

ABSTRACT

PHAGE TYPING OF STAPHYLOCOCCI OF BOVINE ORIGIN AND BIOCHEMICAL CHARACTERIZATION OF BASIC PHAGE PROPAGATING STRAINS

by John J. Solomon

The present investigation was concerned with two major objectives. The first aim was to determine the relationship of staphylococcal strains found in a sampling of bovine herds in Michigan to those commonly involved in human infections, as well as the possible occurrence of a widely distributed strain in the bovine population comparable to phage type 80/81 in human infections. In the second aim, the phage propagating strains of staphylococci were used as representative members of the genus Staphylococcus and a series of biochemical determinations was carried out in order to characterize these strains more fully.

Milk and cervical samples were obtained from 124 cows located on 23 farms in the lower peninsula of Michigan. One hundred sixty-four staphylococcal isolates were obtained from the milk of 92 animals. By subsequent phage typing with the International-Blair and Seto-Wilson phages, it was revealed that there were 30 different phage patterns in 125 sensitive strains. A widely distributed phage type of staphylococcus was not found. The most prevalent type, S2, occurred in 24 samples from 4 farms. The number of different patterns in an individual animal varied from 0 to 3.

The International-Blair phages, normally used to type staphylococci of human origin, were responsible for 62% of the total number of lytic reactions. Phages 42B, 42E, and 44A were the most active of this set. None of the patterns common in human infections was observed, although

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a closely related pattern was found in 6 isolates from 2 farms. In addition, 13 isolates from 4 different herds gave extensive lytic reactions with this series of phages. This evidence suggested a possible cross infectivity or colonization between man and animal.

Differentiation of representative strains from milk was attempted by using a tube dilution technique to measure coagulase quantitatively. Coagulase titers, expressed in reciprocal units, were found to have divergent values, ranging from less than 4 to 2048. Non-typeable organisms were usually low coagulase producers.

Staphylococcus aureus was not commonly found in the reproductive tract samples obtained in this study. Few gram positive cocci were isolated from the cervical samples. Of 15 such isolates, none was found to be coagulase positive and only 1 was sensitive to the phages used. The 1 typeable cervical isolate was not related in phage pattern to the milk isolates obtained from the same animal.

The lack of uniformity in coagulase production by the staphylococci obtained from the clinical samples prompted a systematic study of several biochemical activities of this versatile organism. Using the series of phage propagating strains, the properties measured included quantitative free coagulase by a tube dilution technique; acid phosphatase by a colorimetric procedure using p-nitrophenyl phosphate as a substrate; extracellular protein by a turbidometric trichloroacetic acid method; minimum inhibitory concentration of penicillin; growth capacity in a chemically defined medium; and a qualitative evaluation of mannitol utilization, hemolysin and pigment formation, antibiotic sensitivity, catalase activity, penicillinase production, and gelatin liquefaction.

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These experiments were completed with a manometric determination of oxygen uptake for each strain, as well as the preparation of a family of growth curves in brain heart infusion broth.

The most exceptional strain was #73 which was negative with respect to coagulase, phosphatase, mannitol fermentation, hemolysin formation, gelatinase and penicillinase; it was also sensitive to the antibiotics used, and was lysed by most of the phages. This was followed by strain #70 which not only was catalase negative, and one of the lowest with respect to oxygen uptake and growth rate, but gave the highest coagulase titer (4096). Low coagulase producers were 52A/79, 3B, 3C, 55, 47, 42B and S4. Other notable strains were 80, 53, 54, 75, 77, and 81 which were all penicillinase positive; 44A which gave the highest phosphatase activity; and 29, 52, 47, 73, and 75 which produced no hemolysis in the erythrocytes of the three species used. Human red blood cells were hemolysed only by 3A, 53, and 44A of the International-Blair series, and all but one (S2) of the Seto-Wilson strains.

In general, no one index measured was found to be uniform. Coagulase and phosphatase activity, as well as mannitol utilization invariably occurred together. Variation among the strains was common for all of the properties measured.



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AND BIOCHEMICAL CHARACTERIZATION OF
BASIC PHAGE PROPAGATING STRAINS**

by
John J. ^{Junior} Solomon

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Microbiology and Public Health

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ACKNOWLEDGEMENTS

Sincere thanks are extended to Dr. Charles L. San Clemente for his many helpful suggestions during the course of this study. His constant concern for students and colleagues and his ever present willingness to assist in the solution of any problem were invaluable and very deeply appreciated.

The author also wishes to thank Dr. Donald D. McWade, formerly of the Department of Veterinary Pathology, and Dr. Albert R. Drury, Department of Surgery and Medicine, for their assistance in the clinical phase of this study, and Dr. Delbert E. Schoenhard, Department of Microbiology and Public Health, for his assistance during the study, and the valuable suggestions and criticisms during the preparation of the manuscript.



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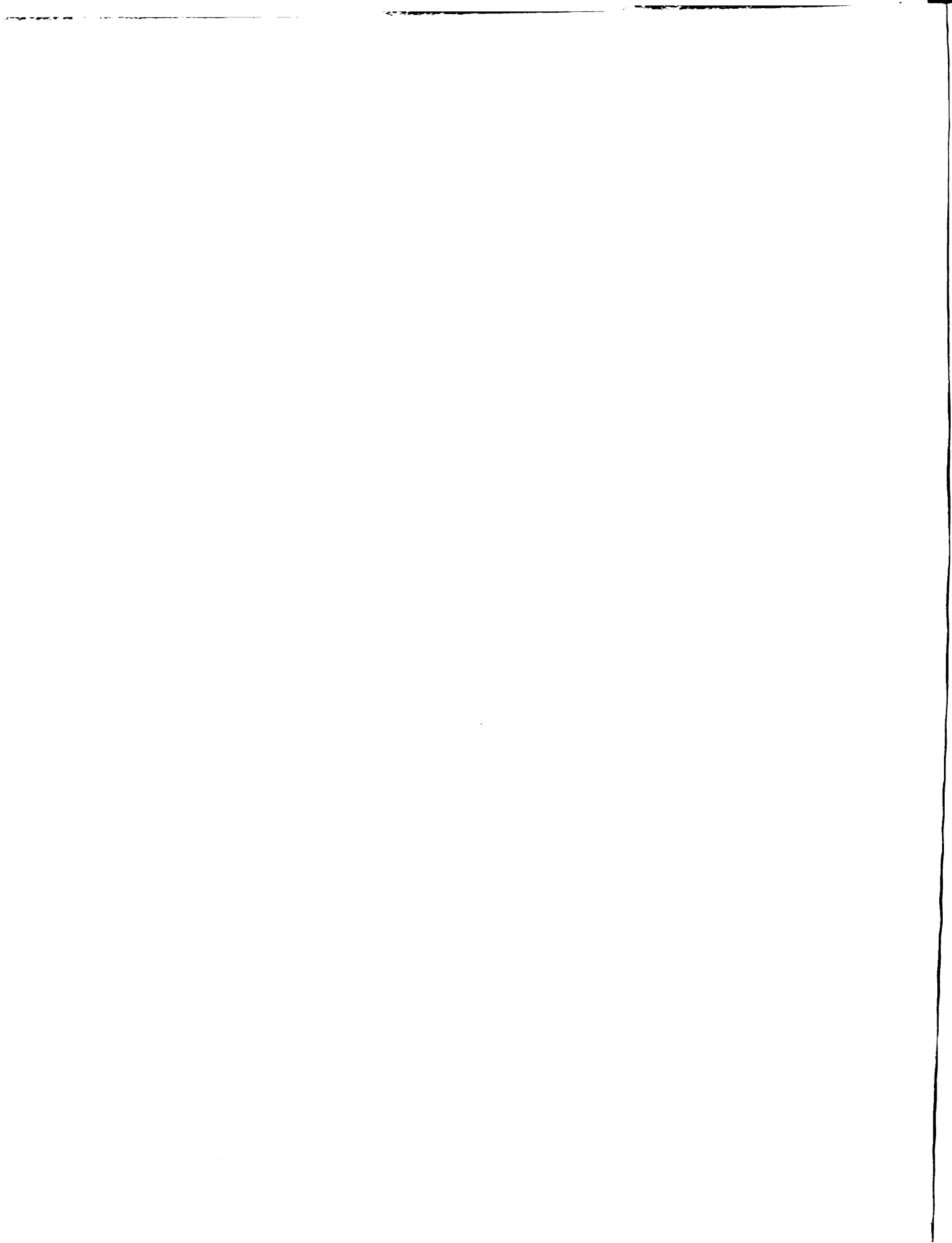
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INTRODUCTION

Staphylococcus aureus has become increasingly important as a human and animal pathogen. In the field of human medicine the staphylococci are frequently involved in skin lesions, burn and post-operative infections, pneumonia, septicemia, diarrhea, food poisoning, and osteomyelitis. Very painful and disabling inflammations are common in severe skin lesions and chronic osteomyelitis caused by this organism. Hospitals have been plagued with endemic and epidemic outbreaks of staphylococcal diseases. The causative organisms frequently fall into a few phage patterns, and often one or two types predominate. The predominating strains appear to have enhanced powers of infectivity and resistance to the antibiotic drugs in common use. One such strain, phage type 80/81, has become known as the epidemic or widely distributed strain because of its common occurrence in hospital nursery outbreaks.

In veterinary medicine the staphylococci are perhaps primarily important in the bovine, although occurring in other species. In dairy animals, S. aureus has become an important cause of mastitis, with a resultant loss of milk and butterfat production, and permanent damage or death to the animals. In some herds it may produce a greater incidence of infection than Streptococcus agalactiae and is often of special concern in herds in which streptococcal mastitis has decreased. It has been reported (Easley et al., 1951; Gunter et al., 1955) that staphylococci are present more frequently in the reproductive tract of cows experiencing difficulties in fertilization. The decrease in reproductive capacity may occur, therefore, as a result of infection with these organisms.

The purpose of this study was to answer several questions. In the survey of farms in Michigan, one object was to determine if a common



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strain of S. aureus occurred in bovine herds as in the case of phage type 80/81 in human infections. A related objective was to learn if cross infection by identifiable strains of staphylococci occurred between man and cow. Phage typing offered a convenient tool for answering both questions. An attempt to achieve an added degree of differentiation was made by measuring quantitative coagulase.

A preliminary survey indicated the presence of a large number of repeat breeder cows and in view of the evidence presented by other workers for the possible incrimination of staphylococci in reproductive failures, a third objective suggested itself; namely, the sampling and culturing of the reproductive tracts of a number of repeat breeders.

Finally, the varied results obtained from the phage typing and coagulase determinations lead to an extensive biochemical characterization of Staphylococcus aureus. The phage propagating strains, both the International-Blair and the Seto-Wilson series, appeared to afford an ideal cross section of the species as indicated by the diversity of phage patterns obtained during the study of the lytic spectrum of the phages.

HISTORICAL

Phage Typing

Twort (1915) was the first to report lysis of micrococci by a transmissible submicroscopic agent. He obtained cultures from calf vaccinia and observed the development of colonies which subsequently became glassy and transparent. A pure culture of micrococcus when touched with a small portion of one of the glassy colonies soon became transparent or glassy. The transparent material was found to pass through porcelain bacteriological filters and to be active in dilute saline or water solutions. Cultures could be carried for long periods without showing the transparencies; however, most pure strains eventually developed such areas. Twort suggested that the phenomenon might be considered an acute infectious disease of the micrococci.

In 1935 Burnet and Lush presented evidence that certain strains of S. aureus could be grouped according to their sensitivity to a series of phages. Williams and Timmins (1938) used the phages isolated by Burnet and Lush to differentiate strains of staphylococcus originating from patients with osteomyelitis. Phage action was indicated by the clearing of broth cultures. Six phage types were found.

Fisk (1942) described a method for the isolation and propagation of bacteriophages carried by lysogenic strains of S. aureus. Lyso-genicity was demonstrated in 19 out of 43 coagulase positive staphylococci by a cross-spotting technique. Several different strains were streaked on a plate and a drop of broth culture from a series of strains was added to each streak. Areas of lysis were harvested and repropagated on the sensitive cultures until confluent lysis occurred.

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Material from the confluent areas was then collected and treated with a low concentration of zephiran. Fisk (1942a) determined the phage susceptibility of 95 strains of S. aureus using 27 different bacteriophages. Cultures to be identified were inoculated on the surface of agar plates and one loopful of each of the test phage solutions was spotted on the seeded area. After suitable inoculation, differentiation of the cultures was accomplished by comparison of their patterns of susceptibility to the phages. Pathogenic staphylococci could be divided into groups as a result of the phage action, and strains isolated from related sources were found to react to the same phages and could be differentiated from other cultures by this method. Fisk and Mordvin (1944) continued the bacteriophage typing of staphylococci using 78 strains of S. aureus obtained from 30 patients. Again it was observed that most cultures from related sources were of the same bacteriophage type.

Wilson and Atkinson (1945) extended Fisk's techniques to permit the differentiation of staphylococci in a manner comparable to that used for the phage typing of typhoid bacilli. The phages were isolated using Fisk's cross-spotting technique and propagated initially in young broth cultures of susceptible host strains. The supernatant fluid was filtered through a Seitz pad and plated on a susceptible strain of staphylococcus. A single plaque was taken up in a small volume of water and again plated on the susceptible strain. The fresh plaque thus obtained was propagated in broth until the titer increased and the resulting phage material was diluted until the highest dilution producing confluent lysis was obtained. This was the test dilution of the phage and was used for typing the staphylococci. Four hundred and sixty strains of S. aureus were subjected to the phage action and 21 patterns

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of lysis noted. Sixty percent of the organisms were successfully typed. The origin of breast abscesses, pemphigus, and outbreaks of food poisoning were traced by this procedure.

A valuable contribution to strain identification was made by the establishment in 1946 of a center for the routine typing of staphylococci employing the method and phages of Wilson and Atkinson in the Central Public Health Laboratories, Colindale, London, England, under the direction of Dr. V. D. Allison (Blair and Carr, 1960). Williams and Rippon (1952) refined the methods for typing staphylococci and subsequently their techniques were established as a basis for further phage typing procedures. The Subcommittee on Phage Typing of Staphylococci of the International Committee on Bacteriological Nomenclature, was established during the Sixth International Congress for Microbiology in Rome in 1953, and recommended that a basic series of 19 phages be used for the routine typing of human staphylococci. The subcommittee felt that rigid rules should not be established, but formulated certain recommended basic procedures. Essentially the methods followed those evolved at the Colindale Laboratories. The 19 basic phages were divided into 4 groups as follows:

- I. 29, 52, 52A, and 79.
- II. 3A, 3B, 3C, and 55.
- III. 6, 7, 42E, 47, 53, 54, 70, 73, 75, and 77.
- IV. 42D.

The broad groups I, II, and III were so numbered because the phages comprising each group generally exhibited some degree of specific action against the staphylococci belonging to the corresponding serological groups established in 1939 by Cowan. In addition to the basic

series other phages were allowed by the committee if an investigator felt they would provide useful information.

The techniques of typing were described in further detail by Blair and Carr (1953), and Jackson et al. (1954). Cultures were assigned to a broad phage group on the basis of their lysis by one or more of the phages within that group. It was often found that lysis was produced by two or more phages from a broad group to give "patterns" of lysis, although some cultures were lysed specifically by a single phage. Identical strains of staphylococci gave identical or closely similar patterns, while unrelated strains showed different lytic reactions.

The Subcommittee on Staphylococcal Phage Typing of the International Committee on Bacteriological Nomenclature at its 1958 meeting revised its list of basic phages. The series included phages originally used by Wilson and Atkinson (1945) or those isolated in or received at the Colindale laboratory. The revised groups included:

- I. 29, 52, 52A, 79, and 80.
- II. 3A, 3B, 3C, 55, and 71.
- III. 6, 7, 42E, 47, 53, 54, 73, 75, and 77.
- IV. 42D.
- Miscellaneous. 187.

Additional phages were again allowed as adjuncts to the basic groups.

Phage Typing of Staphylococci Associated with Bovine Mastitis

Slanetz and Jawetz (1941) isolated staphylococcal bacteriophage from the milk of 7 out of 20 cows which contained staphylococci. The bovine phages resembled those obtained from human sources in many respects, and attempts to show specificity of lysis by these phages on bovine staphylococci failed completely. The broad lytic spectrum

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of the phages prevented their use in epizootic studies.

Macdonald (Williams-Smith, 1948) in 1946 reported on the phage typing of staphylococci from milk and from cases of bovine mastitis using phages provided by Wilson and Atkinson. One hundred twenty-three of 150 strains isolated from milk were susceptible to phage 42D and twelve were lysed by other phages. Fifteen strains could not be typed. All of the strains obtained from cases of mastitis were sensitive, and all 34 belonged to phage type 42D. Williams-Smith (1948) isolated 1,016 strains of staphylococci from bovine milk samples and subjected them to the action of the Wilson series of bacteriophages. Ninety-three percent were typeable. In each of 23 herds the predominant phage type was one of the 42D group. Frequently one phage type predominated in the strains isolated from any one herd. Phage typing did not aid in distinguishing between strains isolated from cows with mastitis and those strains from apparently normal udders.

Price et al. (1954) found a number of characteristic phage patterns among their strains of staphylococci isolated from bovine mastitis. The 42D pattern was again the most prevalent. Staphylococci from heavily infected herds usually gave several different phage patterns. Only one type was commonly found in a particular quarter, however.

Seto and Wilson (1958) utilized the International-Blair, and Jackson phages in typing 102 cultures of S. aureus of bovine origin. Thirty-four cultures were not sensitive to any of the above phages. Of the sensitive strains, the predominant phage type was 44A. Phage 42D and the six phages isolated by Seto et al. (1956) were used to type a larger set of bovine staphylococci. Type S2 was found to be predominant, accounting for 49% of the 429 cultures. The organisms isolated from

small herds tended to be of a single type, while the isolates from a large herd consisted of various types or patterns. More than one phage type of staphylococcus was sometimes obtained from the different quarters of the same animal.

Coles and Eisenstark (1959a) found S. aureus strains of bovine origin to be relatively resistant to the action of phages utilized in typing cultures of human origin. Two such phages, 81 and 42D, however, did produce lysis in a number of the strains. Using a series of phages obtained by adaptation and from lysogenic staphylococci, Coles and Eisenstark (1959) found wide variation of phage type strains of S. aureus within a single herd and more than one type from individual animals. Eighteen different patterns of lysis occurred in 50 cultures obtained from one herd. Not more than one strain of S. aureus was isolated from any one quarter of a cow.

A number of investigators (Edwards and Rippon, 1957; Pagano et al., 1960; Thorne and Wallmark, 1960; and Wallace et al., 1960) have isolated strains of staphylococci from dairy animals and dairy personnel which have identical phage patterns of lysis. The widely distributed or so-called epidemic strain, 80/81, has been isolated from the milk of cows in a number of herds. (Wallace et al., 1960; Fratto and Mann, 1960; and Pagano et al., 1960).

Coagulase

Loeb (1903) first observed in vitro clotting by staphylococci using goose plasma. He found that S. aureus had a specific effect in causing the plasma to coagulate. The phenomenon was independently described by Much (1908) during an investigation of the bactericidal

behavior of human plasma. Using a small inoculum of S. aureus he noted a coagulation of the plasma after overnight incubation, while with larger inocula a much shorter period of incubation was required for the plasma to clot. He found that several aureus strains caused coagulation, whereas albus and citreus strains and several other bacterial species did not. He called the clotting principle a staphylokinase. Gratia (1920) studied the nature of the coagulating agent and found it to be thermostable and to resemble the action of thrombin in producing fibrin from a solution of fibrinogen. He concluded, however, that the substance was not thrombin or any of its precursors. He called the active material staphylocoagulase. Gross (1931) showed that the reaction was readily elicited from culture filtrates of S. aureus, and in 1933 called attention to the fact that blood coagulation was frequently accelerated in staphylococcal infections. This was observed during surgical intervention of staphylococcal osteomyelitis and carbuncles. The clotting occurred more quickly than in streptococcal infections, where secondary bleeding was sometimes a feared complication. In vitro experiments confirmed the plasma clotting ability of the staphylococci. Pyogenic S. albus reacted more slowly and to a lesser extent than S. aureus. A number of other bacterial species were tested, but all were found to be negative. Gross' results thus revealed an important characteristic of the pathogenic staphylococci. Daranyi (1926) had previously demonstrated that pathogenicity for rabbits and the ability to clot citrated blood were parallel characteristics, and concluded (1935) that only staphylococci from pathogenic processes were able to clot plasma. He advocated the use of this test for differential diagnosis of staphylococcal pathogenicity. Cruikshank (1937) likewise demonstrated the

ability of the pathogenic members to coagulate plasma. He investigated a number of characteristics associated with staphylococci and found that the presence of coagulase was the property most closely correlated with pathogenicity for the rabbit. He recommended the use of this property as a test for pathogenicity because of its constancy and ease of demonstration. Other workers, Chapman et al. (1934) confirmed the presence of the plasma clotting property in pathogenic staphylococci, and concluded (Chapman et al., 1938) that it was the most reliable single test for the differentiation of pathogenic and non-pathogenic staphylococci. As a result, the coagulase test has been universally accepted for the recognition of pathogenic staphylococci and has replaced the multitude of other tests used in the past (Elek, 1959). However, even though coagulase has been considered by most to be the best single criterion in estimating the potential pathogenicity of a staphylococcal strain, its exact role in the pathogenesis of infection is not clear. Menkin and Walston (1934) studied the diffusion of trypan blue in rabbits after staphylococcal infections and following the injection of coagulase precipitated from culture filtrates. They found that the coagulase failed to exert a sufficiently powerful local reaction to obstruct lymphatic drainage by means of a fibrinous barrier, and concluded that it did not play a role in mechanical obstruction to lymph flow or the "walling off" reaction induced by staphylococci. Hale and Smith (1945) suggested a possible role of coagulase in the inhibition of phagocytosis by the formation of a fibrin coat around the cocci. The number of coagulase positive staphylococci ingested by rabbit or human polymorphonuclear leucocytes was reported to be significantly lower than coagulase negative cocci. Yotis and Ekstedt (1959) showed

that coagulase positive strains of S. aureus respired more actively in human serum than did coagulase negative strains. Purified coagulase increased the respiration of both strains, but affected the negative strains about twice as much as the positive strains. Ekstedt and Yotis (1960) also found that the virulence of coagulase negative staphylococci was increased when the organisms were suspended in solutions of partially purified coagulase and injected intracerebrally into mice. Cocci or coagulase alone were innocuous. Blobel and Berman (1961) found a marked decrease in plasma proteins following the injection of coagulase preparations into rabbits. Fibrinogen was almost completely depleted in some animals. Intravascular clots containing the enzyme were found in the dilated capillaries and venules of the lungs of rabbits receiving a lethal dose of the coagulase.

Phosphatase

Phosphatase activity was demonstrated in staphylococci in 1932 by Gordon and Cooper who established the presence of a glycerophosphatase in chloroform killed suspensions of S. aureus. Bray and King in 1942 introduced the use of phenolphthalein phosphate as a substrate for the detection of phosphatase activity, and noted that the staphylococci gave strong reactions. They failed, however, (1943) to find differences between S. aureus and S. albus. Barber and Kuper (1951) studied the phosphatase activity of 100 strains of S. aureus using phenolphthalein phosphate in nutrient agar as the substrate. Forty two per cent of the cultures liberated phenolphthalein, which was indicated by the presence of a pink color after exposure to ammonia. One hundred and sixty representative colonies were picked from the 42 plates showing phosphatase positive staphylococci and were tested for coagulase production. All

but one were positive. Staphylococcal colonies obtained from phosphatase negative strains were all found to be coagulase negative. Barber et al. (1951) tested a larger series of staphylococci using the same substrate in a fluid medium. All the coagulase positive strains liberated free phenolphthalein in 24 hours or less. Most of the coagulase negative strains also produced phosphatase; however, a longer period of incubation, from 2 to 7 days, was required. Twenty seven per cent did not produce phosphatase at the end of 7 days incubation. Pan and Blumenthal (1961) obtained similar results, finding that the average coagulase positive strain of S. aureus produced almost 5 times more acid phosphatase than the average coagulase negative strain. Occasionally, coagulase negative strains were more phosphatase active than the coagulase producers.

Hemolysin

The importance of the hemolytic activity of staphylococci was discovered early in the study of these organisms. Krauss and Clairmont (1900) presented evidence that staphylococci laked blood. They used agar plates smeared with blood and noticed that rabbit cells were lysed by S. aureus, but human cells were not affected. In the following year, Meisser and Wechsberg (1901) demonstrated a hemolytic substance in filtrates of broth cultures. They found that the hemolytic substance began to appear on the fourth day in alkaline beef broth, and that both albus and aureus strains produced the lysin, although the virulent strains produced greater quantities than albus or avirulent strains. These results were corroborated later by Kutscher and Konrich (1904), who stated that all pyogenic staphylococci produced hemolysin and that failure to find it was due to a delayed production by some strains. Julianelle (1922) found that all the staphylococcal cultures isolated

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during his investigation were hemolytic, although some strains required a longer period of incubation before showing this property. Pathogenicity studies with mice and rabbits indicated that hemolytic strains were not necessarily virulent. Chapman et al. (1934) agreed with Julianelle after studying some 5,000 strains of staphylococci. They concluded that a knowledge of hemolytic activity alone was insufficient to confirm pathogenicity of the staphylococci. When used in conjunction with other tests, however, Chapman et al. (1938) concluded that hemolysis on rabbit blood agar permitted a simple estimation of the pathogenicity of a strain. Elek and Levy (1950) examined 359 strains of staphylococci for hemolysin production, using blood agar plates and specific neutralization with anti-hemolysin. Three distinct hemolysins were found to be associated with coagulase positive strains. They were identified as the alpha, beta, and delta lysins. Alpha was distinguished by complete lysis of rabbit and sheep erythrocytes and inhibition by alpha antihemolysin. Beta acted only on sheep cells and did not produce complete lysis, while delta caused complete laking of both sheep and rabbit cells. Neither beta nor delta lysins were inhibited by the anti-alpha serum. Delta lysin could also be distinguished from alpha and beta by its action against horse and human erythrocytes (Marks and Vaughan, 1950). One of the three lysins was invariably present in all the coagulase positive strains, either alone or in combination. The alpha-delta pattern occurred most frequently in strains isolated from humans. Some coagulase negative strains were also lytic for sheep and rabbit cells, but could not be inhibited by alpha, beta, or delta antilyns. This type was designated as epsilon hemolysin. Elek (1959) concluded that there are probably only three antigenically different hemolysins produced by coagulase positive staphylococci, and that the production of all three is typical for any strain.

Losses may occur as a result of mutation. Brown (1960) in his study of 400 coagulase producing strains isolated from human lesions, found 99.8% of the strains produced alpha lysin in detectable quantities. Strains from acute lesions in apparently undamaged skin tended to produce more alpha hemolysin than those from burns and post operative wounds; these, in turn, were more active than cocci isolated from the urinary tract. He concluded that although coagulase may well be necessary for the pathogenicity of a strain, the degree of virulence might be most conveniently estimated by its alpha hemolysin production.

Other Physiological Properties

A number of other characteristics of the staphylococci have been associated at one time or another with the ability of this genus to establish pathogenic lesions. The characteristic orange-yellow growth of staphylococci was connected to the yellow appearance of pus as far back as 1882 by Ogston (Elek, 1959). Dudgeon (1908) observed that suppurative and severe inflammatory infections were usually caused by the aureus strain, whereas mild inflammatory processes were due to S. albus. Julianelle (1922) found no relationship between pigmentation and the production of hemolysin. Chapman et al. (1934), however, found that the importance of the hemolysin test depended on an accurate determination of the color produced by the strain. Hemolytic, non-coagulating albus strains were generally innocuous, whereas hemolytic aureus strains were usually pathogenic regardless of their coagulase capacity. Barber (1955) isolated white variants from 6 of 8 strains of S. aureus by cultivation in a fluid medium. The variants all resembled the parent culture from which they were derived, both in the production of coagulase and alpha lysin, and, with one exception, in phage type. Likewise, she

obtained golden variants from white strains of coagulase positive staphylococci which resembled the parent strains.

The use of fermentation reactions with other species led many workers to try this approach with the staphylococci. Dudgeon (1908) tested a number of carbohydrates, but found that most were of little value. Litmus mannite, however, was acidified by most of the staphylococci obtained from pathogenic sources. All of the aureus cultures obtained from bone suppurations were found to acidify the medium. About one-half of the white staphylococci obtained from healing wounds did not ferment the mannitol. Julianelle (1937) studied the fermentative ability of a number of pathogenic and non-pathogenic strains of staphylococci on mannitol. He found that 97% of 62 pathogenic, and 8% of 40 non-pathogenic strains were able to utilize the mannite. Similar results were obtained by Thygeson (1938) in a study of staphylococci causing conjunctivitis in rabbits. He found that 97% of 110 strains could utilize the mannitol, while only 2% of the nontoxigenic strains possessed this ability. Fahlberg and Marston (1959) observed that all of the coagulase positive strains isolated from human pathological lesions in their study were able to utilize mannitol.

The liquefaction of gelatin by staphylococci was also noted early in the study of these organisms. Dudgeon (1908) found that rarely did S. aureus fail to exhibit this ability, and that most of the albus strains were similarly endowed. Stone (1935) used a beef extract gelatin medium and found that the ability to liquefy gelatin was correlated with enterotoxin producing capacity. A negative gelatinase test usually indicated a deficiency in this ability. Cruikshank (1937) found that most of the strains in his study which were pathogenic for rabbits, and about one-half

of the non-pathogenic strains, were able to liquefy gelatin. Richou, et al, (1952) however, could find no relationship between pathogenicity and gelatin liquefaction in staphylococci isolated from the bovine udder. Elek (1959) likewise indicated that gelatinase production was not correlated with pathogenicity.

Catalase was probably demonstrated in the staphylococci by Burnet (1925) when he observed the presence of a diffusible substance which destroyed hydrogen peroxide formed on agar plates by exposure to sunlight or a carbon arc. Scouller (1948) tested 146 cultures classified as staphylococcus, micrococcus, and sarcina for the evolution of gas after the addition of hydrogen peroxide. All were catalase positive. He failed to obtain catalase activity from 116 strains of streptococci and suggested the separation of these organisms from the micrococci by the catalase test. Lucas and Seeley (1955), however, obtained a strain of S. aureus from normal urine which failed to exhibit catalase activity. Cell suspensions and cell extracts tested manometrically, and colonies on solid media of low glucose content failed to release oxygen from hydrogen peroxide, indicating that occasional catalase negative, coagulase positive staphylococci may be encountered.

Abraham and Chain (1940) were the first to show that some bacteria produce an enzyme capable of destroying penicillin. They called it penicillinase. The enzyme was obtained from extracts of E. coli, but not from a penicillin sensitive strain of S. aureus. Kirby (1944) obtained the enzyme from 7 strains of coagulase positive S. aureus, all of which were naturally resistant to penicillin. Penicillinase was not present in extracts of penicillin sensitive strains. Barber (1948) isolated a number of penicillin resistant strains from clinical samples

and found that all produced penicillinase. The resistant strains could not be differentiated from sensitive strains by cultural appearances, biochemical reactions, or pathogenicity to rabbits. El Ghoroury (1952) described a plate method for the detection of penicillinase. Blood agar plates were streaked with a penicillin sensitive strain and penicillin soaked discs placed on the plate surface. A loopful of the strain being tested was then added to the disc. Subsequent growth of the sensitive strain around a disc indicated the presence of penicillinase. All the naturally resistant strains studied were found to produce this penicillin inactivator. Gillissen and Ruda (1957) noted that a direct relationship existed between phosphatase activity and penicillin resistance. Resistant strains produced about 2.5 times as much acid phosphatase as sensitive strains. Markov et al. (1958), however, failed to confirm these results. Resistant and sensitive staphylococci isolated from pathological processes produced about the same amount of phosphatase. Coagulase production was not influenced by the ability to resist penicillin.

Synthetic Medium

Hughes (1932) initiated the development of a synthetic medium for staphylococci. He found that the organisms would not normally grow in a casein hydrolysate medium, but that the addition of a small amount of meat extract promoted heavy growth. Meat extract alone proved to be an adequate medium, but 50 times as much were required. Knight (1935) confirmed the existence of this essential factor and prepared an active fraction from yeast extract which allowed aerobic growth of 10 strains of S. aureus on a medium containing acid hydrolysed gelatin. Hemolysin producers which grew in this medium were able to produce this toxin. It was then found by Fildes et al. (1936) that the acid hydrolysed gelatin

could be replaced by a mixture of 14 amino acids and Knight (1937) found that nicotinic acid or amide could replace the yeast extract in the gelatin hydrolysate basal medium, but not in the amino acid medium. Further work by Knight (1937a), however, demonstrated that vitamin B₁ and nicotinic acid together would support abundant growth of S. aureus in the amino acid basal medium previously utilized. Concentrations of 2 ug of vitamin B₁ and 200 ug of nicotinic acid per liter were sufficient to support good growth. Porter and Pelczar (1941) used the synthetic medium of Gladstone (1937) and studied the effect of biotin on the growth of S. aureus. Certain strains were unable to grow in the synthetic medium without the addition of biotin whereas other strains were not stimulated by this vitamin. Gretter et al. (1955) found that 90 coagulase positive strains grew well when thiamin and nicotinic acid were the only vitamins added to a casein hydrolysate medium. Coagulase negative strains, however, frequently had an absolute requirement for biotin. Thirty-eight out of 46 negative strains failed to grow in the absence of biotin, and the remaining 8 strains attained only 50-60% of maximum growth. Surgalla (1947) described a synthetic medium for the production of staphylococcal enterotoxin; Mergenhagen (1958) obtained the synthesis of hyaluronidase in a defined medium, and Lominski et al. (1950), succeeded in obtaining the production of coagulase in a medium of known composition. The amount of coagulase produced was small, however. Szeto and Halick (1958) likewise reported low coagulase production in their synthetic medium. Marston and Fahlberg (1960), however, using a slight modification of the defined medium of Mergenhagen, obtained coagulase production with two strains of S. aureus which was comparable to a reference medium prepared by dialysis of brain heart infusion broth.

MATERIALS AND METHODS

Milk Samples

Animals with mastitis or reproductive difficulties, or both, were selected from the herd. Strip cup examination was made of the mammary secretion of each quarter prior to collecting the sample for bacteriological examination. The udder was washed with a bactericidal solution with particular attention centered on washing the teat orifices. Approximately 10 ml of milk were collected from each quarter in sterile tubes. A loopful was streaked on tryptose agar (Difco) containing 5% bovine blood from selected animals, and the remainder incubated at 37°C for 24 hours prior to microscopic examination. The plates were incubated for 24 hours at 37°C and hemolytic colonies selected for morphological observation and coagulase determination.

Reproductive Tract Samples

The animals were prepared for sampling by scrubbing the area around the anus and vulva with soap, followed by a rinsing with bactericidal solution. A sterile glass speculum was then inserted into the vagina and the area of the cervix sampled by inserting a sterile swab attached to a flexible metal rod contained in a stainless steel tube. The swab was placed in nutrient broth (Difco) and held overnight at 37°C. Both swab and broth were then streaked on staphylococcus 110 agar (Difco), and the resulting colonies were selected for further study.

Phage Preparation

A set of 24 international phages and their corresponding propagating strains of staphylococcus were obtained from Dr. John E. Blair, Hospital for Joint Diseases, New York City, New York, and the 6 Seto-Wilson phages

and propagating strains from Dr. J. B. Wilson, University of Wisconsin, Madison, Wisconsin. The method used for phage propagation was the soft agar layer procedure recommended by the Communicable Disease Center, Atlanta, Georgia. The final phage preparation was obtained by freezing and thawing the agar which contained the phage-staphylococcal mixture. The free fluid was removed by pipette and assayed by the agar layer technique described by Adams (1950). It was then filtered through a Swinney filter containing approximately one-half thickness of a Seitz filter pad and again assayed. The critical test dilution (C.T.D.) was used to determine the lytic pattern of the staphylococci and was the highest tenfold dilution ($\leq 10^{-3}$) which caused confluent lysis of the propagating strain. The phage solutions were stored at 4°C .

Phage was also prepared in fluid culture by inoculating flasks of trypticase soy broth¹ with equal volumes of concentrated phage and 5-6 hour growth of propagating strain. The flasks were then incubated for 4-6 hours at 37°C on a rotary shaker, and the resulting clear or turbid fluid assayed for phage activity. Titers of 10^8 - 10^{11} particles per milliliter were usually obtained by this method.

Phage Typing

One to 2 ml of a 3-5 hour trypticase soy broth culture of each of the staphylococcal isolates were flooded over the surface of a trypticase soy agar¹ plate previously dried at room temperature. The excess inoculum was removed and the plates again allowed to dry. A drop of phage (C.T.D.) was then added to a marked area of the plate using a 1 ml syringe fitted with a 27 gauge needle. Twenty different phages could be added to one plate. After

¹Baltimore Biological Laboratories, Baltimore, Maryland

overnight incubation, the plates were observed for lytic activity within the marked areas. Phages causing more than 20 plaques were reported as part of the pattern of a strain. Each phage was checked against its own propagating strain during each experiment, and every phage was checked against all propagating strains when initially prepared or prepared anew.

Coagulase Determination

The initial coagulase test on the cultures was made by inoculating 0.5 ml of rabbit or human plasma with 0.5 ml of 16-24 hour culture grown in brain heart infusion broth (Difco). Coagulase was determined quantitatively by using the tube dilution technique of Tager and Hales (1948). The isolates were grown for 5 days at 37°C in brain heart infusion broth. The cells were centrifuged at 2500 rev/min for 10 min and one ml of supernatant fluid diluted serially with an equal volume of 2% peptone-saline solution containing 1:5000 merthiolate. One ml of plasma, diluted 1:5 in physiological saline, was then added to each tube. After thorough mixing, the tubes were incubated in a 37°C water bath. Results were recorded after 4 and 24 hours of incubation and expressed as the reciprocal of the dilution of the supernatant fluid. The endpoint, obtained after 24 hours incubation, was the last tube showing a one plus reaction. Tubes were graded according to the degree of firmness of the clot. Tubes in which the entire contents had coagulated were labeled as 4 plus and those which contained a large organized clot as 3 plus. A small organized clot was indicated by a 2 plus reading, and small unorganized clots as 1 plus.

Rabbit plasma was obtained in the dehydrated form from Difco. Human plasma was prepared from outdated, citrated human blood. Each plasma, diluted 1:5 with saline was first reacted with a coagulase preparation

obtained from a known high coagulase producing staphylococcus. Complementary plasmas were then pooled, dispensed in 10 ml amounts, and frozen.

Mannitol Fermentation

Phenol red broth containing 1% mannitol was inoculated from stock slant cultures of staphylococci. The tubes were incubated for 48 hours at 37°C and the results read at 24 and 48 hours. The formation of acid was indicated by a change in color of the indicator from red to yellow.

Pigment Formation

Pigmentation was determined on nutrient agar containing 10% evaporated milk and 0.5% mannitol. The cultures were first grown in brain heart infusion broth overnight and streaked on the nutrient agar. The plates were incubated at room temperature for one week and observed daily.

Hemolysin Production

The hemolytic patterns of the propagating strains were determined on agar plates with blood from man, rabbit, and sheep. Difco heart infusion agar was prepared to contain 0.2% KH_2PO_4 and 0.03% MgSO_4 . The pH was adjusted to 7.4 and the medium autoclaved. After cooling, 12 to 15 ml of the medium were poured into plates and 0.5 ml of washed red blood cells of the desired species was dispersed in the agar. The cells had been collected in Alsever's solution (Jackson and Little, 1957), washed two times in saline, and prepared as a 50% mixture in saline prior to use. The blood-agar plates were streaked with growth from a 24-hour trypticase soy broth tube and incubated for 48 hours at 37°C in an atmosphere of 30% CO_2 . Results were recorded at 24 and 48 hours.

Antibiotic Sensitivity

An initial concentration of 40 units per ml of sodium penicillin G was prepared in sterile pH 6.0 phosphate buffer. Serial two-fold dilutions were then prepared using penassay broth (Difco) as the diluent, and 0.5 ml of each dilution was dispensed into a set of 13 by 100 mm tubes. The inoculum was prepared by diluting an overnight trypticase soy broth culture 1:1000 in saline and from this a 1:10 dilution in penassay broth. One-half ml of the inoculum was then added to tubes containing each concentration of penicillin. The tubes were incubated at 37°C and observed at 24 and 48 hours for growth.

Penicillinase Production

Penicillinase was determined by the method of Innes (1960). Nutrient agar was seeded with a penicillin sensitive strain of S. aureus, 209p, and poured into plates. A one-fourth inch ditch was cut into the agar and filled with nutrient agar containing 10 units of penicillin per ml. The test organisms were streaked across the ditch and the plates incubated overnight. Penicillinase was estimated by the presence of a V-shaped growth of the sensitive strain near the point where the test strain crossed the penicillin agar.

Extracellular Protein

Extracellular protein (Stadtman et al., 1951) was determined on the supernatant fluid from a 5-day culture in brain heart infusion broth. Two ml of 5% trichloroacetic acid were added to a cuvette containing 2 ml of supernatant fluid. The tubes were shaken and allowed to react for 30 seconds after which the optical density was determined at 540 mμ using a Bausch and Lomb Spectronic 20 spectrophotometer. Supernatant fluid and water in the same proportions were used to adjust the instrument to zero optical density.

Growth Curves

A 6-hour culture in brain heart infusion was adjusted to an optical density of 0.4 at 650 mu, and 6 ml used to inoculate 100 ml of brain heart infusion broth. Flask contents were mixed well and dispensed into test tubes. The tubes were incubated in a 37°C water bath and at one-hour intervals tubes were removed, mixed well, and the optical density determined after setting the instrument with a sterile brain heart infusion broth blank. Optical density was plotted against time, and the growth curves obtained.

Phosphatase

Cellular phosphatase was determined using the method of Barnes and Morris (1957). The cells were grown on brain heart infusion agar slants for 22 hours and harvested by washing the slants with citric acid-sodium hydroxide buffer, pH 5.6. The suspension was then adjusted to an optical density of 0.63 at a wave length of 600 mu with the pH 5.6 buffer. Two and four-tenths ml of cell suspension were then added to 0.6 ml of 0.5% p-nitrophenylphosphate disodium solution. The tubes were incubated for 30 minutes in a 37°C water bath, and 1 ml of 0.01N NaOH added to each to stop the reaction and to develop the color of the p-nitrophenol. The samples were centrifuged and the optical density of the supernatant fluid determined at 400mu. The measurements were repeated after acidification with one drop of concentrated HCl to obtain the optical density values not contributed by the p-nitrophenol.

Catalase

Trypticase soy broth tubes were inoculated from stock slants of the propagating strains and incubated overnight. One loopful was then used

to inoculate 20 ml of nutrient broth contained in Smith fermentation tubes. The fermentation tubes were incubated for 48 hours at 37°C, and 1 ml of a 3% H_2O_2 solution added to each tube. The tubes were stoppered and the amount of gas formed in the closed end was measured after an additional 1 hour incubation period.

Gelatinase

Gelatinase activity was measured by two different procedures. Nutrient gelatin was inoculated from an overnight broth culture and incubated 48 hours at 37°C. Liquefaction of the gelatin was then determined after a 1 hour period of refrigeration. In the second procedure, nutrient agar containing 0.4% gelatin was prepared and poured into petri dishes. An overnight broth culture of the test strain was then streaked on one-third of the plate. After 48 hours incubation, a mercuric chloride solution (15g HgCl_2 dissolved in 100 ml of H_2O and 20 ml concentrated HCl) was flooded over the surface of the agar plate. Gelatinase activity was indicated by the presence of a clear zone around the colonies.

Oxygen Uptake

Oxygen uptake was determined by the conventional manometric technique, and expressed as microliters of oxygen per milligram dry weight of cells per hour. The propagating strains were grown in brain heart infusion broth for two 10-hour periods and washed twice in saline. A suspension of cells in saline was adjusted to an optical density of 1.0, and 1.5 ml added to the main compartment of a Warburg flask containing 0.5 ml of 0.1M, pH 7.0 phosphate buffer. Two-tenths ml of 20% KOH was added to the center well, and 1.0 ml of 0.2% glucose to the side

arm. The flasks were placed in a 37°C water bath and allowed to equilibrate to bath temperature. The side arm contents were then tipped into the main compartment, and manometer readings taken every 10 minutes for a 2-hour period. Control flasks for endogenous measurement containing 1 ml of H₂O in the side arm were run for each strain. Dry weights were determined by drying 5 ml of the cell suspensions overnight at 80°C in tared containers.

Synthetic Medium

A synthetic medium was developed by adding mixtures of amino acids to filter paper discs on the surface of seeded agar plates. The agar plates contained 0.2% glucose, 0.0005% each of thiamine hydrochloride and nicotinic acid, 0.02% MgSO₄·7H₂O, 0.1% Na₂HPO₄·12H₂O, and 1.5% agar, and were seeded by streaking the surface with a 10⁵ dilution of 6 hour broth cultures of propagating strains S3 and 70. The amino acid mixtures were prepared by combining individual acids so that each acid in turn was omitted from a mixture, with the exception of the control, which contained all 16 amino acids. Individual acids (0.2%) were dissolved in 0.15M pH 7.4 phosphate buffer and sterilized by autoclaving. Cystine and tyrosine were first dissolved in a small volume of 1N HCl. One-tenth ml of each mixture was then added to a 1 cm diameter disc on the surface of the seeded agar and the plates incubated for 48 hours. Mixtures which failed to support growth or which allowed poor growth were thus determined and the omitted amino acids of these mixtures were utilized for further development of a liquid medium.

RESULTS

Phage Typing and Coagulase Activity of Clinical Isolates

The lytic activity of the typing phages against all the propagating strains was determined prior to the study of the clinical isolates. The results, shown in Figures 1 and 2, compared favorably with those of other workers (Seto and Wilson, 1958; Blair and Carr, 1960).

Milk and cervical samples were obtained from 124 cows located on 23 farms in the lower peninsula of Michigan. From the 496 quarters sampled, 164 (92 cows) yielded staphylococci which were phage typed. The results are illustration in Tables 1-4. Table 1 indicates the total number of phage patterns obtained on the 23 farms. The number of patterns per herd varied from 0 (non-typeable) to 4, and the total number of patterns from all the herds was 42. However, since 3 of the patterns (Table 3) were common to more than 1 farm and 2 remained unchanged during two visits to the same farm, only 30 of the patterns were different from one another. The various patterns obtained are indicated in Table 2 where the lytic spectrum of the 164 isolates is shown by cow number and quarter. One hundred twenty five of the strains were sensitive to one or more of the typing phages, giving a total of 614 significant lytic reactions. Thirty nine of the strains were not typeable. It is evident from these data, as well as in Table 1, that a number of herds contained strains of more than one phage pattern. Included within this group were several farms which had only a small number of infected animals, as in herds D, J, N, and R. Farms which yielded a larger number of infected animals (P, Q, and S) had about the same number of phage strains as those yielding fewer positive animals.

		PHAGE																							
		I				II				III								IV		Misc.					
		29	52	52 A	79	3A	3B	3C	55	6	7	42 E	47	53	54	70	73	75	77	42 B	VA 4	42 D	44 A	81	80
I	29	+++																							
	52		+++																						+
	52A			+++	+++																				+
	79			+++	+++																				
II	3A					+++																			
	3B						++																		
	3C					+++	+++																		
	55					+++	+	+++																	
III	6									++			+++		++			+++			++				
	7										++														
	42E											+++													
	47												+++					++							
	53													+++	+			+++	++		+++			+	
	54												++	+	++			+			+				
	70									+			+	+	+++				+		++				
	73	++	++	++	+++	++	++	++	++	+++	++	++	++	+++	++	++	+++	++	++	+++	+++		++	++	++
	75																	+							
	77													+					+++		+++				
	42B																			+++					
IV	VA4									+++			+						++		+++			+++	
	42D																					+++			
	44A																						+++		
	81																						+++	+++	
Misc.	80																						+++	+++	

Fig. 1. Significant lytic reactions of the International-Blair series of phage propagating strains of staphylococci

		PHAGE					
		S1	S2	S3	S4	S5	S6
PROPAGATING STRAINS	S1	+++		+++			
	S2		+++				
	S3			+++			
	S4				+++		+++
	S5					+++	
	S6						+++

Fig. 2. Significant lytic reactions of the Seto-Wilson series of phage propagating strains of staphylococci

TABLE 1. The number of phage patterns of strains of Staphylococcus aureus isolated from the udder of selected cows
from 23 herds

Herd	Cows Yielding <u>Staphylococci</u>	Quarters Yielding <u>Staphylococci</u>	No. of Phage Patterns	No. of Organisms Not Typeable
A	3	6	1	5
B	2	5	1	2
C	2	3	1	0
D	4	10	4	3
E	3	3	1	2
F	3	5	1	0
G	2	3	1	2
H	3	5	2	0
I	4	12	1	2
J	2	4	2	2
K	4	11	2	3
L	1	1	1	0
M	3	6	1	1
N	2	2	2	0
O	4	5	1	0
P	9	17	4	5
Q (1)	8	8	3	1
Q (2)	16	31	3	7
R	4	9	3	1
S	6	10	3	0
T	3	3	2	1
U	1	1	0	1
V	2	3	2	0
W	<u>1</u>	<u>1</u>	<u>0</u>	<u>1</u>
Totals	92	164	42	39

TABLE 2. Phage patterns obtained on 23 farms by cow number and quarter

Herd	Cow # and Quarter*	Phage Pattern
A	3c	29/44A/52/52A/80/S1/S4/S6
	1a, 1c 2a, 2b, 2d	Non-Typeable
B	13a, 13b, 13d	S6
	1a 13c	Non-Typeable
C	6a, 6c 7c	S1/S3
D	120c, 120d	S2
	1a, 1c	S5
	3a	42B/42E/54/55/81/S6
	1b 3b	All numbers except 52, 53, and 75
	4b, 4c, 4d	Non-typeable
E	14d	S6
	1c 2c	Non-typeable
F	1b, 1c 2b, 2d 3d	44A/79/S1/S3/S4/S5
G	2a	52A/80/S4/S6
	1d 2b	Non-typeable
H	W9d 24a, 24c 28c	S2
	W9a	VA4/6/42B/47/54/75/S5
I	1a, 1b, 1c, 1d 2d	S1/S3
	3a 4a, 4b, 4c, 4d	
	2b 3b	Non-typeable
J	1d	S6
	1c	42E/54/79/81/S5/S6
	2a, 2c	Non-typeable
K	2b, 2d	S1/S3
	1d 2a, 2c, 3c, 3d 4d	44A/S2/S5
	3b 4b, 4c	Non-typeable

* a--left front quarter
b--right front quarter

c--left rear quarter
d--right rear quarter

1

2

TABLE 2. (Continued)

Herd	Cow # and Quarter	Phage Pattern
L	1c	6/42B/44A/81/S1/S3
M	1a, 1c, 1d 2d 3c	52/52A/80/S4
	2c	Non-typeable
N	1a	44A/S2
	2b	3c/42E/47/70/S2
O	1a 2d 3c 4a, 4b	6/29/42B/42E/44A/S1/S3/S4/S6
P	21c, 21d 72c	S6
	15a 18a, 18d	S1/S3
	4d 5d	29/S1/S3
	1c 21a, 21b 72a	All numbers except 3B, 3C, 53, 55, 79, S2, S4, and S6
	2a, 2b, 2d 3a 4a	Non-typeable
Q	5 8 9	S2
(1)*	10 12 25	42B/42E/S2
	23	All numbers except 3A, 3B, 3C, VA4, 44A, 52, 52A, 53, 55, 70, 73, and 75
	7	Non-typeable
Q (2)**	5c 10a, 10d 18a, 18b 24a 27c 28b 31a 33a 35b 43b 44a	S2
	2d 5a, 5b 6b 18c 24c 25d 27b 45a, 45d	42B/42E/S2
	28a	6/7
	2c 27d 35a, 35c 42a 44d 45c	Non-Typeable
R	1a	VA4/7/53/S2/S5/S6
	1d	3A/3B/3C/6/7/42E/54
	1c 2a, 2c, 2d 3b, 3c	6/7/42B/42E/44A/47/54/70/ 73/75/81/S1/S3
	4a	Non-typeable

* First sampling of herd Q

** Second sampling of herd Q

TABLE 2. (Continued)

Herd	Cow # and Quarter	Phage Pattern
S	Pd Ta	S2
	1b, 1d 2d	Va4/77/S5
	Pb 4c 5a, 5c Tc	3B/42B/42E/54/79/81/S2/S5/S6
T	56c	S6
	2b	52/52A/80/S4/S6
	1d	Non-typeable
U	1b	Non-typeable
V	2a	VA4/6/47/53/S5
	1a, 1b	42D/42E/47/54/79/81/S1/S2/S3/S4/S5/S6
W	1b	Non-typeable

The possible distribution of staphylococci in one animal is illustrated by the patterns of lysis obtained on several farms, as shown in Table 2. Two animals, 1 and 4 on Farm I, were infected in all four quarters with the same phage type staphylococcus, while cow #1 from Herd R carried 3 different phage types in 3 quarters. This was the maximum number of different patterns obtained from any one animal. Most other possible variations between these two extremes could also be found on one of the farms.

Samples were obtained from one farm, Q, during two different occasions, about three months apart. Two of the patterns present during the first sampling were also found to be present during the second visit as shown in Table 2. No record was made of the quarters sampled during the first visit. Cows 5 and 25 carried the same type in the second isolation as in the first. Some of the patterns reported as S2 also had weak lytic reactions with 42B and 42E; thus, cow #10 with an S2 pattern from the second sampling may be identical with the 42B/42E/S2 pattern obtained at the first milking.

Table 3 indicates the frequency of the most common patterns obtained from the 125 phage sensitive organisms. Each of 3 phage patterns was found on 2 or more farms. All were the result of lytic action by the Seto-Wilson series of phages. S2 was the most prevalent type and occurred in 24 samples from 4 farms. This was followed by the S1/S3 pattern in 18 samples from 4 farms and nine S6 patterns in 5 herds. The most active of the 30 phages was S2 which caused lysis in 56 (44.8%) of the 125 sensitive staphylococci, as shown in Table 4. This finding was in agreement with that of Seto and Wilson (1958) who found that S2 was the predominant type in their study, lysing 49% of the sensitive

TABLE 3. Frequency of the most common phage patterns obtained from staphylococci of bovine origin

Herd	Number of organisms of phage type		
	S2	S6	S1/S3
B	0	3	0
C	0	0	3
D	2	0	0
E	0	1	0
H	4	0	0
I	0	0	10
J	0	1	0
K	0	0	2
P	0	3	3
Q (1)	3	0	0
Q (2)	13	0	0
S	2	0	0
T	0	1	0
Totals	24	9	18

TABLE 4. Frequency of lysis by the most active phages upon
125 strains of staphylococci

Phage	No. of Strains Lysed	Percent of Strains Lysed
S2	56	44.8
S1	47	37.6
S3	46	36.8
42E	42	33.6
42B	39	31.2
S5	34	27.2
44A	31	24.8
S6	30	24.0
S4	24	19.2
S4	23	18.4
81	23	18.4

cultures tested. S1 and S3 were the next most active phages in this study, followed closely by 42E, 42B, and S5. Phages 42B, 42E, and 44A were the most active of the International-Blair series of phages used in human medicine. Sixty-two percent of the total number of lytic reactions (614) was due to this series of phages, giving some indication of a possible cross-infectivity or colonization between man and animal. Further, such evidence was indicated by the broad lytic pattern evident in 13 organisms from 4 different farms, D, P, Q, and R, as illustrated by samples 1b and 3b, herd D, and 23 of herd Q in Table 2. In addition, 6 organisms from 2 farms, M and T, gave a 52/52A/80 pattern with the international phages, closely resembling the pattern 52/52A/80/81 found in human infections.¹

Isolates obtained from the cervical samples failed to give significant lytic reactions or phage patterns. From 124 such samples, 24 gave growth on staphylococcus 110 agar. All the isolates were gram positive organisms, but only 15 were mannitol-fermenting gram positive cocci. Subsequent coagulase determinations and phage typing failed to indicate the presence of S. aureus. None of the 15 organisms was coagulase positive, and only one could be phage typed, giving a 3A/75/80/S3 pattern. Milk samples from 3 quarters of the animal yielding the typeable cervical sample were positive. None of the milk isolates, however, was related to the cervical isolate. Two gave a 44A/S2/S5 pattern, and the other was non-typeable. All were coagulase positive.

To further characterize the staphylococci obtained from milk, a quantitative coagulase test was carried out using 100 of the 164 isolates.

¹Personal communication, Dr. Stanley Read, Michigan Department of Health, Lansing, Michigan

The results obtained with some of the more common phage patterns are shown in Figures 3, 4, and 5. The phage type S2 cocci (Fig. 3) obtained from the 4 herds indicated in Table 3 were quite variable in their ability to produce coagulase, ranging from a reciprocal titer of less than 4 to a high of 512. Eleven of the 24 organisms with titers of 128 or greater produced about the same amount of coagulase even though isolated from 3 different farms. The strains isolated from the first sampling of herd Q (cows 5, 8, and 9) gave about the same coagulase titer as most of the organisms isolated during the second sampling, and the S2 organism of cow #5 appears to be the same in each case. The 4 staphylococci obtained from herd H also appear to be identical on the basis of phage type and coagulase production.

Figure 4 represents 8 herds with phage patterns S1/S3 and S6 and shows uniformly high coagulase producing organisms. Only 5 exceptions occurred among the 26 samples. The 4 quarters of 2 cows from herd I are represented in the S1/S3 group. Four of these organisms (from cow #4) show almost identical titers, whereas staphylococci from cow #1 range from a titer of less than 4 to 512. All of the S6 organisms gave high coagulase production although isolated from 4 different farms.

In Figure 5, the coagulase titer of some of the different phage type organisms found in 2 herds is presented. The pattern 42B/42E/81/S2 in the first herd (second visit to herd Q) compares favorably in coagulase production with most of the 42B/42E/S2 organisms in the same herd (first visit), and thus should probably be considered to be the same strain. Variability in coagulase production among the same phage type organisms is again illustrated in the 44A/S2/S5 patterns of the second herd, K, although most of the 6 cultures fall in the higher coagulase range. The primary feature in evidence from Figure 5 is the uniformly low titer of coagulase by the organisms which were not phage

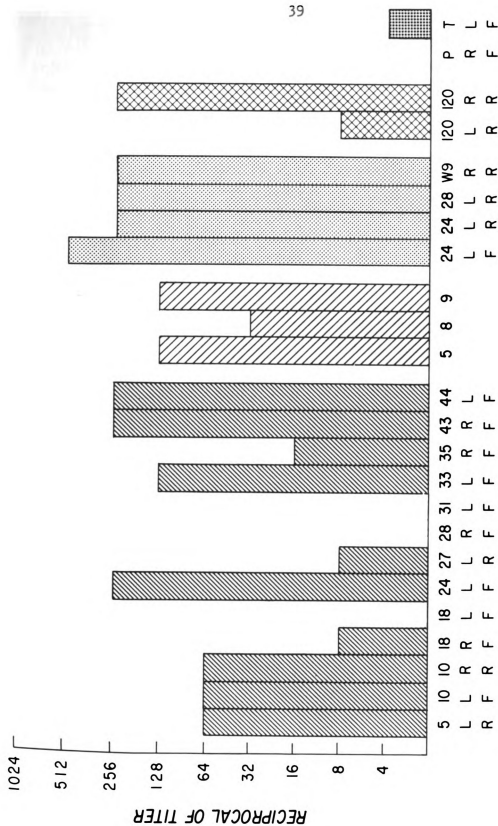


Fig. 3. Coagulase production by the predominant S2 phage types of staphylococci isolated from milk. (Herds Q2, Q1, H, D, and S)

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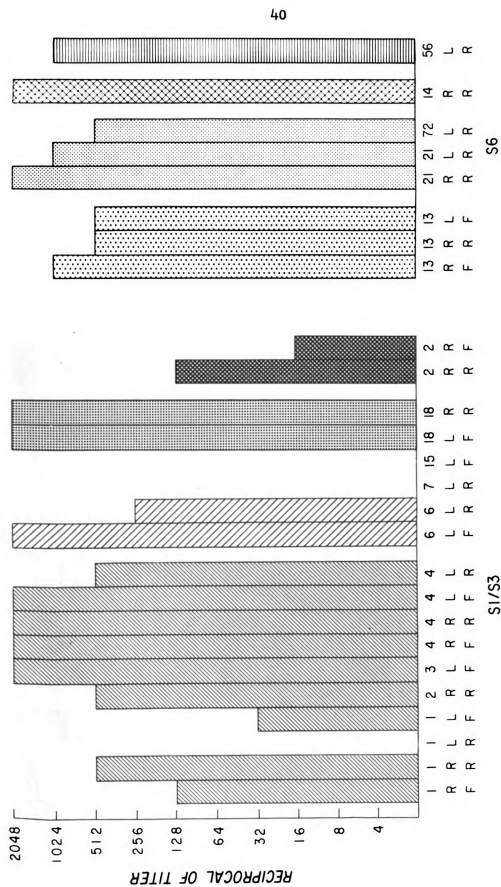


Fig. 4. Coagulase production by phage type SI/S3 and S6 staphylococci isolated from milk.
(Herds I, C, P, K, and B, P, E, T)

1. The first part of the document is a header section containing the title "THE HISTORY OF THE UNITED STATES OF AMERICA" and the author's name "BY JAMES M. SMITH, LL.D."

2. The second part of the document is a preface section containing the following text:

"The history of the United States of America is a subject of great interest and importance to the people of this country. It is a subject which has attracted the attention of the world, and which has been the subject of many valuable works of history and literature."

3. The third part of the document is a table of contents section containing the following entries:

THE HISTORY OF THE UNITED STATES OF AMERICA. BY JAMES M. SMITH, LL.D.

PREFACE.

THE HISTORY OF THE UNITED STATES OF AMERICA. BY JAMES M. SMITH, LL.D.

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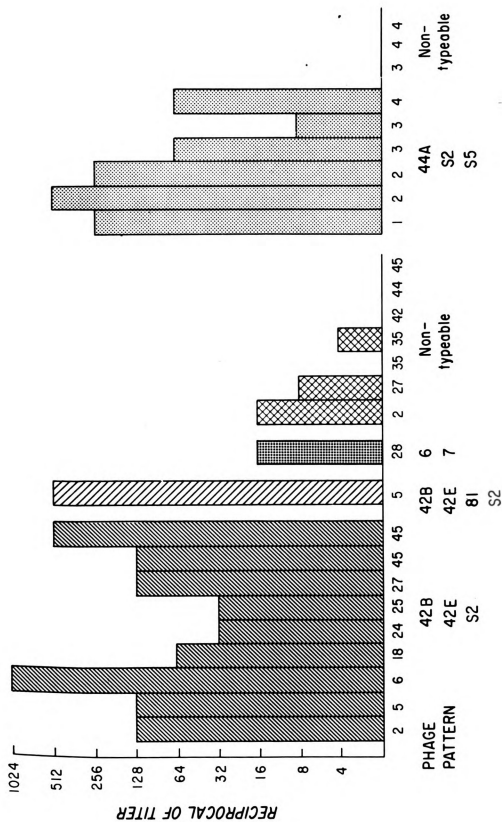


Fig. 5. Coagulase production and phage patterns of staphylococci isolated from 2 herds, Q2 and K

sensitive. Of 10 such strains in both herds only 2 had coagulase titers above 4, and these remained in the low titer range. Twelve other non-typeable organisms, not shown in Figure 5, were also compared in their coagulase producing ability. Five gave titers of less than 4, while 4 were high producers (1024), and 3 were in the medium range (128).

Characteristics of the Phage Propagating Strains

Coagulase

The amount of coagulase produced by the propagating strains after 5 days incubation is indicated in Figure 6, where the reciprocal of the greatest dilution of culture supernatant fluid causing visible clot formation is shown. All the cultures except 52A/79 and 73 were coagulase positive at a dilution of 1:4 or greater. Strain 52A/79 was active at a lower dilution (1:2), while number 73 failed to initiate clot formation at any dilution. It was subsequently tested by direct inoculation into plasma and proved to be coagulase negative. The remaining strains varied considerably in their ability to produce coagulase. Most fell in the intermediate ranges of 32 to 256 reciprocal titer units (RTU). Three of the strains--70, S1, and S3--were very active, ranging from 512 to 4096 RTU. Strain 70 was the highest producer, giving a titer of 4096. Nine strains, in addition to 52A/79 and 73, gave low coagulase activity, with titers of 32 or less. All the group 1 organisms were within this range.

Coagulase production was also determined during aeration of the cultures by continuous mechanical shaking. Representative strains of the high, low and intermediate producers were tested. The results are indicated in Figures 7 and 8 for samples obtained at different time intervals. The titers compared favorably with those obtained during stationary growth, and in most instances coagulase activity was slightly

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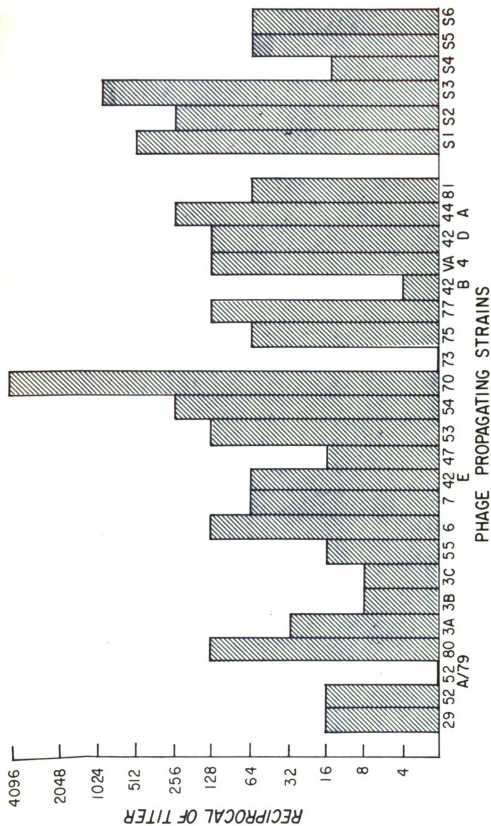


Fig. 6. Coagulase production by the International-Blair and Seto-Wilson strains of staphylococci after 5 days stationary growth

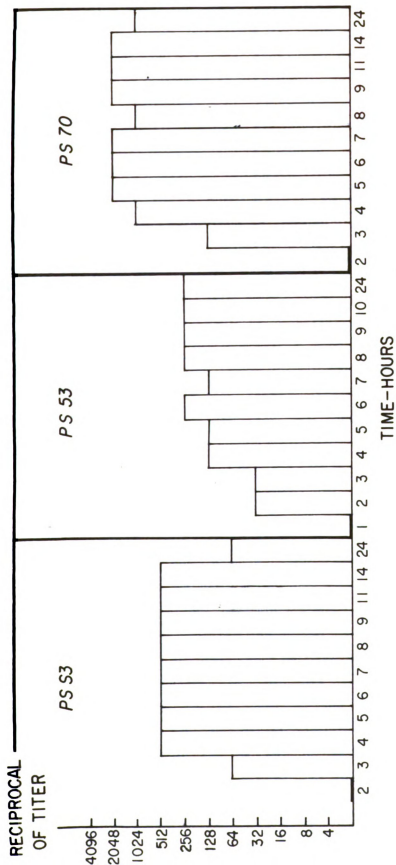


Fig. 7. Coagulase production at various intervals of aerated growth by high and intermediate coagulase producing strains of phage propagating staphylococci

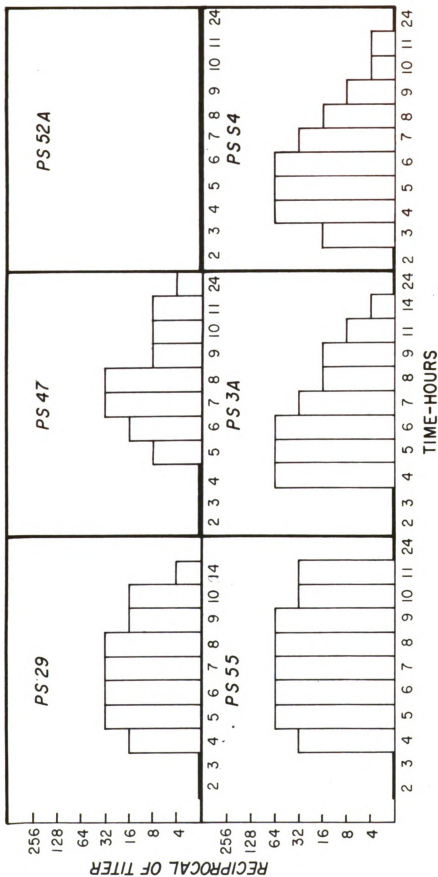


Fig. 8. Coagulase production at various intervals of aerated growth by low coagulase producing strains of phage propagating staphylococci

enhanced. All the strains attained their maximum capacities early in the growth period. The high and intermediate producers maintained their maxima throughout the test period, while the low producers were markedly less active near the end of this period.

Extracellular Protein

The results of the extracellular protein determinations are shown in Figure 9. Most of the strains gave fairly low values for this determination, and four strains (55, 42E, 42B, and S1) failed to show a significant amount. Strain 7 gave the highest reading at an optical density of 0.58. Six strains fell in an intermediate range, showing optical density values between 0.3 and 0.4.

Phosphatase

The amount of phosphatase produced by a fixed amount of cells is indicated for each strain in Figure 10. All strains except 73 were significantly positive. Strain 44A produced the greatest amount of phosphatase. Five other strains were also high producers, but only about one-half as active as 44A. Almost one-half of the cultures gave an optical density reading of less than 0.3.

Catalase

Catalase production is indicated graphically in Figure 11. The significant result to be noted here is the ability of most of the organisms to decompose hydrogen peroxide to about the same extent, with the exception of strain number 70. This strain, which produced the greatest amount of coagulase, failed to show catalase activity.

Growth Curves and Oxygen Uptake

The results of the growth curve determinations are indicated in

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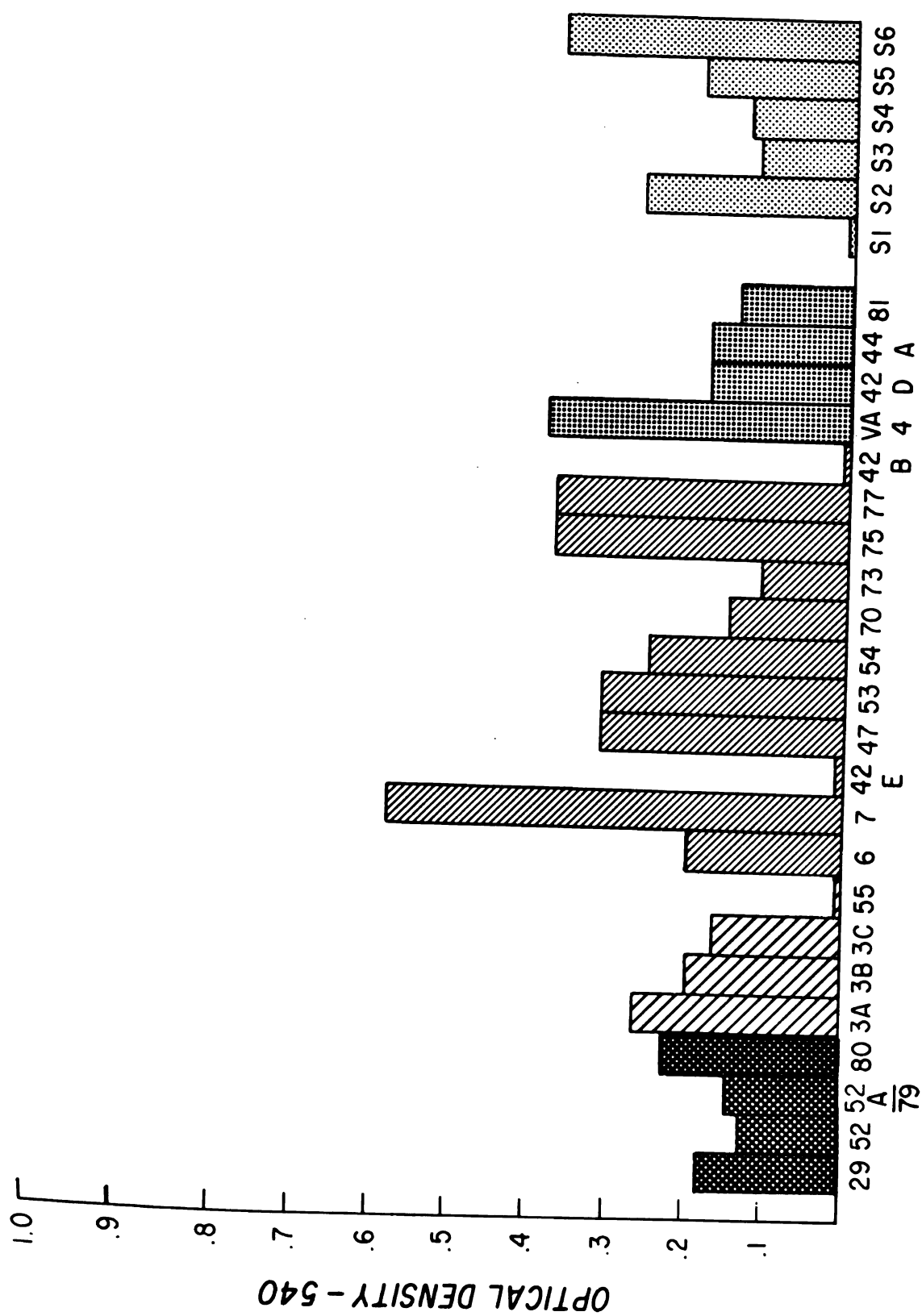


Fig. 9. Extracellular protein of the International-Blair and Seto-Wilson series of *staphylococci*

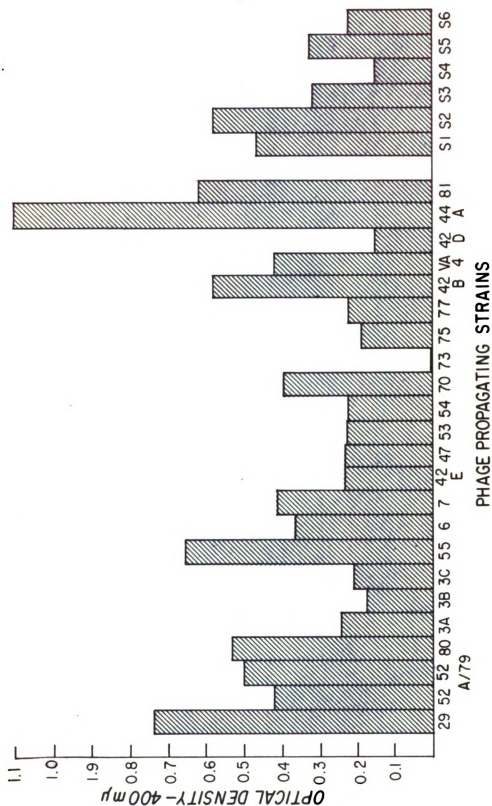
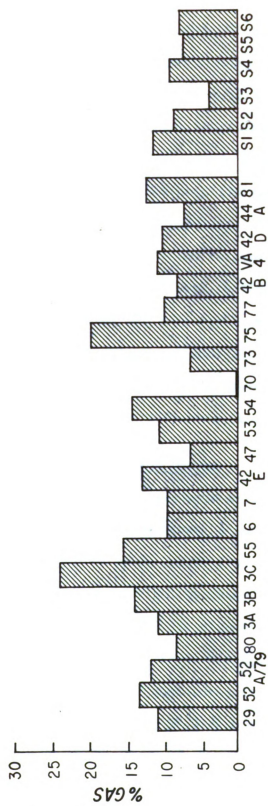


Fig. 10. Cellular acid phosphatase production of the International-Blair and Seto-Wilson series of staphylococci



PHAGE PROPAGATING STRAINS

Fig. 11. Catalase activity of the International-Blair and Seto-Wilson series of staphylococci

figure 12, where three representative curves are drawn to illustrate the data shown in Table 5. Strains 3C, S6, 70, 73, 75, and 77 are exemplified by the lower curve (Δ), and strains 6, S1, 52A/79, 53, 80, and 81 by the top curve (o). The remaining strains tend to fall on the middle sigmoid curve (e). The apparent short lag in the growth curves would have disappeared if a semi-logarithmic plot had been used, and the differences among the growth slopes (Table 5) would be less marked.

Oxygen uptake during the utilization of glucose by washed cells was determined for all the strains and is expressed as microliters of oxygen per mg dry weight of cells per hour in Figure 13. Q_{O_2} values ranged from 23 to 82. Eight strains had a fairly high oxygen consumption, ranging from 70 to 82 microliters. These included 29, 52, 52A/79, 80, 6, 7, 53, and 42D. Five strains, 55, 70, 75, 77 and 42B, gave low values of 23 to 32 microliters. The Seto-Wilson strains were fairly uniform in this measurement.

Growth in a Chemically Defined Medium

A synthetic medium was developed which supported the growth of most of the strains of staphylococci studied. It contained the following nutrients: DL aspartic acid, L cysteine, L glutamic acid, glycine, DL leucine, L lysine hydrochloride, DL phenylalanine, L proline, and DL valine, 0.08% each; glucose, 0.4%; nicotinic acid and thiamine hydrochloride, 0.0005% each; $MgSO_4 \cdot 7H_2O$, 0.02%; and $Na_2HPO_4 \cdot 12H_2O$, 0.5%. One ml of a mineral solution containing boric acid, 0.06g; molybdic acid, 0.02g; $MnSO_4 \cdot H_2O$, 0.03g; $CuSO_4 \cdot 5H_2O$, 0.04g; $FeCl_3 \cdot 6H_2O$, 0.25g; $ZnSO_4$, 0.2g; KI, 0.1g; and H_2O , 1000 ml was added per liter of synthetic medium. The pH was adjusted to 7.4 with 1N NaOH prior to autoclaving. All the propagating strains gave less growth in the synthetic medium than in the control brain heart infusion broth. Plate counts indicated a 10 to 100

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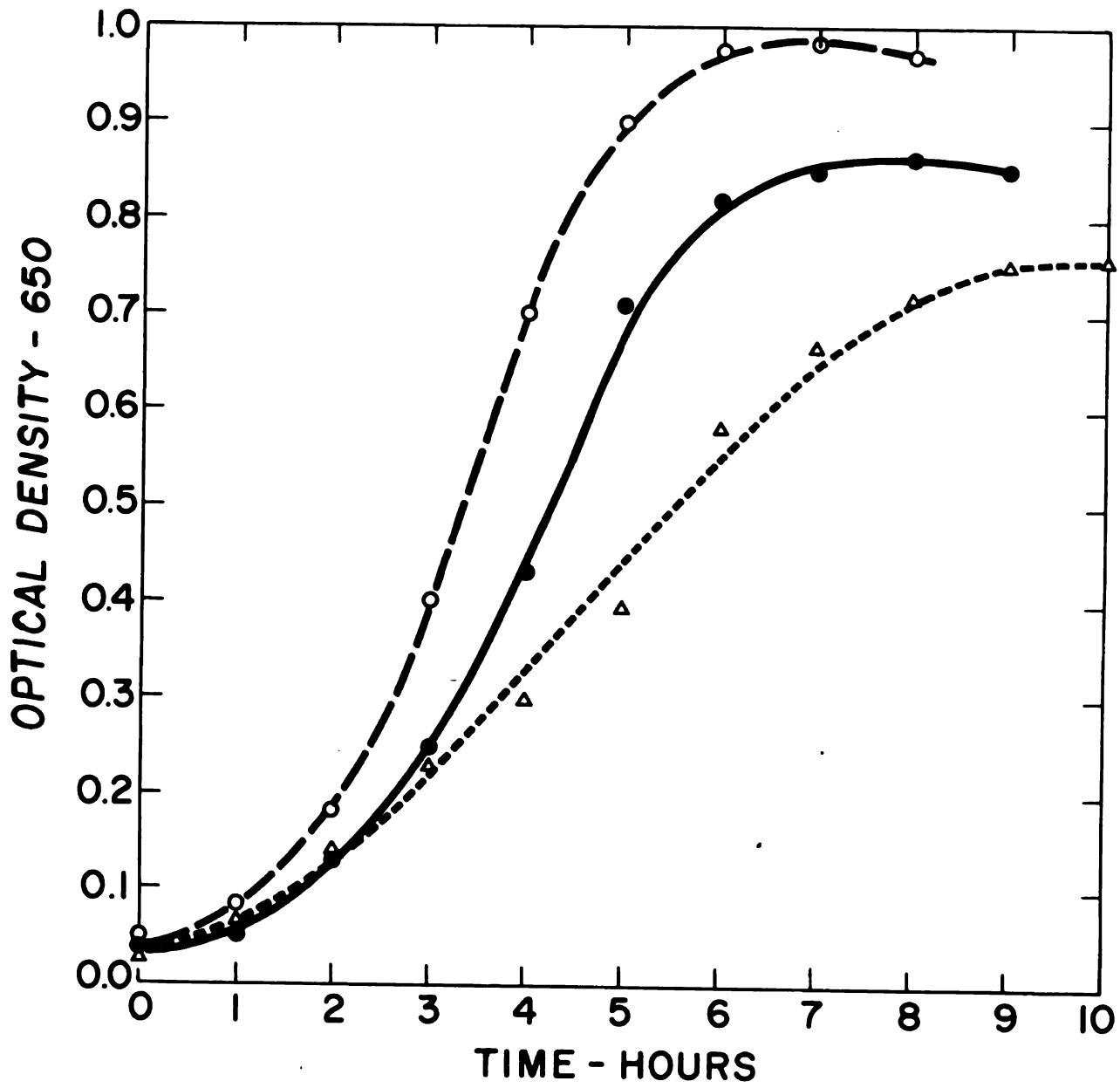


Fig. 12. Representative growth curves of the International-Blair and Seto-Wilson series of staphylococci

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TABLE 5. Some growth characteristics of the International-Blair and Seto-Wilson series of phage propagating strains of staphylococci.

Strain No.	Logarithmic Growth Slope	Maximum Density	Time required to reach maximum density (Hours)
29	2.0	0.88	6.5
52	1.9	0.95	8.0
52A/79	2.7	0.95	5.5
80	3.2	0.98	6.0
3A	2.2	0.81	5.0
3B	2.9	0.80	6.5
3C	2.2	0.73	6.5
55	2.6	0.89	9.5
6	2.3	0.92	5.0
7	2.0	0.82	6.0
42E	2.0	0.86	6.5
47	2.1	0.84	6.0
53	2.8	0.90	5.0
54	2.2	0.85	7.0
70	1.2	0.78	9.0
73	1.2	0.78	9.0
75	1.6	0.65	6.0
77	1.5	0.76	6.5
42B	2.1	0.88	7.0
VA4	1.6	0.85	9.0
42D	1.6	0.93	10.0
44A	2.4	0.83	7.0
81	3.0	0.98	6.0
S1	3.0	0.98	7.0
S2	2.1	0.88	7.5
S3	1.6	0.95	8.0
S4	1.4	0.93	10.0
S5	2.2	0.85	7.0
S6	1.2	0.76	9.0



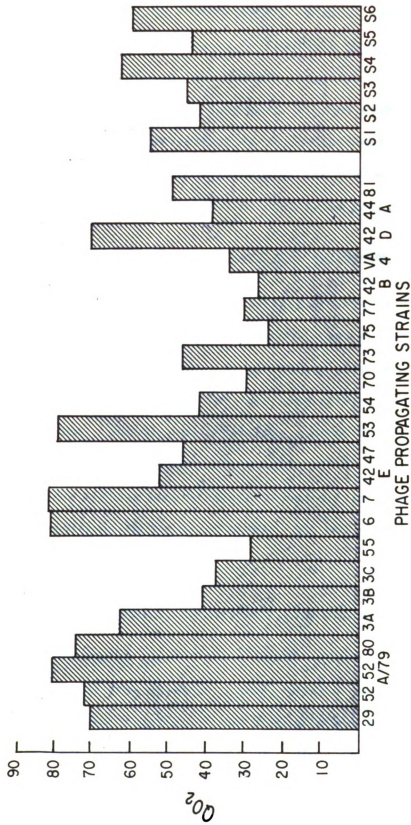


Fig. 13. Oxygen uptake of the International-Blair and Seto-Wilson series of staphylococci

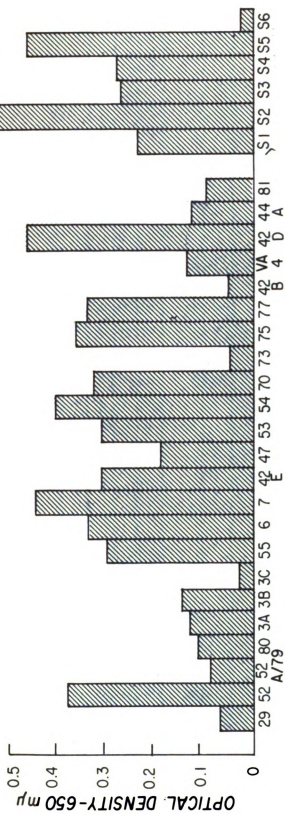
fold decrease in numbers in the defined medium. Coagulase was produced by a number of strains in this medium, but the titer was much less than that obtained with the control broth. Most of the low coagulase producers grew very poorly, as indicated in Figure 14 by strains 29, 52A/79, 3C, 73, and 42B. Strain S6, an intermediate coagulase producer, also failed to grow well in this medium. Subsequent studies with a semi-synthetic casein hydrolysate medium revealed a marked enhancement in growth of this strain after the addition of L tryptophane to the medium. All other strains grew well in the hydrolysate medium without added tryptophane.

Antibiograms, Penicillinase Production, and Minimum Inhibitory Concentration of Penicillin

The antibiograms and penicillinase activity are shown in Table 6. Six strains (53, 54, 75, 77, 80, and 81) were resistant to penicillin, and produced penicillinase. Strain 81 was also resistant to most of the other antibiotics tested. The remaining strains had almost identical antibiograms. The minimum inhibitory concentration of penicillin is shown for all the strains in Figure 15. Five of the 6 resistant strains grew in the highest concentration of penicillin used in the experiment (20 units/ml) while the sixth strain, 53, was inhibited by a concentration of 0.63 units per ml.

Pigmentation, Mannitol Fermentation, and Gelatinase and Hemolysin Formation

The remaining results are grouped in Table 6. Pigmentation of the strains varied from white to orange. Most of the strains were white or yellow; only two strains, 3A and 6, showed a marked orange pigmentation. All of the cultures except number 73 were able to utilize mannitol. Most of the strains were also able to hemolyze the blood of one of the species



PHAGE PROPAGATING STRAINS

Fig. 14. Growth of the International-Blair and Seto-Wilson series of staphylococci in a chemically defined medium

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TABLE 6. Antibigrams and some physiological properties of the International-Blair and Seto-Wilson series of phage propagating strains of staphylococci.

Strain number	52A	29	52	79	80	3A	3B	3C	55	6	7	42E	47	53	54	70	73	75	77	42B	VA4	42D	44A	81	S1	S2	S3	S4	S5	S6
Penicillin	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	R	S	S	R	S	S	S	S	R	S	S	S	S	S	S
Chloramphenicol	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Erythromycin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Tetracycline	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
Dihydro-streptomycin	SS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Penicillinase	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	+	-	-	-	+	-	-	-	-	-	-
Mannitol fermentation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatinase	-	-	-	+	-	-	+	+	+	+	+	-	+	-	-	-	-	-	-	+	+	+	+	-	+	-	+	-	+	-
Rabbit	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemolysin Sheep	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Human	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	+
Pigmentation	W	Y	Y	O	W	Y	O	W	W	W	W	Y	Y	Y	W	Y	W	Y	W	Y	W	Y	Y	Y	Y	Y	Y	Y	Y	Y

Key: S -- sensitive, inhibited by low or medium concentration discs.

SS-- slightly sensitive, inhibited by high concentration discs.

R -- resistant, not inhibited by high concentration discs.

O -- orange yellow

Y -- yellow

W -- gray-white

Note: Oxytetracycline and chlortetracycline gave the same activity as tetracycline.

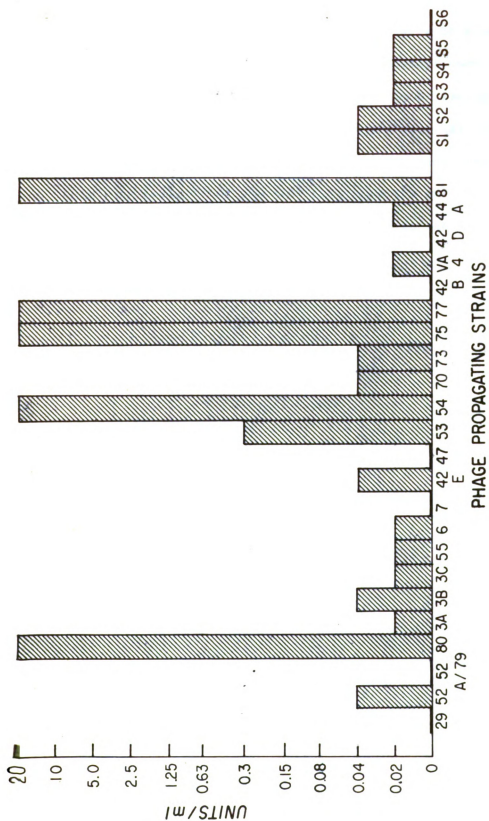


Fig. 15. Minimum inhibitory concentration of penicillin on the International-Blair and Seto-Wilson series of staphylococci

used in the experiment. Only four strains failed to demonstrate lysis: 29, 52, 73, and 75. All of these were also low or negative in coagulase producing ability. Few of the strains caused lysis of human red blood cells. Only three (3A, 53, and 44A) of the International-Blair series and five of the Seto-Wilson strains were capable of laking the human cells. The Seto-Wilson strains showed almost identical lytic spectrums. Strain 52 was slightly less active than the remaining five strains.

Less than one-half of the strains showed the ability to liquefy gelatin. Both of the 80 and 81 strains failed to exhibit this property, as did the two highest coagulase producers.

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DISCUSSION

A number of workers (Coles & Eisenstark, 1959; Edwards & Rippon, 1957; Williams-Smith, 1948) have found staphylococci of phage type 42D to be the predominant type among bovine staphylococci. Our study, using phage at routine test dilution, failed to yield 42D as a significant phage type or as an active phage. Out of a total of 125 sensitive strains only 8 were lysed by phage 42D. On the other hand, phage S2, which was originally derived by propagating phage 42D on staphylococci obtained from milk, was the most active of the 30 phages used, lysing 56 (45%) of the sensitive organisms. This finding compared favorably with that of Seto and Wilson (1958) who found phage type S2 to be the predominant strain in their study, lysing 49% of the sensitive cultures tested. Phage type S2 staphylococcus was also our most predominant strain, appearing in 24 cows on 4 farms. Strain S1/S3 was the second most common organism but was not reported by Seto and Wilson, whereas the S6 strain which appeared in 7% of the Michigan animals also appeared in the Wisconsin study but with a higher frequency (19%).

Seto and Wilson also utilized the international phages to type staphylococci of bovine origin. Over one-half of 102 cultures were lysed by phage 44A, with 36 being lysed by this phage alone. Equivalent results were not obtained in our study of bovine staphylococci, although phage 44A was one of the more active of the international phages. Twenty-five percent of the staphylococci were sensitive to this phage. Only phages 42E and 42B of the international set were more active. However, phage 44A alone, considering the international set separately, lysed only 7 strains, or 5.6% of the sensitive cultures. This value was much lower than the 35% obtained by Seto and Wilson in their study.

Other patterns of lysis, as indicated in Table 2, were irregular and varied and no particular relationship existed between the farms. Within a particular farm, however, frequently one pattern was found in all the animals which were infected. The most notable example perhaps occurred in Herd 1, where 4 animals carried the same organism, and 2 of these were infected in all 4 quarters. While it was possible to have a different type present in each of the 4 quarters of one animal, this was not observed in our study. However, a different type was obtained from each of 3 quarters of 1 cow, and 2 different types were found in 2 or more quarters of 8 animals. Only 1 typical isolate from each quarter was typed. Williams-Smith (1948) and Price et al. (1954) have shown that infection is nearly always due to one type of staphylococcus.

As previously indicated, the same phage type staphylococcus has been isolated both from dairy animals and dairy personnel. In a number of cases the widely distributed or so-called epidemic strain, phage type 80/81, has been obtained from the animal or from dairy products, indicating possible cross-infectivity between human and bovine. The results obtained in the present study also tend to substantiate this possibility. Strain 52/52A/80, similar to a common pattern found in human infections (52/52A/80/81), was isolated from 6 samples obtained from 4 cows on 2 different farms. In addition, the 24 phages used to type staphylococci from human infections were responsible for a large percentage of the lytic reactions occurring in the bovine staphylococci. Sixty-two percent of the total was a result of lysis by this series of phages. The 6 Seto-Wilson phages caused only 38%. This high frequency of lysis by the human series against the bovine staphylococci suggested a possible inter-relationship between the organisms isolated from human and bovine sources. The extensive lytic patterns of a number of the isolated

organisms also supported this conclusion. Thirteen of the bovine staphylococci were lysed by a large number of the human phage series, ranging from 11 to 21 of the 24 phages.

Thirty-nine of the 164 cultures obtained from milk were not sensitive to the action of the 30 phages used in this study. This percentage, 24, compares well with the results of Seto and Wilson (1958), where 21% of 102 cultures were non-typeable. Coles and Eisenstark (1959) reported 49.7% of bovine cultures resistant to the action of the human set of typing phages, but reduced this figure to 18.8% when 8 adapted phages were used. In the present study, the use of the adapted or newly isolated phages of Seto and Wilson gave results comparable to those of Coles and Eisenstark.

A much larger percentage of the organisms isolated from the reproductive tract were not typeable. The high salt content of the isolating medium inhibited the gram negative organisms and few gram positive cocci were obtained. All were coagulase negative, and only one was sensitive to the phages utilized in these experiments. The results suggested, therefore, that S. aureus was not involved in the etiology of the breeding problems encountered in the present study. In addition, the differences in phage sensitivity and coagulase production indicated little or no relationship between the cervical cocci and those obtained from milk.

Further characterization of a number of the organisms isolated from milk was obtained by quantitative coagulase determinations. Particular phage patterns were selected, along with non-typeable organisms. The non-typeable strains for the most part appeared to be low coagulase producers, while there was some variability among the other organisms,

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even among the same phage type obtained from one animal. Normal strain variation could explain the difference in coagulase production, since high, low, and negative coagulase producing variants have been found within the same strain (Smith et al., 1952). In addition, the use of routine test dilutions of phage may result in the loss of lytic reactions and phage types, causing an incomplete differentiation of strains. Such strains would therefore appear to be identical by the phage typing procedure, but would be expected to show differences in other characteristics.

The phage patterns of the propagating strains indicated a variety of phage types and further biochemical studies were carried out on this group as representative of the genus Staphylococcus. Coagulase activity differed markedly among the strains. All were coagulase positive except number 73. This strain has been reported previously as coagulase negative (Rippon, 1956), although at one time consisting of a mixture of coagulase positive and negative staphylococci. Only the coagulase negative strain was lysed by phage 73 and hence became the propagating strain for this phage. Coagulase production in the remaining cultures ranged from less than 4 to 4096 RTU, suggesting a need for quantitative studies of this property in pathogenic strains. This was further indicated in the determination of the plasma clotting ability during timed intervals of growth. Low producing strains attained their highest titers during the early growth periods but failed to maintain maximum activity for long periods. In contrast, the high coagulase producers gave maximum titers throughout the growth period, after reaching this maximum. Low coagulase producing strains, therefore, might easily be reported as coagulase negative if tested at sub-optimal periods of production.

Coagulase production was correlated in all cases with the ability to ferment mannitol. Coagulase negative 73 was the only strain which failed to utilize this carbohydrate. Alpha, or rabbit cell, hemolysin also correlated well with plasma clotting activity. Seven strains failed to lyse rabbit erythrocytes, including number 73 and three low coagulase producers. Beta, or sheep cell, hemolysin was occasionally found alone, but usually in combination with the alpha lysin, or, as in the case of most of the Seto-Wilson strains, in combination with the lytic capacity for both human and rabbit cells, an alpha-beta-delta pattern. Elek and Levy (1950) reported this as the most common pattern among staphylococci pathogenic for animals. Most of the Seto-Wilson strains, originally obtained from milk, showed this pattern. Hemolysis of human red cells was rare except in the Seto-Wilson strains and never occurred alone.

Phosphatase activity likewise was correlated with coagulase production, though not quantitatively. All strains except number 73 were positive for both tests. Six of the strains were resistant to penicillin. Four of these, however, were low phosphatase producers. The results of Gillison and Ruda (1958), who found higher phosphatase activity in penicillin resistant strains, were therefore not corroborated in the present study. Markov et al. (1958) found essentially no differences in the average amounts of acid phosphatase produced by penicillin sensitive and resistant strains.

Extracellular protein was low in most strains and appeared to bear no relationship to the other parameters studied. High coagulase producing strains 70, S1, and S3 were low in extracellular protein while some of the low or intermediate coagulase producers were high in protein,

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as in strains 3A, 7, 47, and 75. Rogers (1954) observed that the formation of extracellular protein did not reflect the formation of coagulase, and Bernheimer and Schwartz (1961) could not relate most of their extracellular protein species to well known products of staphylococcal growth.

Most of the strains grew in the synthetic medium utilized in these studies, but growth was always sub-optimal. Low coagulase producing strains gave the poorest growth, indicating a potential method for differentiating between high and low coagulase producers.

The remaining characteristics, for the most part, did not appear to be correlated with the more common indices of pathogenicity, primarily coagulase, phosphatase and alpha hemolysin activity. Some of the characteristics were fairly constant within the various groups of the propagating strains. All the organisms of group 1, for example, had a high oxygen uptake, and no gelatinase activity. Two of the strains possessed hemolytic activity and had high growth rates while the other two were non-hemolytic and average in rate of growth. The Seto-Wilson strains were largely average or low in growth rate and gave almost identical patterns of hemolysis. Q_{O_2} values were fairly high for three of the strains (S1, S4 and S6) and about average for the other three (S2, S3 and S5). Most were able to liquefy gelatin and were pigmented, though not a characteristic orange-yellow. Only two of the 29 strains possessed this pigmentation and neither was high in coagulase or phosphatase activity. All the strains except 70 produced catalase.

From the results, it is obvious that several of the strains possess one or more characteristics which are significantly different from those of the remaining strains. The outstanding strain in this respect was 73 which gave negative tests for hemolysin, mannitol, gelatinase,

coagulase, and phosphatase, and yet was lysed by most of the phages normally specific for coagulase producing strains. Strain 44A, on the other hand, was positive for these tests and gave fairly high coagulase titer and exceptionally high phosphatase activity. Strain number 70 was also unique, being the highest coagulase producer and the only strain not giving catalase activity. Such marked strain differences suggest the need for further quantitative studies on the staphylococci.

SUMMARY

Milk and cervical samples were obtained from 124 cows on 23 farms in the lower peninsula of Michigan. Four hundred and ninety-six quarters were sampled and 164 (92 cows) yielded staphylococci which were typed with 24 International-Blair and 6 Seto-Wilson phages. Thirty different phage patterns were obtained. No one phage type was found to be widely distributed. S2 was the most prevalent type, occurring in 24 samples from 4 farms. This was followed by the S1/S3 pattern in 18 samples from 4 farms and nine S6 patterns in 5 herds. The most active of the 30 phages was S2 which caused lysis in 44.8% of the 125 sensitive staphylococci. This finding was in agreement with that of Seto and Wilson (1958) in their study of bovine staphylococci in Wisconsin.

There was some indication of possible cross infectivity or colonization between man and animal. Phage patterns found in human infections were not obtained with the bovine staphylococci; however, six organisms from two farms gave a pattern of lysis which closely resembled a pattern common in human infections. In addition, the phages used to type staphylococci of human origin were quite active against the bovine isolates. Sixty-two percent of the total number of lytic reactions were due to this series of phages. Thirteen strains were lysed by a large number of the human phage series, ranging from 11 to 21 of the 24 phages.

Cervical samples failed to yield a significant number of staphylococci. None of 15 isolates was coagulase positive, and only one could be phage typed. Staphylococcus aureus apparently was not involved in the etiology of the breeding problems encountered in the present study.

Coagulase production in the milk isolates varied from a titer of less than 4 to 2048. In some instances there was a marked difference

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in coagulase activity in strains giving the same phage pattern. Non-typeable organisms were usually low coagulase producers.

The propagating strains were utilized as representative of the genus Staphylococcus and studied more intensely. Coagulase production was similar to that found in the clinical isolates. All were positive except strain 73. Low producers did not maintain maximum titers during extended incubation periods.

Mannitol utilization, and alpha hemolysin and phosphatase activity correlated well with coagulase activity. Pigmentation, gelatinase production, and extracellular protein content did not. All the strains gave evidence of catalase activity except #70. This strain, however, was the highest coagulase producer.

A synthetic medium was prepared which supported the growth of most strains. Low coagulase producers failed to grow well in this medium, suggesting a possible method for distinguishing such strains.

Six strains (53, 54, 75, 77, 80 and 81) were resistant to penicillin and all produced penicillinase. There appeared to be no correlation between penicillin resistance and high phosphatase activity.

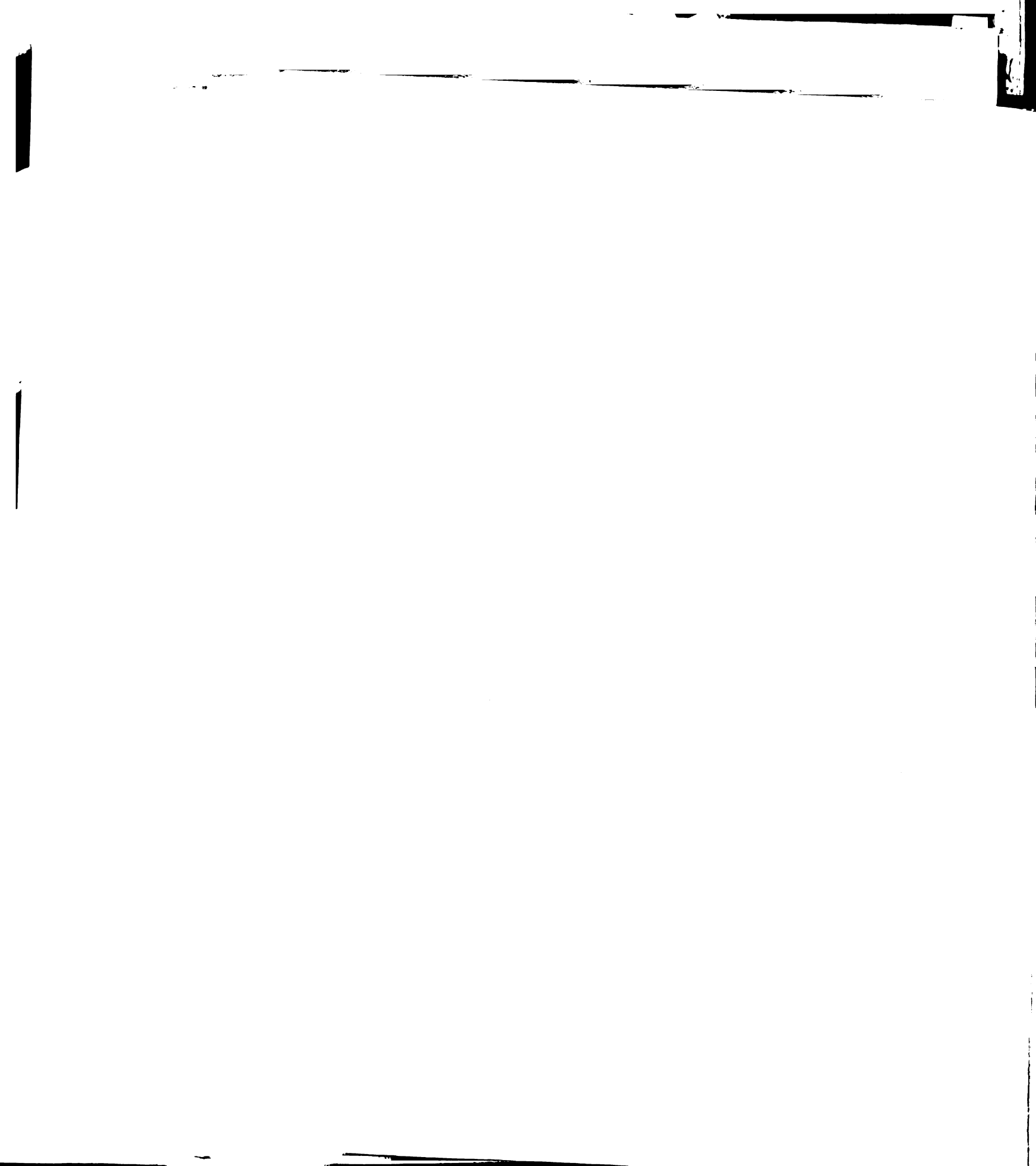
Q_{O_2} values varied from 23 to 82. Group I organisms gave uniformly high results. The high coagulase producers usually gave low to average Q_{O_2} values as did the highest phosphatase producer.

The study of growth rates yielded three representative curves for the 30 strains. Six strains attained a high maximum density in a short period of time, and 6 strains required a longer period of incubation to reach a lower maximum. The remaining strains were represented by a normal or average growth curve.

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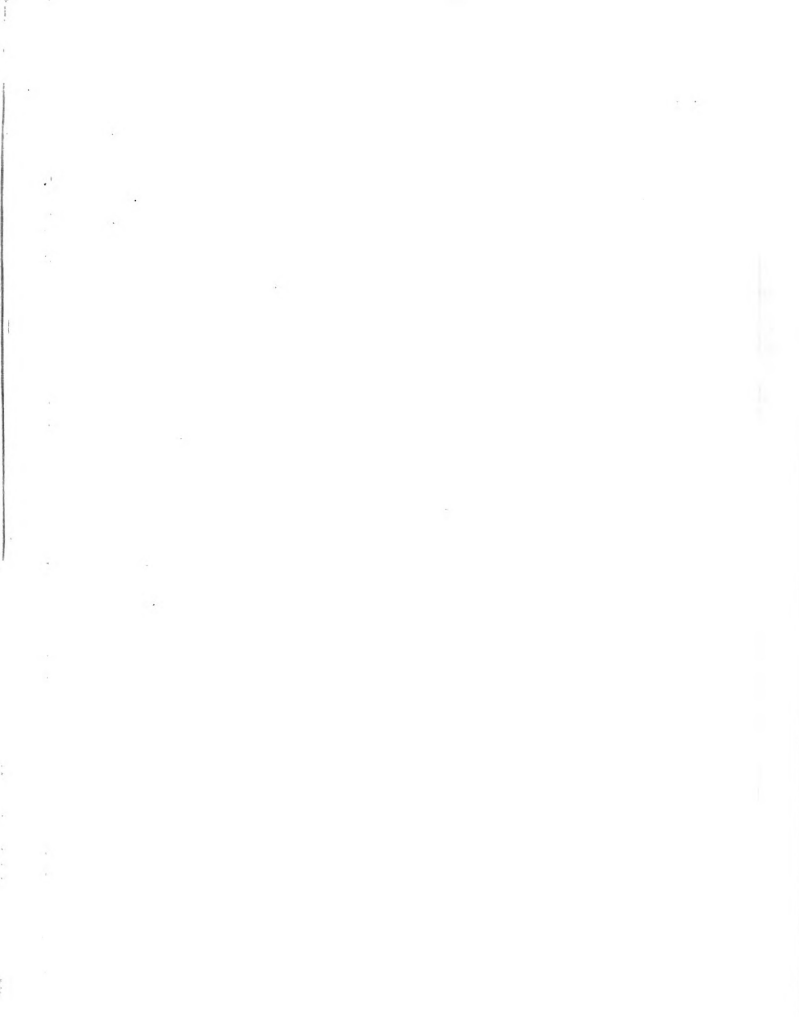
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