A fractionation procedure for studies of the synthesis of cell wall mucopeptide and of other polymers in cells of *Staphylococcus aureus*. *J. Gen. Microbiol.* 22:249-258.
13. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty per cent end points. *Am. J. Hyg.* 27:493-497.
14. Reimann, H. A. 1935. *Micrococcus tetragenus* infection. I. Review of the literature, report of a non-fatal case with septicemia, meningitis and arthritis and bacteriologic studies. *J. Clin. Invest.* 14:311-319.

15. Reimann, H. A. 1936. *Micrococcus tetragenus* infection. II. Description of variant forms. *J. Bacteriol.* 31:385-405.
16. Roberson, B. S., and J. H. Schwab. 1960. Studies on preparation of bacterial cell walls and criteria of homogeneity. *Biochim. Biophys. Acta* 44:436-444.
17. Roberson, B. S., J. H. Schwab, and W. J. Cromartie. 1960. Relation of particle size of C polysaccharide complexes of group A streptococci to toxic effects on connective tissue. *J. Exptl. Med.* 112:751-764.
18. Schuster, G. S., J. A. Hayashi, and A. N. Bahn. 1967. Toxic properties of the cell wall of gram-positive bacteria. *J. Bacteriol.* 93:47-52.
19. Schwab, J. H., and W. J. Cromartie. 1957. Studies on a toxic cellular component of group A streptococci. *J. Bacteriol.* 74:673-679.
20. Schwab, J. H., W. J. Cromartie, and B. S. Roberson. 1959. Identification of a toxic cellular component of group A streptococci as a complex of group specific C polysaccharide and a protein. *J. Exptl. Med.* 109:43-54.
21. Schwab, J. H., and S. H. Ohanian. 1967. Degradation of streptococcal cell wall antigens in vivo. *J. Bacteriol.* 94: 1346-1352.
22. Spencer, A., M. Vera, V. Cozzi, and J. Vigouroux. 1952. Subacute bi-bacterial endocarditis. *Rev. Med. Chili* 80:363-366.
23. Spicer, S. 1953. An antibiotic agent from a *Micrococcus tetragenus* variant. *Antibiot. Chemotherapy* 3:254-255.
24. Stubinger, K. 1934. Die Ursache der verschiedenen Virulenz von Tetragenusstämmen. *Arch. Hyg. Bakteriol.* 112:70-83.
25. Tobin, W. R. 1943. Pneumonia caused by *Micrococcus tetragenus*. *J. Am. Med. Assoc.* 121:41.
26. Westphal, O., O. Lüderitz, and F. Bister. 1952. Über die Extraktion von Bakterien mit Phenol/Wasser. *Z. Naturforsch.* 7b:148-155.
27. Wreshner, H. 1921. Untersuchungen über die biologische Bedeutung der Kapsel beim *Micrococcus tetragenus*. *Z. Hyg. Infektionskrankh.* 93:74-86.
28. Wynne, E. S., and J. O. Norman. 1953. On the concept of "direct antagonism" in bacteria. *J. Infect. Diseases* 93:243-246.
29. Zeligman, I., and S. J. Sinesi. 1953. Carbuncle caused by *G. tetragena* treated with oxytetracycline (Terramycin). *Arch. Dermatol. Syphilol.* 68:382-384.