JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1978, p. 142-147
0095-1137/78/0008-0142$02.00/0

Vol. 8, No. 2
Printed in U.S.A.

Human Infections Caused by Thiamine- or Menadione-Requiring *Staphylococcus aureus*

J. F. ACAR,[1] F. W. GOLDSTEIN,[1] AND P. LAGRANGE[2]

Hôpital Saint-Joseph, Université Pierre et Marie Curie,[1] and Institut Pasteur,[2] Paris, France

Received for publication 21 February 1978

Stable dwarf forms of *Staphylococcus aureus* have been identified in clinical specimens as the sole or predominant isolate in eight cases. These organisms have been shown to be menadione or thiamine dependent, i.e., cultivation in the presence of one of these agents has permitted growth of colonies which appear typical of *S. aureus*. In vitro resistance to aminoglycosides was overcome by cultivation in the presence of menadione or thiamine. Menadione- or thiamine-requiring *S. aureus* can be considered as causative agents in severe human infections. Special care must be taken if they are to be identified in pathological specimens. Their antibiotic sensitivity testing should be done comparatively on supplemented and nonsupplemented media.

Staphylococcus aureus growing as dwarf colonies on usual media have been documented by many investigators after in vitro exposure of the bacteria to adverse environmental conditions such as chemicals, antibiotics, or aging (4, 8-13, 16, 19, 21-24, 26-29, 37, 39-41, 43-45). In some cases, these organisms have been shown to have increased nutritional requirements, requiring supplementation with carbon dioxide, hemin, menadione, thiamine, or pantothenate (6, 10, 12, 13, 18-20, 22, 23, 26-28, 31-36, 38-41). Isolation of such strains from clinical material was first reported by Hale in 1951 (20). Thiamine-requiring strains isolated by Sompolinsky et al. as the causative agent of bovine mastitis were never isolated from human subjects (33-35). Menadione-requiring strains similar to those that were selected in vitro by Sasarman et al. (27, 28) have been isolated by Borderon and Horodniceanu from a clinical specimen (6).

The present study concerns eight strains of *S. aureus* which were isolated as dwarf colonies from clinical specimens and which require thiamine or menadione to grow normally. The problems related to the detection of these strains in a clinical laboratory and the methods for testing their antibiotic sensitivity are presented here.

MATERIALS AND METHODS

Bacterial strains. From June, 1975, to May, 1976, among 1,110 strains of *S. aureus* isolated from clinical material, 15 isolates were observed on initial culture to grow as dwarf colonies on our routine media, Trypticase soy agar (TSA; Baltimore Biological Laboratories) and TSA with 5% horse blood. Eight of these isolates were stable on serial subculture with these same media and required either thiamine or menadione to grow as normal-appearing staphylococcal colonies. *S. aureus* was identified on the basis of microscopic morphology, coagulase, phosphatase, deoxyribonuclease, catalase production, and biochemical tests according to Baird-Parker (3). *S. aureus* 209 P (Institut Pasteur) was used as control.

Supplementation of dwarf colony variants. Dwarf colonies were subcultured by using one or more of the following media: TSA, TSA with 5% horse blood, chocolate agar with 1% IsoVitaleX (Baltimore Biological Laboratories), Mueller Hinton (MH) agar with disks containing 5 μg of thiamine-hydrochloride (Hoffman-LaRoche) or 1 μg of menadione bisulfite (E. Merk AG). Incubation was carried out for 18 and 48 h aerobically, aerobically with 5% CO_2 supplement, and anaerobically (H_2-CO_2, GasPak).

Quantitative supplementation was performed on MH agar containing either thiamine or menadione at a 10-fold concentration ranging from 0.01 to 100 μg/ml. The colony size was measured after 18 and 48 h on each plate.

Antibiotic sensitivity testing. Disk susceptibility testing was performed on MH agar by the ICS method (15). Dwarf colony variants and reverse mutants of each strain were tested separately. Zones of inhibition for kanamycin, gentamicin, erythromycin, lincomycin, and vancomycin were read after 24 and 48 h of incubation at 37°C. A separate test was performed at 30°C with an oxacillin disk to detect methicillin-resistant strains (2).

MIC. Minimal inhibitory concentrations (MICs) of the same antibiotics were determined by an agar dilution method by using MH agar supplemented with 5 μg of thiamine per ml or 1 μg of menadione per ml. Standardized bacterial inocula (10^2 to 10^3 colony-forming units per spot) were deposited on the surface of the agar plates with an automatic Steers replicator device. Dwarf colony variants and the reverse mutants were tested simultaneously. Results were read after 24 and 48 h of incubation.

Effect of supplementing substances on antibiotic sensitivity. (i) Paper strip method. Two

filter paper strips were placed at right angles on an MH agar seeded with 10^7 colony-forming units per ml by the technique of Bonifas (5) and Dye (14). One paper contained the supplementing substance, and the other contained one of the antibiotics tested by MIC. Concentrations of solutions used for filter paper impregnation were (in μg/ml): thiamine, 400; menadione, 100; oxacillin, 100; kanamycin, 200; gentamicin, 200; erythromycin, 200; lincomycin, 200; and vancomycin, 400.

Checkerboard method. MICs of kanamycin for a thiamine- and a menadione-requiring strain were determined by a checkerboard arrangement as described previously (1). Thiamine or menadione at concentrations ranging from 0.01 to 100 μg/ml were tested with kanamycin at concentrations ranging from 0.1 to 100 μg/ml in MH broth. Results of growth after 24 and 48 h of incubation at 37°C were drawn as an isobologram on a logarithmic scale.

RESULTS

Bacterial strains and supplementation. Three menadione- and five thiamine-requiring *S. aureus* were isolated from specimens obtained from human subjects (Table 1); in most of the cases, dwarf colony variants were recovered as predominant bacteria in mixed cultures with the reverse mutants (Fig. 1). All the strains had normal microscopic morphology and produced coagulase, deoxyribonuclease, and catalase.

Menadione-requiring strains grew very poorly after 48 h on all of the nonsupplemented media, except on the plate containing the 1-μg menadione disk where satellite growth with normal-sized colonies occurred around the disk (Fig. 2). Reverse mutants growing normally could be observed. Quantitative supplementation tests showed adequate colonial morphology with menadione concentrations ranging from 0.1 to 2 μg/ml. At concentrations higher than 10 μg/ml, growth was inhibited.

Thiamine-requiring strains grew poorly on MH agar but had normal appearance on chocolate agar containing IsoVitaleX. On TSA and TS blood agar, intermediary-sized colonies could be observed, probably because these media contain a small amount of thiamine. Reverse mutants were isolated from six of the eight strains tested, and three of these strains showed the phenomenon of autosatelliting. All of the primary isolates had a heterogenous appearance, with a range from normal to tiny variants being observed (Fig. 3).

Satellite growth around a disk containing 5

TABLE 1. *Menadione- or thiamine-requiring S. aureus isolated from patients*

Origin	No. of isolates	Supplementing substance
Blood	1	Menadione
Osteomyelitis	1	Menadione
Subcutaneous abscess	1	Menadione
Cerebrospinal fluid	1	Thiamine
Blood	2	Thiamine
Osteomyelitis	2	Thiamine

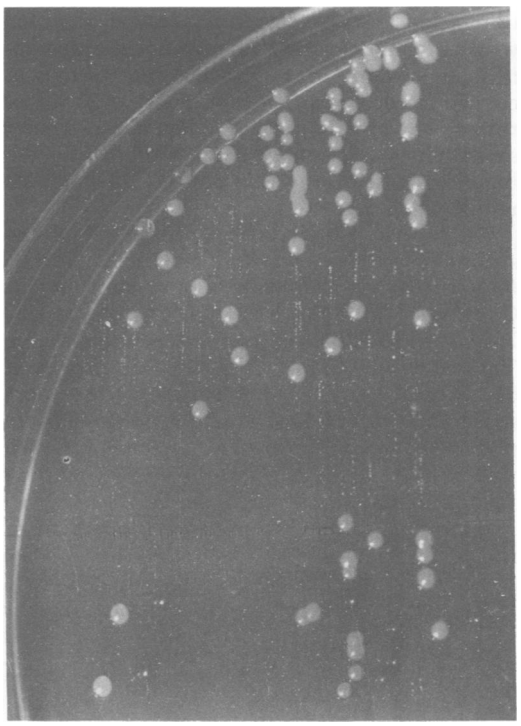


FIG. 1. *Menadione-requiring S. aureus, TSA, 48-h growth. Some reverse mutants are noted together with dwarf colonies.*

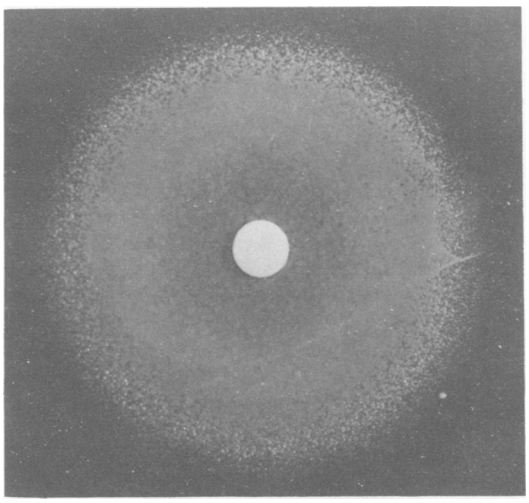


FIG. 2. *Supplementation test, MH agar, 18-h growth. Satellite growth of menadione-requiring strain around a disk containing 1 μg of menadione.*

μg of thiamine was very similar to that observed with a menadione disk. Quantitative supplementation showed adequate colonial morphology at concentrations ranging from 0.05 to 25 μg of thiamine. Growth in 5% CO_2 in air or under anaerobic conditions did not significantly enhance growth of any thiamine- or menadione-requiring strains.

Antibiotic sensitivity testing. All of the strains tested were sensitive by disk sensitivity testing to oxacillin, lincomycin, and vancomycin. One strain was highly resistant to erythromycin. Results of MIC determinations are presented in Table 2. Oxacillin inhibited all of the strains at 0.5 μg/ml. Dwarf colony variants were more susceptible to oxacillin when tested on nonsupplemented media. On media supplemented with thiamine or menadione, dwarf colonies and their reverse mutants had similar MICs.

Kanamycin and gentamicin inhibited all of the reverse mutants at 1 μg/ml (except for one strain which was highly resistant to kanamycin). In each case, dwarf colony variants had higher MICs to kanamycin and gentamicin when tested on nonsupplemented media. On media supplemented with thiamine or menadione, MICs of dwarf colonies were lower but did not reach the normal level of the reverse mutants.

Except for one strain which was highly resistant to erythromycin, all of the strains were inhibited by 0.2 μg of erythromycin per ml, 0.5 μg of lincomycin per ml, and 1 μg of vancomycin per ml. No significant differences relating to colonial morphology or media supplementation were observed with these antibiotics.

Paper strip method. Results obtained by this method showed that the more a strain is supplemented with required metabolites, the greater is its sensitivity to kanamycin and gentamicin (Fig. 4). The checkerboard method confirmed this qualitative observation. MICs of kanamycin were higher when thiamine or menadione concentrations were decreased. The ratio of MIC on supplemented media to MIC on nonsupplemented media was 1/16 for the menadione-requiring strain and 1/4 for the thiamine-requiring strain. The graphic expression of the MICs obtained by the checkerboard method is similar to the figure obtained by the paper strip technique (Fig. 5).

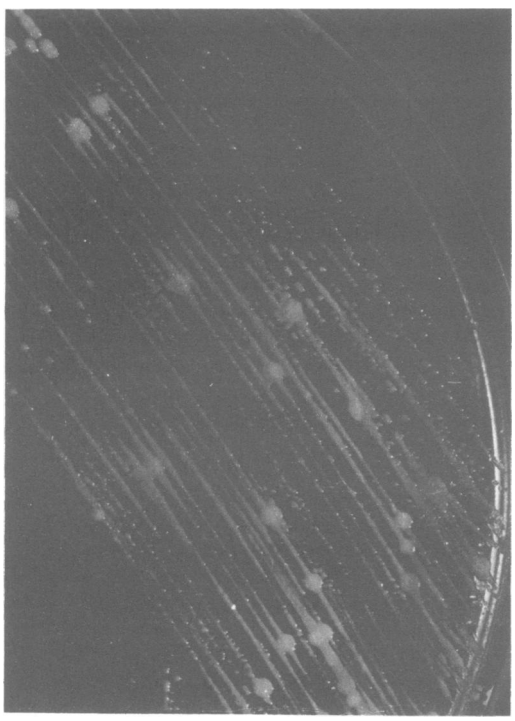


FIG. 3. *Primary isolation of a thiamine-requiring strain showing the heterogeneous appearance of the culture and the satellite growth of dwarf colonies around some reverse mutants (TSA, 18-h growth).*

DISCUSSION

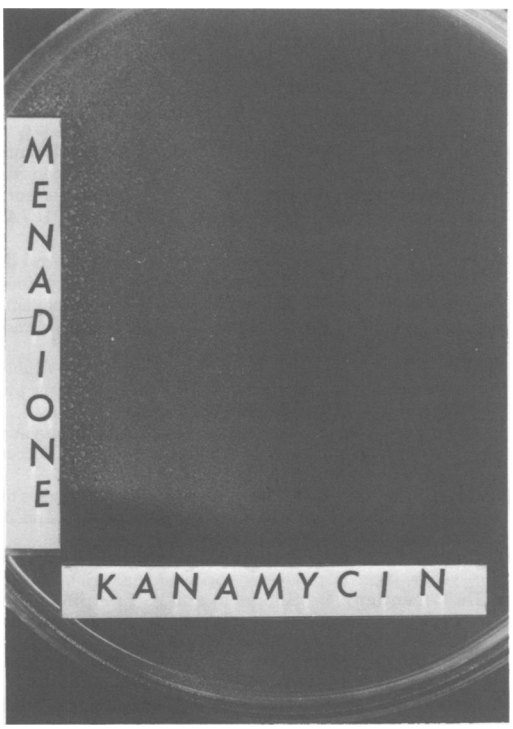
S. aureus growing as dwarf colonies on usual media has been isolated in pure culture or as the predominant isolate thought to be causing infection from eight patients.

Delayed growth, a high rate of reverse mutation, supplementation by rich media, and presence in mixed cultures with normal-sized colonies are the factors which explain why metabolically deficient *S. aureus* are often overlooked in a clinical laboratory. Choice of adequate media for the isolation of such strains from clinical specimens is problematic. Systematic use of enriched media such as chocolate agar containing IsoVitaleX or brain heart agar with IsoVitaleX and menadione can be recommended as acceptable routine media in situations in which deficient strains might be present. These include sterile cultures when *S. aureus* are seen on the Gram stain, cultures obtained from patients with known *S. aureus* infections who are receiving antibiotic therapy, or those who have chronic staphylococcal infections, especially osteomyelitis.

Previous reports considered dwarf colony variants of *S. aureus* having minimal or no virulence when tested in experimental animal infections (12, 21, 26, 37, 39–41). However, a recent study by Musher et al. (24) of small-colony variants selected in vitro by gentamicin clearly demonstrated the virulence of these strains in two different animal models. In this context, the isolation of such strains from well-documented

TABLE 2. *MICs of drugs tested against S. aureus*

Strain	Colonial morphology	Supplementing substance	MIC (μg/ml) of:					
			Oxacillin	Kanamycin	Gentamicin	Erythromycin	Lincomycin	Vancomycin
T1	Dwarf		0.1	4	1	0.06	0.2	0.5
	Dwarf	Thiamine	0.5	2	0.5	0.1	0.5	0.5
	Revertant		0.5	1	0.2	0.1	0.5	1
T2	Dwarf		0.06	4	0.5	0.06	0.1	0.2
	Dwarf	Thiamine	0.2	1	0.1	0.1	0.1	0.2
	Revertant		0.2	0.5	0.1	0.06	0.06	0,5
T3	Dwarf		0.06	>32	1	0.1	0.1	1
	Dwarf	Thiamine	0.1	>32	0.2	0.2	0.2	1
	Revertant	Thiamine	0.1	>32	0.06	0.1	0.2	1
T4	Dwarf		0.02	4	4	0.06	0.1	0.5
	Dwarf	Thiamine	0.1	1	0.5	0.2	0.2	0.5
	Revertant		0.1	0.5	0.5	0.1	0.5	1
M1	Dwarf		0.01	32	8	>32	0.1	1
	Dwarf	Menadione	0.06	4	1	>32	0.5	1
	Revertant		0.06	0.5	0.2	>32	0.5	1
M2	Dwarf		0.1	16	8	0.1	0.2	0.5
	Dwarf	Menadione	0.2	2	0.5	0.2	0.2	0.5
209P (control strain)			0.06	0.5	0.1	0.1	0.2	0.5
		Thiamine	0.1	0.5	0.06	0.06	0.5	0.5
		Menadione	0.06	0.2	0.06	0.1	0.2	0.5



human infections (20) is of great interest. In some cases, the nutritional requirements of these strains have been determined, with CO_2, hemin, menadione, thiamine, or pantothenate being required for growth (6, 9, 12, 13, 18–20, 22, 23, 26–28, 31–36, 38–41). Thiamine-requiring *S. aureus* reported by Sompolinsky et al. in bovine mastitis (33–35) have not been isolated from human subjects, whereas menadione-requiring *S. aureus* have been described by Borderon and Horodniceanu in one clinical specimen (6). The strains presented in this study were repeatedly isolated as the only causative agent of severe human infections and must be considered as pathogenic.

Factors which select these strains in the host have not been fully determined. Aminoglycosides which are able to select deficient strains in vitro (6, 9, 22–24, 27, 28, 39, 43) may select similar strains in vivo (6, 17, 42). Two of the menadione- and three of the thiamine-requiring strains were isolated from patients during or

FIG. 4. *Paper strip method, menadione-requiring strain. Strips of filler paper contain either menadione (100 μg/ml) or kanamycin (200 μg/ml). Increased resistance to kanamycin can be observed (MH agar, 18-h growth).*

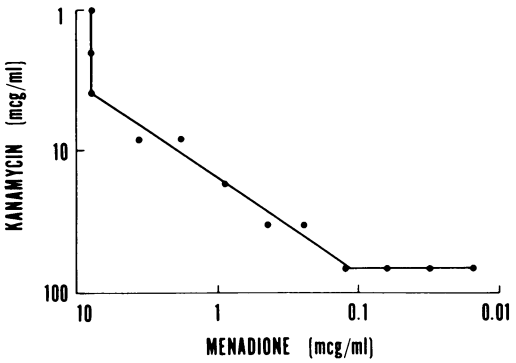


FIG. 5. *Graphic representation of the checkerboard technique showing that the MIC of kanamycin increases as the concentration of menadione in the medium decreases.*

immediately after gentamicin therapy. Another possibility is that deficient strains are selected in the presence of structural analogs of essential nutrients (34). One menadione-requiring strain was isolated from a patient who was receiving warfarin (anti-vitamin K) after surgery. Two of the thiamine-requiring strains were isolated from patients recently treated with trimethoprim-sulfisoxazole for another infection, and one was isolated from a patient who was taking high doses of barbiturates. A thiamine-requiring *Escherichia coli* was recently identified in infected urine from a patient on long-term barbiturate therapy (7). Trimethoprim and the barbiturates have in common the same pyrimidine nucleus as the pyrimidine moiety of thiamine. Further studies are necessary to prove the role of these substances in selection of thiamine-requiring strains and the mechanism which could be involved.

Whatever are the factors selecting thiamine- or menadione-requiring strains, another difficult question to resolve is, what are the factors which enable the persistence of these strains in the host? Thiamine and menadione are present in the serum at concentrations of about 0.01 μg/ml, respectively (31), which are sufficient levels for the in vitro multiplication of the bacteria. Deficient bacteria have a lower metabolism and a reduced rate of replication which could cause decreased sensitivity to antibiotics, because most of them require a normally growing bacteria for optimum inhibitory activity or increased resistance to the antibacterial mechanism of the host and the ability to survive in a hostile environment.

As reported by others (6, 27, 28), the menadione-requiring strains are resistant to aminoglycosidic antibiotics. Thiamine-requiring strains, which were thought to be fully sensitive (34), appeared in this study to be more susceptible to penicillin and less susceptible to aminoglycosides than the normal strains. When sensitivity determinations were performed on supplemented and nonsupplemented media, deficient strains were not as sensitive to aminoglycosides as the reverse mutants, even when tested on supplemented media.

It is difficult to predict which is the most valid method for sensitivity testing because it is not known whether the deficient strain in the host is supplemented or not. Previous reports (6, 17, 42) and some personal observations (unpublished data) of treatment failure with aminoglycosidic antibiotics when dwarf colony variants of *S. aureus* are involved seem to correlate with the in vitro results.

ACKNOWLEDGMENT

We are indebted to Daniel M. Musher of the Baylor College of Medicine for his careful revision of this manuscript.

LITERATURE CITED

1. **Acar, J. F., F. W. Goldstein, and Y. A. Chabbert.** 1973. Synergistic activity of trimethoprim-sulfamethoxazole on Gram-negative bacilli; observation in vitro and in vivo. J. Infect. Dis. **128**(Suppl.):470–477.
2. **Annear, D.** 1968. The effect of temperature on the resistance of Staphylococcus aureus to methicillin and some other antibiotics. Med. J. Aust. **1**:444–446.
3. **Baird-Parker, A. C.** 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. J. Gen. Microbiol. **30**:409–427.
4. **Barbour, R. G. H.** 1950. Small colony variants ("G" forms) produced by Staphylococcus pyogenes during the development of resistance to streptomycin. Aust. J. Exp. Biol. Med. Sci. **28**:415–420.
5. **Bonifas, V.** 1952. Determination de l'association synergique binaire d'antibiotiques et de sulfamides. Experientia **8**:234–235.
6. **Borderon, E., and T. Horodniceanu.** 1976. Mutants déficients à colonies naines de Staphylococcus: etude de trois souches isolées chez des malades porteurs d'ostéosynthèses. Ann. Microb. Inst. Pasteur **127A**: 503–514.
7. **Borderon, E., T. Horodniceanu, J. Buissière, and J. P. Barthez.** 1977. Mutants déficients à colonies naines de Escherichia coli: etude d'une souche thiamine-déficiente isolée d'une uroculture. Ann. Microb. Inst. Pasteur **128A**:413–417.
8. **Browning, C. H., and H. S. Adamson.** 1950. Stable dwarf-colony forms produced by Staphylococcus pyogenes. J. Pathol. Bacteriol. **62**:499–500.
9. **Chinn, B. D.** 1936. Characteristics of small colony variants of Shigella para-dysenteriae Sonne and Staphylococcus aureus. Proc. Soc. Exp. Biol. Med. **34**:237.
10. **Chiu, Y. M., and S. A. Harmon.** 1971. Genetic studies of kanamycin resistance in Staphylococcus aureus. Jpn. J. Microbiol. **15**:417–423.
11. **Colien, F. E.** 1935. A study of microbic variation in a yellow pigment-producing coccus. J. Bacteriol. **30**:301.
12. **De Repentigny, J., S. Sonea, and A. Frappier.** 1964. Selection of thymineless mutants by growing Staphylococcus aureus pathogenic strains in the presence of aminopterin and thymine or thymidine. Rev. Can. Biol. **23**:451–454.
13. **Devriese, L. A.** 1973. Hemin dependent mutants isolated from methicillin resistant Staphylococcus aureus

strains. Antonie van Leeuwenhoek J. Microbiol. Serol. **39**:33–40.

14. **Dye, W. E.** 1955. An agar diffusion method for studying the bacteriostatic action of combinations of antimicrobial agents. Antibiot. Annu. **1955-1956**:374–382.
15. **Ericcson, H. M., and J. C. Sherris.** 1971. Antibiotic sensitivity testing. Acta Path. Microbiol. Scand. **217**(Suppl.).
16. **Gause, G. F., G. V. Kochetkova, and G. B. Vladimirova.** 1961. Biochemical changes accompanying impaired respiration in staphylococci. Nature (London) **190**:978–980.
17. **Godeau, P., J. C. Pechère, and D. Sicard.** 1972. Evolution de la sensibilité à la gentamicine d'une endocardite aiguë à Staphylocoque post-abortum. Ann. Med. Interne **123**:225–228.
18. **Goudie, J. G., and R. B. Goudie.** 1955. Recurrent infection by a stable dwarf-colony variant of Staphylococcus aureus. J. Clin. Pathol. **8**:284–287.
19. **Hale, J. H.** 1947. Studies on Staphylococcus mutation: characteristics of the "G" (gonidial) variant and factors concerned in its production. Br. J. Exp. Pathol. **28**:202–210.
20. **Hale, J. H.** 1951. Studies on Staphylococcus mutation: a naturally occurring "G" gonidial variant and its carbon dioxide requirements. Br. J. Exp. Pathol. **32**:307–313.
21. **Hoffstadt, R. E., and G. P. Youmans.** 1932. Staphylococcus aureus dissociation and its relation to infection and to immunity. J. Infect. Dis. **51**:216–242.
22. **Kaplan, M. L., and W. E. Dye.** 1976. Growth requirements of some small-colony-forming variants of *Staphylococcus aureus*. J. Clin. Microbiol. **4**:343–348.
23. **Lacey, R. W.** 1969. Dwarf colony variants of Staphylococcus aureus resistant to aminoglycoside antibiotics and to a fatty acid. J. Med. Microb. **2**:187–197.
24. **Musher, D. M., R. E. Baughn, G. B. Templeton, and J. N. Minuth.** 1977. Emergence of variant forms of Staphylococcus aureus after exposure to gentamicin and infectivity of the variants in experimental animals. J. Infect. Dis. **136**:360–369.
25. **Patte, J. C., H. Hirsch, and Y. A. Chabbert.** 1958. Etude des courbes d'effet bactériostatique des associations d'antibiotiques. Ann. Inst. Pasteur (Paris) **94**:621–635.
26. **Quie, P. G.** 1969. Microcolonies (G variants) of Staphylococcus aureus. Yale J. Biol. Med. **41**:394–403.
27. **Sasarman, A., M. Surdeanu, V. Portelance, R. Dobardzic, and S. Sonea.** 1971. Classification of vit. K-deficient mutants of Staphylococcus aureus. J. Gen. Microbiol. **65**:125–130.
28. **Sasarman, A., M. Surdeanu, J. Sabados, V. Greceanu, and T. Horodniceanu.** 1968. Menaphthone-requiring mutants of Staphylococcus aureus. Rev. Can. Biol. **27**:333–339.
29. **Schnitzer, R. J., L. J. Camagni and M. Buck.** 1943. Resistance of small colony variants (G forms) of a Staphylococcus toward the bacteriostatic activity of penicillin. Proc. Soc. Exp. Biol. Med. **53**:75–78.
30. **Sebrell, Jr, W. H., and R. S. Harris, ed.** 1954. The vitamins: chemistry, physiology, pathology, vol. I–III. Academic Press Inc., New York.
31. **Sherris, J. C.** 1952. Two small colony variants of Staphylococcus aureus isolated in pure culture from closed infected lesions and their carbon dioxide requirements. J. Clin. Pathol. **5**:354–355.
32. **Slifkin, M., L. P. Merkow, S. A. Kreuzberger, C. Engwall, and M. Pardo.** 1971. Characterization of CO^2 dependent microcolony variants of Staphylococcus aureus. Am. J. Clin. Pathol. **56**:584–592.
33. **Sompolinsky, D., M. Cohen, and G. Ziv.** 1974. Epidemiological studies on thiamine-less dwarf-colony variants of *Staphylococcus aureus* as etiological agents of bovine mastitis. Infect. Immun. **9**:217–228.
34. **Sompolinsky, D., Z. E. Geller, and S. Segal.** 1967. Metabolic disorders in thiamineless dwarf strains of Staphylococcus aureus. J. Gen. Microbiol. **48**:205–213.
35. **Sompolinsky, D., I. Gilskin, and G. Ziv.** 1969. Pantothenate-requiring dwarf colony variants of Staphylococcus aureus as the etiologic agent in bovine mastitis. J. Hyg. **67**:511–516.
36. **Suganuma, A.** 1965. Fine structure of Staphylococcus aureus. Ann. N.Y. Acad. Sci. **128**:26–44.
37. **Swingle, E. L.** 1935. Studies on small colony variants of Staphylococcus aureus. J. Bacteriol. **29**:467–490.
38. **Thomas, M. E. M., and J. H. Cowlard.** 1955. Studies on a CO^2-dependent Staphylococcus. J. Clin. Pathol. **8**:288–291.
39. **Wilson, S. G., and C. C. Sanders.** 1976. Selection and characterization of strains of *Staphylococcus aureus* displaying unusual resistance to aminoglycosides. Antimicrob. Agents Chemother. **10**:519–525.
40. **Wise, R. I.** 1956. Small colonies (G variants) of staphylococci: isolation from cultures and infections. Ann. N.Y. Acad. Sci. **65**:169–174.
41. **Wise, R. I., and W. W. Spink.** 1954. The influence of antibiotics on the origin of small colonies (G variants) of Micrococcus pyogenes var. aureus. J. Clin. Invest. **33**:1611–1622.
42. **Worms, R., and A. Lockhart.** 1959. Un aspect particulier des phénomènes de variation microbienne: les microcolonies ou colonies G. Pathol. Biol. **7**:2153–2163.
43. **Yegian, D., G. Gallo, and M. W. Toll.** 1959. Kanamycin resistant Staphylococcus mutant requiring heme for growth. J. Bacteriol. **78**:10–12.
44. **Youmans, G. P., and E. Delves.** 1942. The effect of inorganic salts on the production of small colony variants by Staphylococcus aureus. J. Bacteriol. **44**:127–136.
45. **Youmans, G. P., E. H. Williston, and M. Simon.** 1945. Production of small colony variants of Staphylococcus aureus by action of penicillin. Proc. Soc. Exp. Biol. Med. **58**:56–57.