

THE DIFFERENTIATION OF BOVINE STAPHYLOCOCCI

BY

BACTERIOPHAGE

by

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ABSTRACT

A preliminary study was carried out on the optimum time for incubating phages produced by staphylococci. This was done by comparing the growth of each of several strains of Staphylococcus with the growth of the same strain plus its homologous phage in trypticase soy broth at 37°C at 15-minute intervals for nine hours, with an additional final colorimetric reading at 22 hours. A titer of the concentration of phage particles in the lysed culture was made at one hour and thereafter at half-hour intervals until the reading on the colorimeter remained stationary for one hour. The optimum time proved to be different for different groups of phages. In one group the titer remained at a high level overnight; in a second the titer was lowered appreciably; and in a third group the titer was practically lost. On the basis of this finding the time of incubation in the studies reported hereafter was varied from 2 to 6 hours, the time in each case depending on the reaction with the individual phage.

Three hundred and twenty-five isolates were obtained from udders of cows in nine areas adjacent to Winnipeg. Of these 166 were typable by using the 21 phages in the basic set together with six others. Most of the typable isolates were susceptible to phage 81. The majority of these isolates were susceptible also to one or more of four other phages-42B, 47B, 80 and 82. One phage-type was predominant in each area, but different predominant phage-types occurred in different areas. The majority of the isolates could not be grouped on the basis of the basic set alone.

Seven of 45 isolates obtained from pooled (cans) milk in one of these areas were typable. Two of the types from the pooled milk were the same as two of the 18 types obtained from individual cows in that area. Two other types were different from the types isolated from the milk of individual cows. In a second area investigated none of the isolates from the pooled milk was typable.

One of the seven isolates from bulk (tank) milk obtained from the latter two areas was typable. This type was the same as the predominant type isolated from herds in the first of the two areas.

In trials with 30 bottles of milk staphylococci were not found.

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## INTRODUCTION



## INTRODUCTION

Staphylococci are widespread. Their presence in air and in the immediate environment of man is common. Under normal conditions they may be present in the nose, the throat or on the skin of the human and certain domestic animals. Due to their high adaptability staphylococcal infections have become of increasing concern, particularly during the past decade. They can adjust themselves to new environments such as human tissue bathed in antimicrobials, and most pathogenic strains presently predominant are antibiotic-resistant (16).

Of the acute infections in man staphylococcal infections present the most urgent need for basic treatment, especially following operations (2). This need results from the fact that penicillin-resistant strains have steadily risen from 50 to 80% of the strains currently carried by selected groups of individuals within hospitals, and to a lesser extent in the population at large (16). Staphylococcal

infections are endemic in the general population, since outbreaks have been reported repeatedly (2,19).

Immunization against staphylococcal diseases has not proven promising. Further, the development of strains resistant to antibiotics and drugs, currently accepted as effective, limits optimism that a permanent effective antibiotic or synthetic drug will appear in the immediate future. Therefore, control remains of primary importance. A prime requisite in this control is the ability to differentiate pathogenic from non-pathogenic strains.

The heterogeneity of this group of microorganisms confounds both the medical man and the biologist. In the medical field, a narrow view widely held is based on the dogma of the fixity of bacterial species, allowing plasticity within the species as regards virulence but failing to recognize variation due to genetic instability. The biologist, caring little about disease, aims to fit a species into a phylogenetical scheme and is bedevilled by the overlapping of characters such as coagulase reaction, toxin production,

pigment formation and tolerance to high salt concentration. However, methods of differentiating strains on the basis of one or more of these characters have not proven completely satisfactory (22). Bacteriophage typing appears to be stable and consistent. It provides an arbitrary method for the segregation of strains. The staphylococci that cause an epidemic in one area always fall into one phage pattern (17,19).

This presentation deals with an attempt to establish types of staphylococci by the bacteriophage method. It involves the flora in milk aseptically drawn from the cow and in milk at various stages of handling. The finding at a stage of handling of a type not present in the aseptically drawn sample could provide a basis for measuring sanitation in the handling of milk, since the new type could represent contamination. Conceivably, as an alternate probability, the new type could represent a genetical variant.

HISTORICAL

### HISTORICAL

The first investigation involving results that seemed to suggest that strains of staphylococci could be divided into groups according to their susceptibility to a series of bacteriophages was reported by Epstein and Fejgin (6). These investigators noted that staphylococci from lesions and from other sources did not respond in the same way to the phages studied. Burnet and Lush (3) divided different staphylococcal phages into different groups according to lytic action on different staphylococci. Williams and Timmins (24) attempted to use this phenomenon to differentiate the strains of staphylococci causing acute osteomyelitis.

However, it was Fisk (7,8) who initiated the present practice of phage typing of staphylococci. This investigator demonstrated that lysogenic staphylococci are widespread. He used the cross culture method to obtain different phages from the lysogenic strains of staphylococci and found that the reaction of these phages on different strains of staphylococci is selective. This reaction of a

staphylococcal strain to a given phage is not readily altered by changes in environment. Soon this method of typing proved of value in two small epidemiological studies (9,11).

Except for the addition of new phages and certain modifications in technique, the current methods used in phage typing of staphylococci are based on those used by Wilson and Atkinson (25). These workers demonstrated that phages could be separated from cocci by filtration. The earlier method depended upon inhibition of the cocci by the quaternary ammonium disinfectant, zephiran (7). They isolated 18 phages and devised a method for routine testing of different types of staphylococci on the basis of phage pattern. They divided their typable staphylococci into 21 types.

A comprehensive review of the methods currently in use has been reported by Williams and Rippon (23).

The main use of phages from human staphylococci has been in tracing the sources of epidemics of infections in man, but their use has been extended to the typing of staphylococci of animal origin.

MacDonald (12) was the first to report the

typing of staphylococci of bovine origin by phages. This investigator, working with phages obtained from Wilson and Atkinson, reported that the majority of the coagulase positive strains isolated from 280 samples of accredited milk, and all the 34 strains from bovine mastitis, were fully susceptible to phage 42D.

Smith (20), noting the value of the phage-typing in the investigation of outbreaks of staphylococcal infections in human subjects, attempted to ascertain whether the method of Wilson and Atkinson (25) might be of value in the epidemiological study of staphylococcal infections in animals. This investigator obtained 21 phages from G. S. Wilson, and isolated 11 additional phages. He reported that 25 of these could be grouped, as follows:

3A Group: Phages 3A, 3B, 3C and 51.

6 Group: Phages 6, 7, 42B, 47 and 47C.

29 Group: Phages 29, 29A, 31, 42C, 44, 52 and 52A.

42D Group: Phages 42D, E174/16, 14/94, 1363/14,  
129/16, 30/16 and P42D/E193.

88 Group: Phages 88 and 88A.

He showed that 93.3% of 1016 isolates obtained from

milk were typable by this grouping, whereas the remaining 6.7% proved to be susceptible to the 32 test phages. Over 83% were susceptible to one or more of the 42D group, and 9.5% to one or more of the 29 group. He divided the typable staphylococci from bovinemilk into 25 types.. Strains isolated from the milk of cows suffering from acute or chronic mastitis did not differ in phage type from the strains isolated from the milk of cows with apparently normal udders. One phage type was predominant in the strains of staphylococci examined from one herd but in other herds the predominant types were different. Different phage types in different quarters of the same cow were not unusual but strains isolated from one quarter were generally of the same type, with few exceptions.

With a larger number of phages, Smith (20) typed a greater percentage of strains of staphylococci than did MacDonald (12) who used only the phages obtained from Wilson and Atkinson. Further, the percentage of strains fully susceptible to 42D alone (now allocated to Group IV) was not as large as that reported by MacDonald (12).

Price et al (14) studied the phage types of



staphylococci isolated from cows in England. These investigators divided most<sup>of</sup> the 896 isolates from 276 quarters of 15 herds into 12 fairly characteristic patterns when phages at routine test dilutions were used. These were designated by the letters A to M, as follows;

Code Letter	Phage susceptibility pattern determined with phages at routine test dilution
A	42D, 7/42D/44/44A, and other combinations of these four phages
B	Numerous phages of groups I and III
C	Lysed by one or more phages of group I and also by 42B
D	7 or 7/52A+
E	3C/7/29/42B/52/52A/54 (and 7/52/54+)
F	7/42B/54 +

Code Letter	Phage susceptibility pattern determined with phages at routine test dilution
H	47A
I	42C/42D/44/44A/54 +
J	54 +
K	6/47/53/54 +
L	6/7/47/53/54 +
M	53/54

Three hundred and thirty nine colonies fell into pattern C; 183 into A; and 181 into L. In heavily infected herds infections were due to more than one phage type. Two phage types obtained from one quarter were uncommon, though different types from different quarters of the same cow were encountered quite frequently. This finding was in agreement with that obtained by Smith (20).

Barnum and Fuller (1) used the bacteriophage typing of staphylococci initiated by Smith (20), to

differentiate pathogenic from non-pathogenic strains in milk, along with other characteristics of the strains, in studies on the epidemiology and the efficacy of treatment and control measures.

These investigators isolated phages from 42 lyso-genic cultures of staphylococci. Using these phages, they tested 648 isolates of staphylococci from milk, and found that most of the isolates belonged to one of five groups. One hundred and ninety five belonged to Group A; 179 to Group A<sub>1</sub>; 76 to Group B; 45 to Group B<sub>1</sub>; 17 to Group B<sub>2</sub>; 123 were not susceptible to any of the 42 phages; and 13 were unclassifiable by these phages. Further, this method of phage typing did not distinguish between strains isolated from healthy and diseased udders. This result confirmed the finding of Smith (20). However, it did afford definite evidence that cows served as carriers of strains that could cause infection in the mammary gland. One phage group of staphylococci was predominant in one herd and other groups in different herds. This is in agreement with the results obtained by other workers (14).

Edwards (4) found that most strains from 35 cows of one herd were lysed by phage 42D at routine test dilution, but also were lysed by stronger concentrations

of phages 54, 77 and 79. Later this investigator working with Rippon (5) reported similar findings, in this case stronger concentrations of a larger number of phages proving to be lytic. They divided the pathogenic staphylococci isolated from these herds into five types, as follows:

Type	Diluted Phage	Phage Pattern undiluted
1	42D	29/52/79/42D/54/77
1a	42D	42D/6/7/42E/47
2	—	3A/3B/3C/71
3	52B	7/75/77/52B
4	52A	52/52A
5	78	54/76/77/78

All the above types were found in cases of clinical mastitis in cows. This finding confirmed the results of Price et al (14) mentioned earlier. These results likewise confirmed those of Smith (20) and Price et al (14) that one type was predominant in each herd but the predominant type in one herd differed from that in

another.

In a study of 57 isolates of staphylococci from milk of a mastitis-affected herd, Rountree et al (17) found that 33 were lysed by phage 42D or by other phages not attacking human strains. Twenty two isolates belonged to phage pattern 29/42D/52.

Thatcher and Simon (21) reported that the predominant type of staphylococci isolated from dairy products in Canada was 42D.

In Wisconsin, Seto and Wilson (18) isolated six staphylococcal phages designated as S1, S2, S3, S4, S5, and S6. The phages were propagated on micrococci of bovine origin. These investigators found that of 379 coagulase positive staphylococcal cultures tested 93% were sensitive to one or more of the above six phages and to phage 42D. All cultures of phage type S2 were sensitive to phage 44A-- this type was reported by McLean (13) to be the predominant type in staphylococci isolated from milk in Australia-- and 80% of the 44A phage type cultures were sensitive to phage S2. Thus, the predominant type of staphylococci of bovine origin isolated in Wisconsin was the same as that in Australia but differed from the predominant type of bovine origin isolated in England (20) and in Canada (21). In the

latter two countries the predominant type was 42D.

In a subsequent study in Wisconsin, Reid and Wilson (15) found the same predominant phage type of staphylococci in cows with acute mastitis, with chronic mastitis and with clinically normal udders as that reported by Seto and Wilson (18). Forty-four per cent of the typable isolates belonged to phage type 44A, and 30% belonged to type 42D. Further, type 44A staphylococci occurred more frequently in udders of acute and chronic mastitis-affected cows than did type 42D. However, the 42D type occurred slightly more frequently in normal udders than did the 44A type. A greater number of strains was phage typable from cows with acute mastitis than from those with chronic mastitis or from those with normal udders.

## MATERIAL AND METHODS

## MATERIAL AND METHODS

### Milk Samples

The milk samples used in this study were collected under the supervision and with the cooperation of Dr. Norman E. Stanger, Assistant Pathologist of the Animal Pathology Laboratory located on the University campus. The first set of samples was obtained aseptically from each of the four quarters of each cow. Before sampling, the udders of the cow were washed with a warm chlorine compound followed by 70% ethanol. The second set was obtained from cans of pooled milk from each herd; the third set from bulk tank which contained the milk from a number of herds; and the fourth from the bottled milk after pasteurization.

These cows were from herds in the vicinity of Winnipeg, namely: St. Vital, Tuxedo, the University of Manitoba, Haywood, Grunthal, Gunton, Kenton, Stonewall, and West Kildonan. Can samples, tank



samples and bottled milk samples were obtained from herds in the first two areas.

#### Isolation of Staphylococci

Each sample was streaked onto a plate containing bovine blood in tryptose agar base and incubated at 37°C overnight. Typical staphylococcal colonies were isolated on nutrient agar slopes and incubated at 37°C overnight. The cultures from these slopes were then sub-cultured in trypticase soy broth at 37°C for 4-6 hours, at which time they were typed.

#### Sources of Bacteriophages

The phages used comprised the basic set of 21 recommended by the International Committee on Phage Typing of Staphylococci (10), as follows:

Gr. I. 29, 52, 52A, 79, 80.

Gr. II. 3A, 3B, 3C, 55, 71.

Gr. III. 6, 7, 42E, 47, 53, 54, 73, 75,  
77.

Gr. IV. 42D.

Misc. 187.

and the following five phages obtained from and by the courtesy of Dr. E. T. Bynoe, Laboratory of Hygiene, Ottawa, Ontario:

42B, 47B, 81, 82, 971.

One additional phage designated phage W was obtained from the Manitoba Provincial Bacteriological Laboratories, Department of Public Health and Welfare, Winnipeg by courtesy of Dr. L. P. Landsdown.

#### Culturing the Propagating Strains of Staphylococci

Each strain of Staphylococcus from the freeze-dried vial was suspended in 0.5 ml nutrient broth, and sub-cultured on a blood-agar plate and in a tube of broth, and incubated at 37°C overnight. If growth was obtained on the blood-agar plate, a typical staphylococcal colony was picked and streaked on a nutrient agar slope in a screw-capped tube, and stored at 4°C. However, if staphylococci failed to grow on the blood-agar plate, a transfer from the broth culture was streaked on a second blood-agar plate and after incubation at 37°C a discrete colony was cultured on an agar slope. This was incubated at 37°C overnight and stored at 4°C.

The cultures thus obtained were replated on blood-agar plates at three-week intervals and the new isolates cultured at 37°C overnight on nutrient agar slopes and stored at 4°C. Each propagating strain of Staphylococcus was designated by placing PS before the number of the homologous phage. Thus, the propagating strain for phage 3A was labelled PS3A.

For use in propagating a phage, a propagating strain was sub-cultured from the agar slope in trypticase soy broth. This broth culture was discarded after two weeks, at which time a fresh culture in broth was prepared from the agar slope.

#### Broth Method of Propagating Bacteriophages

A propagating strain of Staphylococcus was sub-cultured in 8 ml trypticase soy broth at 37°C for two hours. One milliliter of the homologous phage was then added and incubation at 30°C continued for 2-6 hours, depending on the phage. Generally a maximum titer was obtained when the broth started to clear up. The lysed culture was centrifuged at 4,000rpm., and the supernatant titered. The method of titering will be given later.

The supernatant was stored at 4°C.

If the supernatant had a titer less than 1/1,000, it was used as phage for repeating the process described immediately above until the supernatant gave a titer of 1/1,000 or more. The supernatant with the required titer was passed through a sintered glass filter (Hysil, grade 5), and the filtrate titered and checked for lytic activity on the other propagating strains. The lytic spectrum will be referred to later.

#### Plate Method of Propagating Bacteriophages

Freshly prepared trypticase soy agar was poured in 15 cm Petri dishes to a depth of 5 mm. The plates with the lids slightly opened were then dried at 37°C for one hour and with the lids on for 10 hours. Each plate was flooded with one of the overnight trypticase soy broth cultures and allowed to stand at 20°C for five minutes. Excess broth was drawn off with a sterile Pasteur pipette, and the agar surface dried for one half hour with the lid slightly opened. The homologous phage was spread over all but a small segment of

the agar surface. This segment served as a control on the growth of the bacteria. Incubation was either at  $37^{\circ}\text{C}$  for 3-6 hours or at  $30^{\circ}\text{C}$  for 10-15 hours.

Incubation at  $30^{\circ}\text{C}$  generally gave higher titers. However, at either temperature close observation should be carried out at three hours and after to detect overgrowth of lytic areas by secondary colonies. The control segment was then removed with a sterile knife, and the preparation held at  $-20^{\circ}\text{C}$  overnight. This was followed by leaving the plate slightly tilted at  $20^{\circ}\text{C}$  to thaw. The fluid expressed on thawing was drawn off with a Pasteur pipette and centrifuged at 4,000 rpm. A portion of the supernatant was titered and the remaining portion stored at  $4^{\circ}\text{C}$ . If a satisfactory titer was obtained (1/1,000 or higher) the supernatant was passed through a sintered glass filter. The filtrate was titered, and checked for lytic activity on other propagating strains. If the supernatant failed to give a satisfactory titer, the process was repeated using the supernatant as phage.

The plate method was found to be essential

for the propagation of phages 29, 52, 52A and 79 and to be superior for phage 187. However, it was much more convenient in terms of labour and equipment to propagate the others by the broth method, which method was used for the propagation of the larger group.

#### Optimum Incubation Time for Phage Production

During the propagation of phages, it was observed that time of incubation was important in obtaining high titer. Over incubation very often gave an unsatisfactory titer (less than 1/1,000). The following experiment was set up in an attempt to find the optimum time of incubation.

Two test tubes of trypticase soy broth were sub-cultured with a propagating strain of Staphylococcus in the same way as in the propagation of phage by the broth method. To one tube 0.5ml of homologous phage suspension having a titer of 10 was added. The other tube served as a control on the growth of the propagating strain. These cultures were incubated in a water bath at 37°C. To test for growth and lysis, readings at 15-minute intervals on the percent light transmittance

with a Beckman colorimeter (Model C) were taken. One hour after the phage had been added, an aliquot was taken from the tube containing phage and the aliquot centrifuged at 4,000 rpm and titered. Thereafter, aliquots were taken every half hour, and the aliquots centrifuged and titered until the percent transmittance remained stationary or decreased steadily for one hour. The remaining portion of the culture containing the phage was then incubated at 37°C overnight. After incubation, it was centrifuged and the supernatant titered.

#### Titration of Phage-Containing Broth

Serial ten-fold dilutions of the filtrate were prepared. Freshly prepared trypticase soy agar was poured in Petri dishes and dried at 37°C overnight. Each plate was then flooded with a six-hour culture of the homologous propagating strain, and the excess liquid drawn off. The surface of the medium was permitted to dry, the lid being slightly opened. When dry, the surface of the medium was spotted with one drop of each dilution on different areas of the plate. Incubation was at 30°C overnight. The routine test dilution (R. T. D.) was the highest dilution at which the

phage produced confluent lysis on the propagating strain. A titer of 1/10 indicated 10 times as strong as the routine test dilution.

### Testing of Bacteriophages

At the outset it was essential to test each phage for contamination or slight variations otherwise undetected. This was carried out by comparing its lytic activity with the standard lytic spectrum issued by the Staphylococcal Reference Laboratory, Colindale, England, and the Laboratory of Hygiene, Ottawa, Canada.

Each new phage was tested on the following propagation strains for lytic activity:

PS29, PS29A, PS42B/47C, PS42C, PS42E,  
PS44A, PS47, PS51, PS52, PS53, PS54,  
PS80, PS2009.

The undiluted phage suspension was spotted on each propagating strain, and the phage was titered on those strains which were lysed. The highest dilution of the phage suspension that gave 50 or more plaques on the homologous propagating strain



was given the arbitrary number of 5. The lytic activity of this phage on the other propagating strains listed was graded relative to this reaction, as follows:

- 5 = maximum titer.
- 4 =  $10^1$  -  $10^2$  of maximum titer.
- 3 =  $10^3$  -  $10^4$  of maximum titer.
- 2 =  $10^5$  -  $10^6$  of maximum titer.
- 1 = Occasional or very weak lysis.
- 0 = Inhibition.

#### Typing of Isolates

Routine test dilutions (R. T. D.) in trypticase soy broth were prepared for each of the 27 phages used. Each R.T.D. was checked twice weekly by spotting it on its homologous propagating strain. If the spot showed less than 50 plaques, this R.T.D. was discarded and new R.T.D. was prepared from stock phage suspension.

Isolates growing in the trypticase soy broth mentioned earlier were checked for purity and morphology by Gram staining. Only those cultures showing cocci, less than one micron and arranged in grape-like clumps were typed.

A freshly prepared and dried trypticase soy agar plate was flooded with one of the six-hour broth cultures referred to earlier. Excess liquid was drawn off with a sterile Pasteur pipette, and the medium surface permitted to dry with the lid slightly opened. The plate was placed on a labelled spinned wheel as shown in Fig. 1, and each of the 27 phages was spotted with a loop on the proper area. One loopful of phage suspension contained approximately 0.005 ml. After the spotted areas dried, the plate was incubated at 30°C overnight with the plate inverted. The lytic activity of the different phages on the isolate were recorded, as follows:

++ = confluent lysis, or 50 or more plaques.

+ = 20 - 50 plaques.

± = weak lysis or less than 20 plaques.

If confluent lysis occurred with 3A, 3B and 3C only the result was recorded as 3A/3B/3C. If on the contrary in addition weak lysis occurred with some of the other phages the result was recorded as 3A/3B/3C+.

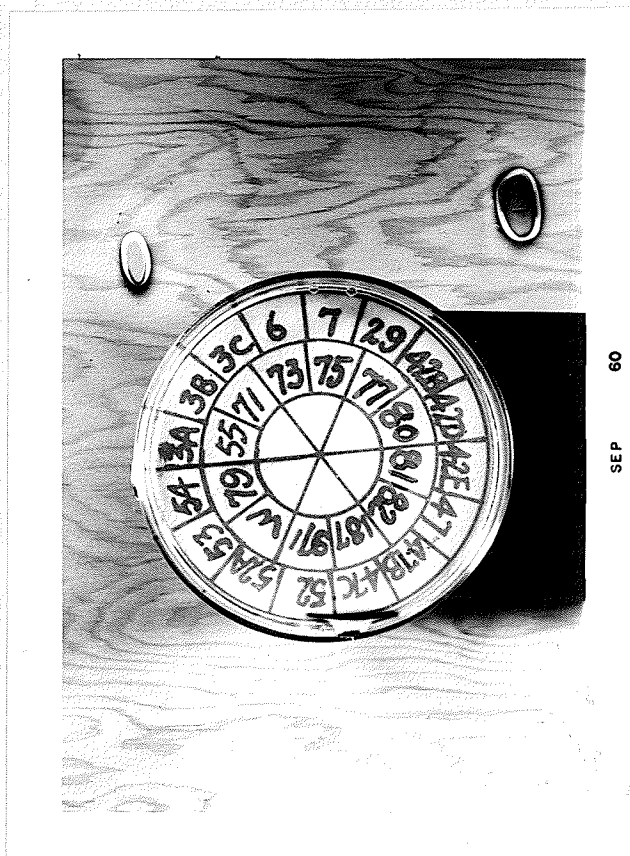


Fig. 1.

Spotting of isolates with phages.  
 Trypticase soy agar plate, flooded with broth  
 culture and dried, was placed on the spinning  
 wheel. Each phage was spotted on the proper  
 area.

## EXPERIMENTAL

## EXPERIMENTAL

### Optimum Time of Incubation for Phage Production

The phages studied (3A, 3B, 3C, 6, 7, 42, 47, 51, 53, 54, 55, 71, 73, 75, 77, 80, 81 and 82), could be divided into three groups according to the relationship between time of incubation and concentration of phage particles produced. The first group included phages 3A, 3B, 7, 42E, 47, 71, 73, 80, 81 and 82, and is represented in Fig. 2 by phage 3A. It may be observed that the staphylococci increased in number for about two hours after which time lysis has a greater effect than increase in number of cells. The titers of the aliquots showed that at one hour the phage particles had increased in number, and the number of phage particles kept on increasing for three to five hours, when a slight drop in titer occurred. This titer remained approximately the same thereafter, even after incubation overnight. The detailed data concerning colorimetric readings and titers of the aliquots on the individual phages may be found in appendix A.

The second group included phage 77, 75 and 51 and is represented in Fig. 3 by phage 77. In this group,

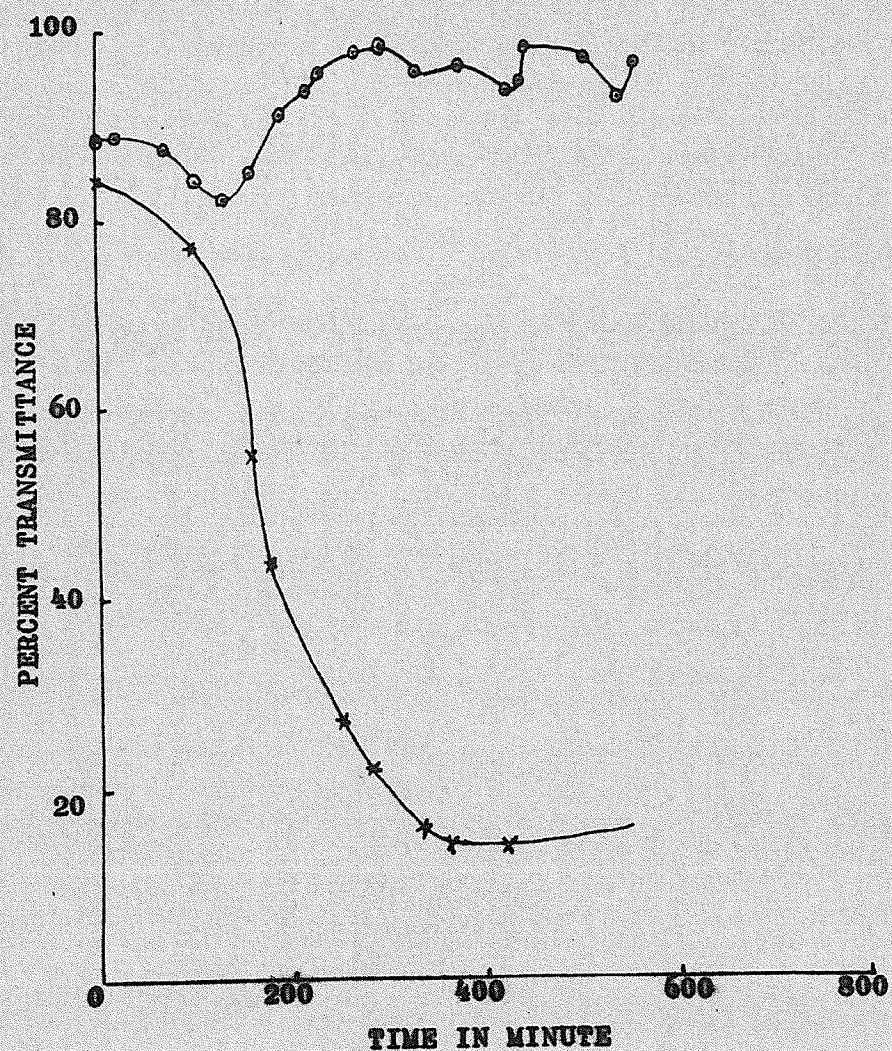


Fig. 2. Lysis of a strain (3A) of Staphylococcus aureus by its homologous phage in trypticase soy broth at 37°C, measured with a Beckman Colorimeter.

\*—\* Growth of Staphylococcus aureus.

o—o Lysis of Staphylococcus aureus by its homologous phage.



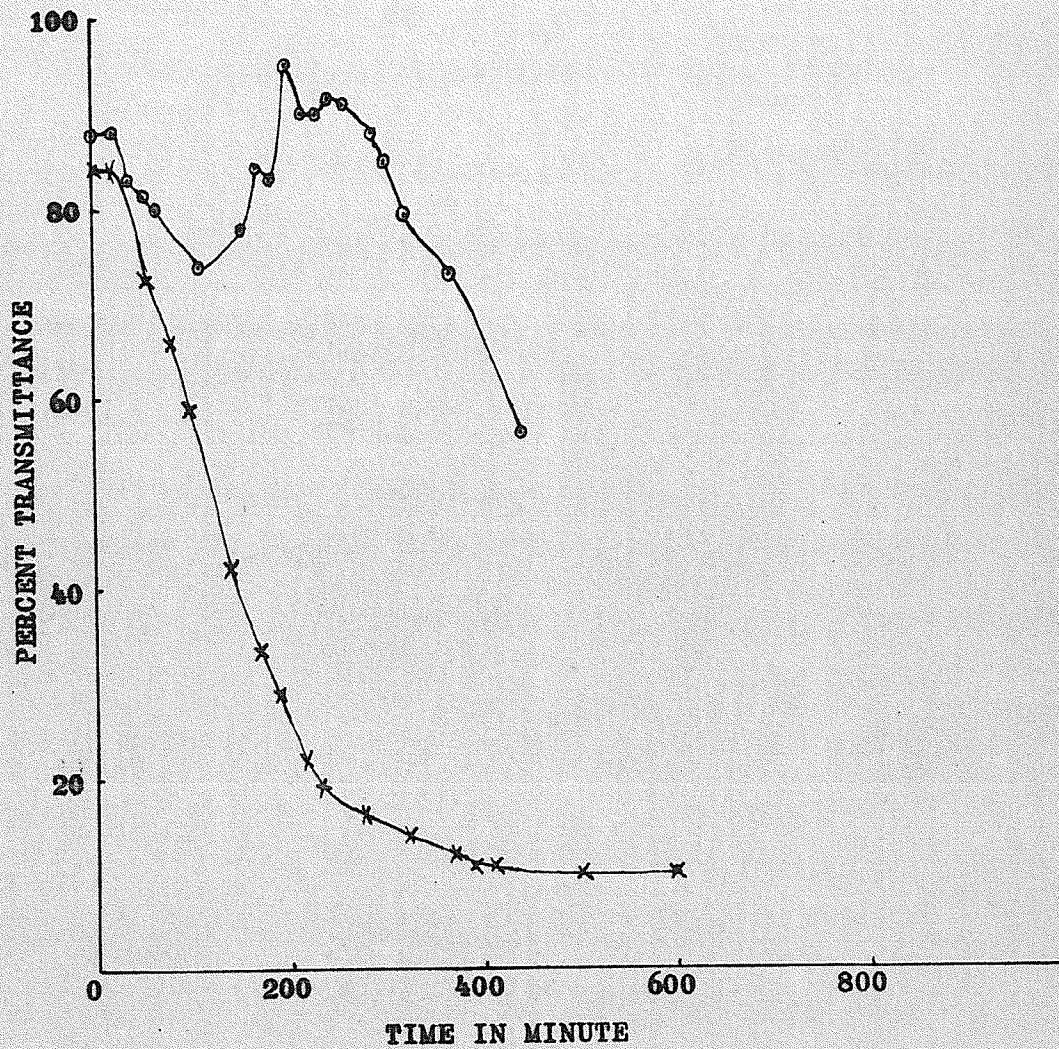


Fig. 3. Lysis of a strain (77) of *Staphylococcus aureus* by its homologous phage in trypticase soy broth at 37°C, measured with a Beckman Colorimeter.

x — x Growth of *Staphylococcus aureus*.

o — o Lysis of *Staphylococcus aureus* by its homologous phage.

the first two to three hours of incubation gave approximately the same result as did the first group. However, with each phage of this group soon after the maximum titer had been reached, the staphylococci increased and the phage particles decreased rapidly as shown by the titers of the aliquots. After incubation overnight no reaction or only very weak lysis occurred when the undiluted supernatant was spotted on the homologous strain.

The third group included phages 3C, 6, 42D, 53, 54 and 55 and is represented in Fig. 4 by phage 3C. In this group the result during the first two to three hours of incubation was much the same as in the previous two groups. However, when the maximum titer had been reached, it remained stationary for two to four hours. Then, the titer decreased slowly, and if incubation was continued overnight, a satisfactory titer ( $10^3$  or more) was not obtained.

The data on these three groups indicated that the optimum time of incubation for the production of these phages was from two to six hours.



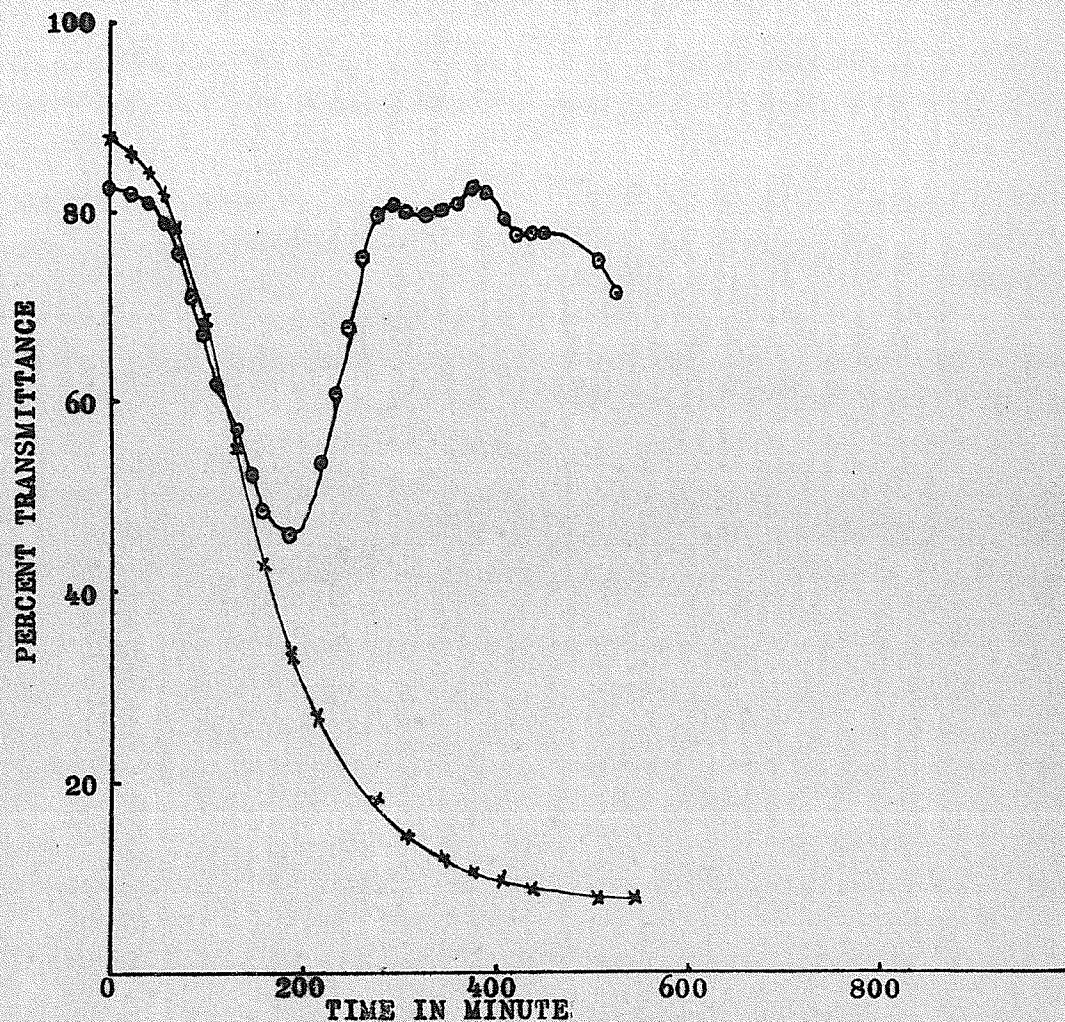


Fig. 4. Lysis of a strain (3C) of *Staphylococcus aureus* by its homologous phage in trypticase soy broth at 37°C, measured with a Beckman Colorimeter.

x — x Growth of *Staphylococcus aureus*  
 o — o Lysis of *Staphylococcus aureus* by its homologous phage.

Phage-Types of Staphylococci--All Areas

Three hundred and seventy-seven isolates were typed during the course of this study. Three hundred and twenty-three were from milk taken directly from cows, 47 from pooled samples, and seven from bulk milk. In three trials (30 milk samples) with bottled pasteurized milk staphylococci were not encountered. One hundred and seventy-two of the isolates from milk obtained directly from udders were lysed by one or more of the phages used. However, the number of isolates susceptible to these phages varied greatly--from 83.3% (West Kildonan) to 0% (St. Vital). Only seven of the 47 isolates from pooled samples were susceptible to one or more of the phages. Six of the seven isolates from bulk milk were not susceptible to any of the phages.

Several isolates were susceptible to practically all the phages and were considered to be nontypable. Most of the typable isolates could not be grouped in the basic set recommended by the International Committee on Phage Typing of Staphylococcus (10). Further none of the isolates was susceptible to phage 42D alone, but a few were susceptible to it as well as to certain others.

TABLE I

Phage-Types of Staphylococci from Udder Milk

Source of milk	No. of isolates	No. susceptible to phage 81	No. not susceptible to phage 81	No. of non-typable
University	40	24	1	15
Grunthal	35	22	0	13
Mastitic strains	20	13	2	5
Stonewall	15	5	2	8
Haywood	12	4	0	8
Kenton	10	0	5	5
W. Kildonan	6	0	3	3
Gunton	5	0	2	3
Total	143	68	15	60

Phage Type of Staphylococci from Seven Areas

From herds in seven areas in Manitoba 7400 udder samples of milk were obtained aseptically. From these samples 123 staphylococcal isolates were obtained. As may be observed in Table I, 68 or 47.5% of these isolates were susceptible to phage 81. Forty-three percent of the isolates susceptible to phage 81 were susceptible also to the following phages 42B, 47B, 80 and 82, and some others to certain phages of this group. The phage susceptibility pattern of phage 81 and the other four phages are presented in Table II. Only one of the 68 isolates was susceptible to phage 81 but not to the other four. This isolate belonged to group III (42E/73/81~~4~~).

Isolates from herds in two areas, Kenton and Gunton, were not susceptible to phage 81 but were susceptible to phage 77. The majority was susceptible also to phage 47B. Three types were recognized, 77; 47B/77; and 3B/6/7/42B/42D/47B/77. The first two types could be included in Group III of the basic set. Five or 33.3% of the isolates were not susceptible to any of the phages. Most of the isolates from the herd in West Kildonan were weakly lysed by many phages. Considering only strong lysis, one of the

TABLE II

Susceptibility to Certain Other Phages by Staphylococci  
from Milk Lysed by Phage 81

Source of isolates	No. of isolates lysed by phage 81	Phage			
		42B	47B	80	82
University	1	++	++	-	-
	21	++	++	++	-
Grunthal	18	++	++	++	++
	3	-	-	++	-
	1	-	-	++	++
Mastitic strains	6	++	++	-	-
	1	-	-	++	++
	2	++	++	++	++
Stonewall	5	++	++	++	++
Haywood	4	++	++	++	++
Total	67	57	57	55	31

++ = 50 or more plaques.

- = no reaction.

isolates was type 52A+; one type W+; and one type 52A/80/82/971+. Two isolates were strongly lysed by practically all of the phages and one was not sensitive to any. They were considered nontypable. Of 20 additional isolates from mastitis-infected cows 13 were susceptible to phages 6/53/77/81. This may be considered a broad type, including in it the basic group III and phage 81. This type may be divided into three subtypes on the basis of susceptibility of each isolate to other phages.

Twenty typable isolates from the university herd belonged to 6/7/42B/47/47B/73/54/80/81/W+. These phages except 80, 81, 42B and 47B belonged to Group III. Phage 80 belonged to Group I and the remaining ones were not listed in the basic set. One of the typable isolates was type 52A; one, type 42E/73/81; one, type 42B/47B/54/81/W+; one, type 42B/81+; and one, type 42B/47B/77/80/81. None of these isolates was sensitive to phages in Groups II and IV, except three isolates which were susceptible to practically all the phages. These three plus 12 not susceptible to any phage gave 15 nontypable ones. Three phage types were found from <sup>the</sup> Grunthal herd. Most of the phages that lysed these isolates were the ones not listed in the basic set. These isolates were sensitive

to only one phage in Group I, two in Group II, one in Group III but none in Group IV. Five of the seven typable isolates from the herd at Stonewall were strongly lysed by phages 3B/3C/42B/47B/77/80/81/82. They may be subdivided into two subtypes according to susceptibility to other phages, namely: 3B/3C/42B/42E/47/47B/54/73/77/80/82/W and 3B/3C/42B/47B/77/80/81/82/971. The remaining two typable isolates were type 47/47B/54/77 which is practically the same as Group III. All of the four typable isolates from the Haywood herd were susceptible to phages 42B/47B/71/77/80/81/82.

It was found that in each area one phage-type was predominant, but different predominant phage-types occurred in different areas. The predominant type in the university herd was 6/7/42B/47/47B/73/54/80/81/W+ and comprised 71.4% of the typable isolates; type 3B/3C/42B/47B/80/81/82/971 in Grunthal accounted for 81.8% of the typable ones. In the Kenton herd the five typable isolates belonged to type 77, three of these being susceptible also to phage 47B. The two typable isolates from the Gunton herd belonged to phage-type 3B/6/7/42B/42D/47B/77, and all the typable ones from the Haywood herd belonged to type 42B/47B/71/77/80/81/82. Four of the five typable isolates

from the herd in West Kildonan were susceptible to phage 52A but were lysed also by many other phages. Presumably, the predominant type was 52A, but the isolates could be divided into three subtypes on the basis of lysis by other phages.

#### Types of Staphylococci from Two Other Areas

None of the five isolates taken from 12 milk samples from the herd in St. Vital was typable. One hundred and seventy-five staphylococci were isolated from 1024 milk samples obtained from 13 herds in Tuxedo. Of the 175 isolates 92 were not typable and 83 typable. The majority of the typable isolates were susceptible to phage 81 and to four other phages, namely: 42B, 47B, 80 and 82 as was the case in most of the isolates referred to in the previous section. Further, most of these isolates were lysed in addition by phage 42D. It should be noted that isolates in the other areas susceptible to these phages were not susceptible to phage 42D.

In many cases the isolates were found to have rather complex phage-susceptibility. Eighteen fairly characteristic types were recognized. They are referred to as type A to type R, as may be observed



TABLE III

Phage-Types of Staphylococci from Udder Milk--Tuxedo Herds

Code	No. of letter isolates	Phage-type
A	23	3A/3B/42B/42D/42E/47B/53/54/77/80/81/ 82/971+
B	19	77+
C	7	3B/3C/42B/47B/77/80/81/82/971+
D	7	3A/3B/3C/42B/42D/47B/54/77/80/81/82/971
E	4	3B/3C/42B/42D/47B-77/80/81/82/971+
F	3	73/54+
G	3	52A
H	2	42B/47B/80/81
I	2	3B/3C/42B/42D/47B/80/81/82/971+
J	2	42E
K	2	3B/47B/54/77/80/82/W/
L	2	42B/47B/80/81/82
M	2	47/47B/52A/54/73/80/81/82/W
N	1	80/81/82/42B
O	1	6/7/47
P	1	6/7/42B/47B/73/80/81/W
Q	1	3B/47B/54/80/82/971
R	1	3B/3C/42B/47B/80/81/82/971

in Table III. The predominant type (27.7% of the typable ones) was susceptible to many phages--3A/3B/3C/42B/42D/42E/47B/53/54/77/80/81/82/971+, and the next type occurring often (22.8% of the typable isolates) was susceptible to 77+. These two types comprised approximately half of the typable isolates.

It may be observed from Table III that types A, C, D and E were susceptible to phages 47B, 77, 80, 81, 82 and 971. One could consider A, C, D and E to be subtypes of a broad type, 47B/77/80/81/82/971. This would reduce the number of types from 18 to 15 in this group of isolates. Types I and R were quite similar to this broad type. The main difference was that the isolates were not susceptible to phage 77. If the broad type were taken to include 47B, 80, 81, 82 and 971 phages, the others A, C, D, E, I and R could be considered to be subtypes of type 47B/80/81/82/971. This would reduce the number of phage-types in this group of isolates to 13. As will be referred to later, the broad type, 47B/77/80/81/82/971 was the predominant type isolated from pooled samples.

Isolates from Pooled and Bulk Milk Samples

Forty-seven isolates were obtained from 52 pooled samples (cans) from herds in Tuxedo and St. Vital. Two of these isolates were from St. Vital herd and were nontypable. Seven of 45 isolates from Tuxedo herds were typable, and 38 untypable. The typable ones represented six different types, as shown in the following table:

TABLE IV

Phage-Types of Staphylococci from Pooled Milk

No. of isolates	Phage-type
2	42D/77
1	3B/3C/42B/42D/47B/77/80/81/82/97L+
1	3B/3C/42B/47B/77/80/81/82/97L+
1	42B/77
1	3B/3C/6/7/42B/42E/47B/77/80/81/82/97L/W+
1	3A/3B/3C/42B/47B/77/80/81/82/97L

It may be observed also that four of these isolates were susceptible to phage 81. In fact, they belonged to the broad type, 47B/77/80/81/82/971 which was the predominant type isolated from Tuxedo herds. Type 3B/3C/42B/42D/47B/77/80/81/82/971+ in this group of isolates was the same as type E from isolates in Tuxedo; and type 3B/3C/42B/47B/77/80/81/82/971+ was the same as type C (Table III). Further, these seven isolates were susceptible to phage 77. Three isolates were susceptible to phage 42D and belonged to type 42D/77. A much higher percentage (84.6%) of nontypable isolates was encountered in these pooled samples than was the case in milk taken directly from the udder in the Tuxedo herds (52.6%).

Only one of seven isolates from three bulk (tank) samples was typable. It belonged to type 3A/3B/3C/42B/42D/42E/47B/53/54/77/80/81/82/971+, which was the predominant type in Tuxedo herds.



## DISCUSSION

## DISCUSSION

Staphylococci of phage type 81 were common in milk from the areas studied. This is in close agreement with the findings by other workers in Canada (1). Staphylococci lysed by this phage were lysed by other phages also, especially phages 42B, 47B, 80 and 82. Therefore, in Manitoba at least these five phages should be considered as a group in the typing of staphylococci of bovine origin.

Of interest is the fact that in this study staphylococci of phage-type 42D was encountered only rarely. This has been reported to be the predominant type of bovine origin in Canada (21). The finding that phage type 42D was not the predominant type could mean that shifting of types has occurred, or that type 42D has become "immune" to this phage in the Winnipeg area.

Classification into the broad phage groups recommended by the International Committee on

Phage Typing of Staphylococci (10) was of little value in the differentiation of the strains of Staphylococcus isolated in this study. Most of the typable isolates were susceptible to phages in more than one of the groups, and more often to phages not listed in the basic set. Indeed, phages not listed in the basic set were of greater value than the phages listed in it. Most of the typable isolates were susceptible to unlisted phages together with phages in Group III. In addition, phage 80 was found to be associated more often with Group III phages than with Group I phages, in which group phage 80 is placed in the basic set.

Since fewer than 60% of the isolates were typable by these 27 phages, a different set of phages might be of greater value for typing staphylococci of bovine origin. The phages should be isolated and propagated on bovine staphylococci. Seto and Wilson (18) have shown that 93% of the bovine staphylococci studied were typable by phages propagated on micrococci of bovine origin. However, different phage types

might be expected in different areas. This would present a problem in obtaining a standard set for general use. It might be possible to prepare a better set of phages by selection of isolates of bovine origin from a large number of isolations in many different areas, and by adapting some of the more specific phages by the use of bovine staphylococci.

It may be observed that variation from a type appears to take place or in other words that two phage-types of staphylococci arise from one origin. This is apparent from the typable isolates from Tuxedo herds. Phage-types A, D, C and E (Table III) were susceptible to phages 47B, 77, 80, 81, 82 and 971 and strongly or weakly lysed by others. The main difference separating these four types was the degree of lysis by the other phages, differences in which could have been due to variations in environmental conditions. Because of this probability it was suggested that these types A, C, D and E should be considered sub-types of type 47B/77/80/81/82/971/. Probably this would be the same for the two phage-types isolated from the Kenton herd. Types 77 and 47B/77 might have the same origin.



Two of the phage-types isolated from Tuxedo herds appeared in the pooled samples, in addition to two other types. The latter two types were susceptible to phages 42B, 42D and 77. Since many of the isolates from the herds studied were susceptible to these two phages and since phage 42D is of bovine type (21), one hesitates to conclude that the presence of these two types of staphylococci indicates contamination of the milk during handling. However, their appearance in the pooled milk remains unexplained.

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## APPENDIX



## APPENDIX A

Colorimetric Readings on the Growth and Lysis of Strains of Staphylococci, and the Titers of the Lysed Cultures.

Time	Colorimetric Reading								
	3A			3B			7		
	G	L	T	G	L	T	G	L	T
0	88	89		80	75		86	90	
20	88	89		79	72		86	87	
40	87	90		73	70		85	85	
55	86	89	10 <sup>2</sup>	69	64	10 <sup>2</sup>	78	83	NO
70	84	87		65	62		79	79	
85	81	85		61	61		74	75	
100	77	84		57	63		70	71	
115	74	83	10 <sup>2</sup>	52	67	10 <sup>3</sup>	65	67	
130	68	82		48	76		60	65	
145	62	82		44	81		55	64	10
160	55	85		41	84		49	64	
175	50	89	10 <sup>3</sup>	38	92	10 <sup>4</sup>	45	69	10 <sup>2</sup>
190	43	91		35	97		40	72	
205	40	93		32	98		35	74	10 <sup>3</sup>
220	35	93		29	98		32	76	
235	31	95	10 <sup>4</sup>	27	98	10 <sup>4</sup>	29	77	10 <sup>4</sup>
250	28	96		25	97		27	78	
265	24	97		22	96		24	80	
280	22	98		20	95		21	82	
295	19	98	10 <sup>5</sup>	19	97	10 <sup>4</sup>	18	82	10 <sup>4</sup>
310	17	96		18	97		16	80	
330	16	95	10 <sup>4</sup>	16	97		14	81	
350	14	94		15	97		13	84	
365	14	95	10 <sup>4</sup>	14	97	10 <sup>4</sup>	12	87	10 <sup>4</sup>
380	14	96		14			12	88	
395	14	97	10 <sup>4</sup>	13			11	90	
410	16	95		12			10	89	
425	17	93		12			9	86	
440	16	94	10 <sup>4</sup>	12			9	87	10 <sup>4</sup>
455	16	98		12					
510	16	96							
525	17	95							
540	16	92							
555	16	96	10 <sup>4</sup>						
Overnight			10 <sup>4</sup>			10 <sup>4</sup>			10 <sup>3</sup>

G = Growth

L = Lysis

T = Titer

Readings on per cent transmittance.

time	Colorimetric Reading								
	42E			71			73		
	G	L	T	G	L	T	G	L	T
0	92	91		76	89		99	94	
20	94	91		73	87		98	93	
35	84	92		71	86		97	92	
50	98	94		69	86		98	93	
65	97	95	10	66	85	none	96	92	10 <sup>2</sup>
80	99	95		62	82		95	93	
95	97	95		60	83		95	96	10 <sup>3</sup>
110	94	93		55	83		90	96	
125	97	94	10 <sup>3</sup>	51	82		88	94	
140	93	93		48	83	10 <sup>2</sup>	85	98	10 <sup>4</sup>
155	92	93		45	86		83	96	
170	92	95		42	84		80	98	
185	85	93	10 <sup>3</sup>	38	83	10 <sup>3</sup>	76	97	10 <sup>4</sup>
200	84	93		36	85		73	97	
215	79	93		33	87	10 <sup>4</sup>	70	97	
230	73	95		31	88		68	99	10 <sup>4</sup>
245	68	92	10 <sup>4</sup>	28	90		64	97	
260	60	95		26	91		61	97	10 <sup>4</sup>
275	56	93		23	88	10 <sup>4</sup>	55		
290	49	96		21	90		52		
305	43	93	10 <sup>4</sup>	20	92		51		
320	38	95		19	94	10 <sup>4</sup>	49		
335	32	90		17	92		45		
350	29	93		17	92		43		
370	25	95	10 <sup>4</sup>	16	92	10 <sup>4</sup>	34		
390	22	93		14			26		
410	19	95		14			22		
440	15	93	10 <sup>4</sup>						
470	13								
500	10								
520	8								
540	9								
overnight			10 <sup>4</sup>			10 <sup>4</sup>			10 <sup>3</sup>

Time	Colorimetric Reading								
	80			81			82		
	G	L	T	G	L	T	G	L	T
0	74	68		91	92		93	94	
20	74	69		93	93		94	95	
40	72	67		93	95		93	95	
55	70	64	none	95	96	10	94	94	$10^2$
70	71	63		94	96		94	95	
85	69	62		94	96		94	95	
100	64	63		93	96		93	96	
115	59	58		93	97	$10^2$	92	95	$10^2$
130	53	52		91	96		90	94	
145	49	50		90	96		89	95	
160	44	49		88	96		87	94	
175	40	50	10	85	96	$10^3$	84	93	
190	37	50		80	95		81	93	
205	33	50		76	94		79	93	
220	28	50		73	94		75	93	
235	26	51	$10^2$	68	95	$10^4$	73	93	$10^3$
250	22	51		64	95		68	93	
265	21	54		58	95		64	96	
280	19	56	$10^3$	54	97		58	95	
295	17	63		46	96	$10^4$	52	93	$10^3$
310	16	71		42	94		46	93	
330	14	79		35	93		40	93	
350	13	83	$10^5$	31	91	$10^4$	37	93	
365	12	85		27	93		33	94	
380	11	88		25	95		30	96	
395	10	88		23	95		27	94	
410	9	89	$10^5$	20	94	$10^4$	24	92	$10^3$
425	9	89		17	92		22	94	
440	9	88		16	95		21	96	
455	9	87		14	97	$10^4$	18	96	$10^4$
510	9	87	$10^5$	12	95		17	95	$10^4$
525	9	90		11	95		14	95	$10^4$
540	9	92		10	93				
555	9	90	$10^5$	9	93	$10^4$			
overnight			$10^4$			$10^4$			$10^4$

Time	Colorimetric Reading								
	51			75			77		
	G	L	T	G	L	T	G	L	T
0	92	93		75	74		84	88	
20	89	89		74	72		84	88	
35	79	84		64	66		72	83	
50	81	89		67	65		73	81	
65	78	88	10 <sup>2</sup>	64	63	none	71	80	none
80	75	88		61	58		66	78	
95	73	88	10 <sup>3</sup>	55	56	10	59	74	none
110	68	93		50	58		52	74	
125	63	92	10 <sup>4</sup>	45	63	10 <sup>2</sup>	47	75	10
140	60	86		41	67		42	72	
155	57	86	10 <sup>4</sup>	37	68	10 <sup>3</sup>	38	78	10 <sup>2</sup>
170	56	86		34	68		33	84	
185	50	84	10 <sup>3</sup>	30	69		29	83	10 <sup>3</sup>
200	47	84		28	67		25	95	
215	43	77	10 <sup>2</sup>	25	69	10 <sup>3</sup>	22	90	10 <sup>4</sup>
230	39	75		22	71		19	90	
245	33	70		21	73	10 <sup>3</sup>	18	91	10 <sup>4</sup>
260	29	67	10 <sup>2</sup>	19	75		17	89	
275	23			17	75	10 <sup>3</sup>	16	89	10 <sup>4</sup>
290	19			16	78		16	88	
305	18			14	78	10 <sup>3</sup>	14	85	10 <sup>4</sup>
320	17			12	79		14	84	
335	16			11	81		14	79	10 <sup>3</sup>
350	15			11	80	10 <sup>3</sup>	14	77	
370	15			10	80	10 <sup>3</sup>	12	73	10 <sup>3</sup>
390	15			9	75		11	67	
410	14			9	72		11	62	10 <sup>2</sup>
440	13			8	72	10 <sup>2</sup>	10	56	10
470	11			8	69		11		
500	10			7	66	10 <sup>2</sup>	10		
overnight			very weak reaction			weak reaction			none

Time	Colorimetric Reading								
	5C			6			42D		
	G	L	T	G	L	T	G	L	T
0	88	82		81	90		90	89	
20	86	82		83	86		89	88	
40	84	81		82	85		87	88	
55	82	79	none	80	83	none	87	87	none
70	78	76		77	80		85	86	
85	73	71		74	76		84	84	
100	68	67		70	71		82	83	
115	62	62		65	67		79	80	
130	55	57	none	60	61	none	73	76	
145	49	52		55	57		67	75	10
160	43	49	10	50	53		63	77	
175	38	47		44	50	10	58	81	10 <sup>2</sup>
190	33	46	10 <sup>2</sup>	40	46		51	86	
205	29	49		36	44		45	88	10 <sup>3</sup>
220	27	54	10 <sup>2</sup>	33	43	10 <sup>2</sup>	43	90	
235	24	61		30	43		40	93	10 <sup>3</sup>
250	22	68	10 <sup>3</sup>	28	44		39	93	
265	20	75		25	47	10 <sup>2</sup>	36	91	10 <sup>4</sup>
280	18	80	10 <sup>4</sup>	23	48		33	92	
295	16	81		19	52	10 <sup>3</sup>	32	91	10 <sup>4</sup>
310	14	80	10 <sup>4</sup>	18	57		33	90	
330	13	80		16	60	10 <sup>3</sup>	33	89	
350	12	80		14	64		31	88	
365	11	81		14	65	10 <sup>4</sup>	29	88	10 <sup>4</sup>
380	11	82	10 <sup>4</sup>	13	66		28	87	
395	10	82		13	65	10 <sup>4</sup>	27	86	
410	10	79		12	65		25	86	
425	9	78		11	63	10 <sup>4</sup>	24	85	10 <sup>4</sup>
440	9	78	10 <sup>4</sup>	11	60	10	22	84	
455	9	78		11	57		22	84	
510	8	75		10	55		20	82	10 <sup>4</sup>
525	8	71	10 <sup>4</sup>	10	52	10 <sup>3</sup>	20	81	
540	8			10	51	10 <sup>2</sup>	18	81	10 <sup>3</sup>
overnight			10						10 <sup>2</sup>

Time	Colorimetric Reading								
	53			54			55		
	G	L	T	G	L	T	G	L	T
0	81	69		96	98		85	86	
20	77	68		97	88		86	86	
35	71	63		92	90		83	87	
50	65	58		95	87		82	86	
65	59	53	none	94	86	10	82	86	none
80	54	51		93	90		80	87	
95	49	51		91	88		77	84	
110	45	51		86	86		70	84	10
125	41	50	10	84	87	$10^2$	67	86	
140	34	46		79	86		61	84	
155	30	45	$10^2$	74	87	$10^3$	57	84	$10^2$
170	27	47		71	90		53	88	
185	25	69	$10^3$	63	86	$10^3$	47	85	$10^3$
200	22	81		58	89		47	89	
215	19	86	$10^4$	51	89	$10^4$	44	90	$10^4$
230	17	87		46	90		42	90	
245	15	87	$10^4$	41	90		39	89	$10^4$
260	14	83		37	89		37	90	
275	13	86	$10^4$	33	90	$10^4$	35	92	$10^4$
290	12	83		26	91		32	93	
305	11	85		24	89		29	92	$10^4$
320	10	87		20	89	$10^4$	27	92	
335	10	86		20	88		23	90	$10^4$
350	10	85	$10^4$	19	88		22	90	
370	10	82		17	87	$10^4$	19	91	$10^4$
390	10	82		14	86		17	90	
410	10	75	$10^4$	13	88		16	92	$10^4$
440	10	75		11	87	$10^4$	13	88	
470	10	75		10	86		11	89	$10^4$
500	9	73	$10^3$	9	83	$10^3$	10	83	
overnight			10			$10^2$			10

## APPENDIX B

## Phage-Types of Staphylococci from Milk

Source of isolates	No. of isolates	Strongly lysed (++) by	Weakly lysed by (+ or ±)
Gunton	2	3B,6,7,42B,42D, 47B,77.	none
Grunthal	18	3B,3C,42B,47B, 80,81,82,971.	none
	3	47,80,81.	3B,42B,82,W.
	1	47,80,81,82	3B,42B,W.
Haywood	4	42B,47B,71,77, 80,81,82.	none
Kenton	2	77	none
	3	47B,77	none
Stonewall	3	3B,3C,42B,42E, 47B,54,73,77 80,81,82,W.	none
	2	47,47B,54,77.	none
	2	3B,3C,42B,47B, 77,80,81,82,971.	42E.
Tuxedo	23	3A,3B,42B,42D, 42E,47B,53,54, 77,80,81,82,971.	3C,W.
	19	77.	3B,6,7,73,W.
	7	3B,3C,42B,47B, 77,80,81,82,971.	42E,42D,54.
	7	3A,3B,3C,42B, 42D,47B,54,77, 80,81,82,971.	none
	4	3B,3C,42B,42D, 47B,77,80,81, 82,971.	3A,42E.
	3	73,54.	971.

Source of isolates	No. of isolates	Strongly lysed (++) by	Weakly lysed (+ or ±) by
Tuxedo	3	52A.	none.
	2	42B, 47B, 80, 81.	none
	2	3B, 3C, 42B, 42D, 47B, 80, 81, 82, 971.	73, 77.
	2	42E.	none
	2	3B, 47B, 54, 77, 80, 82, W.	none
	2	42B, 47B, 80, 81, 82.	none
	2	47, 47B, 52A, 54, 73, 80, 81, 82, W.	none
	1	80, 81, 82, 42B.	none
	1	6, 7, 47.	none
	1	6, 7, 42B, 47B, 73, 80, 81, W.	none
	1	3B, 47B, 54, 80, 82, 971.	none
	1	3B, 3C, 42B, 47B, 80, 81, 82, 971.	none
University of Manitoba	20	6, 7, 42B, 47, 47B, 73, 54, 80, 81, W.	82.
	1	52A.	none
	1	42E, 73, 81.	80, W.
	1	42B, 47B, 54, 77, 81, W.	80.
	1	42B, 81.	77, 971.
	1	42B, 47B, 77, 80, 81.	none



Source of isolates	No. of isolates	Strongly lysed (++) by	Weakly lysed (+ or ±) by
West Kildonan	1	52A.	3B,6,7,42B,42D, 47B,54,80,81,82, W
	1	W.	3B,6,7,42B,80,82.
	1	52A,80,82,971.	3B,6,7,42B,47B.