

EFFECT OF SILICATE ON GRAM STAINING AND VIABILITY OF PNEUMOCOCCI AND OTHER BACTERIA*

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(Received for publication, December 23, 1955)

In the course of preparing suspensions of heat-killed pneumococci in a phosphate buffer at pH 7.4, it was observed that the bacteria uniformly and promptly became Gram-negative instead of remaining Gram-positive. Conversion to Gram negativity was traced to the presence of silicate in the NaOH solution that had been used in the preparation of the phosphate buffer. Contamination by silicate had occurred during storage of the stock solution of 5 N NaOH in a soft glass bottle over a period of several months. Following this observation, study was made of the effect of silicates on the staining properties and viability of a number of species of Gram-positive and Gram-negative bacteria. The present paper summarizes some of these experiments and shows that while silicate affects the Gram staining of various Gram-positive bacteria, its action on pneumococci and certain strains of streptococci which may be related to pneumococci is in some ways unique. Reversal to Gram positivity of pneumococci and a few strains of streptococci which have been made Gram-negative by suspension in silicate solutions, cannot be accomplished simply by washing in water as in the case of the other Gram-positive bacteria which become Gram-negative in silicate solution, but requires the application of alkaline solutions of various salts. Furthermore, the killing action of silicates appears to be limited to pneumococci and certain streptococci of the *viridans* group.

Materials and Methods

Preparation of Bacterial Suspensions.—Unless otherwise noted suspensions for staining reactions were prepared by centrifuging cultures that had been grown at 37°C. for 16 to 18 hours in fresh meat infusion broth containing 1 per cent neopeptone. The cells from 100 ml. of culture were taken up in 2 ml. of distilled water and "flash-killed" by discharging them into 18 ml. of distilled water in a boiling water bath. The bath was then held at 100°C. for 20 minutes. In the case of pneumococcus flash killing at 100°C. is advantageous since suspensions that are uniformly Gram-positive can be prepared reproducibly. It should be

* These studies were sponsored by the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, and supported in part by the Office of The Surgeon General, Department of the Army, Washington, D. C.

noted that the temperature used in flash killing differs from that in the original description of Dubos (1) in which a temperature of 75°C. was employed. Silicates affect the Gram staining of pneumococci and certain other Gram-positive bacteria whether living or killed by heat at various temperatures from 56°C. to 100°C.

For treatment with silicate 1 ml. of the 5 times concentrated bacterial suspension was spun down in the centrifuge and resuspended in 5 ml. of silicate solution.

Gram Stain Technique.—New glass slides were washed in soap and water, rinsed thoroughly first in tap water, then in distilled water, and placed in 95 per cent ethanol. The slides were wiped dry with cellucotton tissue just before use. The bacteria were smeared on the glass slides over small circular areas by means of a platinum loop, dried in air, and fixed by heating gently over an open flame. After cooling, the slides were flooded for 5 minutes with a mixture composed of 5 parts 1 per cent aqueous solution of crystal violet and 1 part 5 per cent sodium bicarbonate. They were then rinsed once and flooded for 2 minutes with an iodine solution containing 1 per cent iodine and 2 per cent potassium iodide. The slides were then washed in distilled water and immersed 4 times (during a period of about 4 seconds) in a mixture composed of equal parts of acetone and 95 per cent ethanol. They were then washed in distilled water, drained, and flooded for 45 seconds with a dilute solution of fuchsin made by dissolving 0.1 gm. basic fuchsin (95 per cent dye content) in 100 ml. distilled water. The slides were then washed in distilled water and blotted dry. Smears of flash-killed but otherwise untreated cells were always made on the same slides to which had been applied cells treated with silicates or other materials.

Solutions of Silicates.—After it had been found that a number of different silicate solutions had the same effect upon the Gram stain, that is in causing Gram-positive cells to become Gram-negative, the remaining experiments both on staining effects and viability were carried out with sodium silicofluoride, Na_2SiF_6 . This compound has the advantage that when the reaction of an aqueous solution is brought from about pH 3 to neutrality by addition of NaOH, the silicate remains in solution for a period of time sufficient for the necessary manipulations to be carried out. Solutions of sodium orthosilicate and sodium metasilicate are very alkaline and when the pH is lowered by addition of acid they polymerize rapidly and flocculate.

In experiments on the Gram stain sodium silicofluoride was used in a concentration of 0.1 per cent and for its effect on bacterial viability at 0.2 per cent. Except when indicated the pH of the solutions was about 7.0. The salt was dissolved in water and the pH adjusted by addition of NaOH and the use of an inside indicator. In order to guard against fluctuations of pH the solutions were held at room temperature for 20 minutes before use.

Experiments on Bacterial Viability.—Five ml. aliquots of broth culture that had been incubated for 16 to 18 hours at 37°C. were sedimented in the centrifuge, resuspended in 5 ml. of 0.2 per cent Na_2SiF_6 pH 7.0 to 7.4, and held at room temperature. Control microorganisms were suspended in distilled water. After variable periods of time, most commonly 2 hours, dilutions of the suspensions were prepared in the basal medium of Adams and Roe (2) which preserves viability of the cells but does not permit growth. Broth is unsatisfactory for viability experiments because flocculation occurs rapidly upon addition of silicates to broth, and activity is lost. Viable counts were made in duplicate pour plates of nutrient agar containing 5 per cent horse blood after incubation at 37°C. for 24 hours.

EXPERIMENTAL

Effect of Silicate on Gram Staining of Pneumococci.—The effect of silicate in converting pneumococci to Gram negativity was tested on 18 strains, including both encapsulated (S) and non-encapsulated (R) forms. In all cases the heat-

killed organisms suspended in 0.1 per cent Na_2SiF_6 , pH 7.0 to 7.4 for 20 minutes at room temperature lost the ability to retain gentian violet after application of iodine; that is to say, they were converted to Gram negativity. Repeated washing of the silicate-treated cells in distilled water failed to remove the silicate and to permit normal staining of the treated cells. On the other hand, if the silicate-treated cells are suspended in solutions of any one of a variety of salts at alkaline pH, their capacity to give a Gram-positive reaction is restored.

TABLE I
Effect of Silicates on Gram Staining of Pneumococci

Silicate solution	Concentration	Cells converted to Gram negativity in 20 min.
	<i>per cent</i>	<i>per cent</i>
Sodium metasilicate	0.1	80
	0.01	30
Sodium orthosilicate	0.1	60
	0.01	20
Sodium silicofluoride	0.1	100
	0.01	80
Magnesium silicofluoride	0.1	70
	0.01	60

All silicates were obtained from Fisher Scientific Company, New York: sodium silicofluoride and sodium metasilicate were c.p., sodium orthosilicate and magnesium silicofluoride were technical grade.

Because of the uniform behavior of so many different strains of pneumococci most of the following studies were carried out with an encapsulated strain of type I, strain SV-I.

Effect of Various Silicates upon Gram Staining of Pneumococcus.—Table I shows the effect of a number of silicates upon the reaction of pneumococcus type I, strain SV-I to the Gram stain. All of the silicates tested caused conversion to Gram negativity at the concentrations shown. As noted above solutions of sodium orthosilicate and sodium metasilicate polymerize and flocculate rapidly when the pH is lowered toward neutrality so that the concentrations of active silicate as shown in Table I are not maintained throughout the course of the experiment. On the other hand sodium silicofluoride yields a relatively stable aqueous solution at neutral pH that can be used over a period of at least 24 hours without marked loss of activity.

The right hand column in Table I shows the per cent of cells that stained Gram-negative at concentrations of 0.1 per cent and 0.01 per cent silicate.

These figures are estimates made from a survey of a number of microscopic fields under oil immersion lens. Sodium silicofluoride appeared to be somewhat more active than the other silicates tested and because of its greater stability in solution was chosen for subsequent experiments. Upon the appearance of the flocculum of polymerized silicate that occurs when solutions of sodium silicate are adjusted toward neutrality, the solutions have lost most of their activity in causing conversion to Gram negativity. However, when the flocculated silicate is removed by centrifugation, dissolved in Na_2CO_3 , brought to neutrality and applied to pneumococci, it will again convert them to a Gram-negative state.

Although solutions of silicate in water lose activity upon standing, an opposite effect is observed in the case of phosphate buffers made with KH_2PO_4 and solutions of NaOH which contain dissolved silicate. Freshly made buffer solutions at pH 7.6 do not cause alteration in the reaction of pneumococci to the Gram stain, and no effect is manifest if prior to use the buffer solution is allowed to stand at room temperature for $1\frac{1}{2}$ hours. However, if the buffer is held at room temperature or in the icebox for 24 hours and then applied to pneumococci, the bacteria become Gram-negative. This property of the buffer is not lost on prolonged storage at icebox temperature. On the other hand, if the buffer is placed in a boiling water bath for 15 minutes, and then cooled, it loses its effect on Gram staining of pneumococci. Activity is restored upon standing for 24 hours.

The amount of silicon present in active solutions prepared from different batches of contaminated NaOH varied between 43 and 108 μg . per ml. This is comparable to the concentrations of silicon in silicates (as shown in Table I) that have been found to cause pneumococci to stain Gram-negatively. Concentrations of freshly dissolved Na_2SiF_6 , pH 7.0 to 7.4, below approximately 0.03 to 0.01 per cent often caused only a portion of the bacterial cells to become Gram-negative, so that stained smears showed a variable mixture of Gram-positive and Gram-negative forms depending upon the concentration of bacterial cells and the time of exposure to silicate. Under conditions of incomplete conversion of the population to Gram negativity, individual cells or chains of cells may show an intermediate violaceous color as contrasted to Gram-positive cells which are stained a deep purple and Gram-negative cells which stain a bright pink on the addition of the dilute fuchsin used as a counter stain. Repeated washing in distilled water fails to restore Gram positivity to pneumococci converted to negativity by treatment with silicate.

In addition, silicate-treated, flash-killed pneumococci retain their morphology for prolonged periods on storage in water at 4–8°C. This may be related to the phenomenon of "mummification" described by Policard (3) in the case of tissue cells exposed to silicate. Cells that have not been treated with silicate tend to disintegrate on prolonged storage in the icebox.

Silicate-treated pneumococci are stained normally by crystal violet alone and are indistinguishable from similarly stained, untreated cells. After addition of the iodine mordant and application of acetone-alcohol, the silicate-treated cells are readily decolorized in contrast to untreated cells.

Influence of pH of Silicate Solutions on Gram Staining of Pneumococci and Effect of pH and Heat on Reversal to Gram Positivity.—Aqueous solutions of 0.5 per cent Na_2SiF_6 were adjusted to various pH values between 4.0 and 9.8 and then applied to flash-killed pneumococci for a period of 20 minutes at room temperature. Over the range of pH 4.0–8.0 all of the cells were converted to Gram negativity but at pH 9.8 most of them remained Gram-positive. Because of this observation it was of interest to determine whether cells converted to Gram negativity by silicate could be restored to positivity by exposure to solutions of various salts at alkaline reaction. Restoration to Gram positivity of silicate-treated pneumococci can be accomplished readily in solutions of sodium borate, sodium phosphate, sodium carbonate, sodium veronal, or sodium arsenate that have been adjusted to pH values in the range of pH 7.4 to 11.0 and higher. At pH values between 7.0 and 9.0 restoration to Gram positivity was accelerated by heating the suspensions to 56°C. or 65°C. However, above pH 10 restoration occurs very rapidly in all salt solutions tested and acceleration by heat was not observed. Of the few salts tested, sodium arsenate appeared to be most active in restoring Gram positivity. Suspensions of silicate-treated pneumococci heated at 56°C. for 1 hour in $\text{M}/50$ sodium arsenate at pH 7.0 showed partial restoration. At pH 8.0 in the same salt, the cells were uniformly restored to positivity. Less complete or slower restoration to positivity under similar conditions was observed with the other salts mentioned above.

It is apparent from these observations that pneumococci converted to Gram negativity by silicate can be restored to positivity by being suspended in alkaline solutions of various salts and that the process of restoration is accelerated by heating at moderately alkaline reactions. The “restored” cells do not appear to react differently to the Gram stain as compared to untreated control cells.

Effect of Silicate on Gram Staining of Gram-Positive Bacteria Other than Pneumococci.—Table II shows the action of silicate on Gram staining of a number of bacterial species belonging to several genera. All 18 strains of pneumococcus behaved similarly in that silicate converted them to Gram negativity and they remained Gram-negative after the silicate-treated suspensions had been washed in water. Gram positivity was restored after the pneumococci had been suspended in alkaline solution. Seven strains of *Streptococcus viridans* were studied. The behavior of five of these was similar to that of the pneumococcal strains. One strain (Mørch 1498/41) was not converted to Gram negativity by silicate. With respect to *Streptococcus viridans*

it should be noted that this is not a valid species and under this designation is included a heterogeneous collection of streptococcal strains that have in common the property of causing alpha hemolysis in blood agar plates.

The single strains of Group A, C, and D *Streptococcus hemolyticus* tested were converted to Gram negativity by silicate and restored by washing in water. *Streptococcus MG* and *Streptococcus salivarius* type 1 showed no alteration in Gram staining in the presence of silicate. *Micrococcus leisodeikticus* became Gram-negative upon application of silicate but was restored to positivity by washing in water. A strain of *Staphylococcus albus*, on the other hand, showed no tinctorial alteration in the presence of silicate.

One strain of *Clostridium welchii* was studied. It became Gram-negative in silicate but restoration to Gram positivity occurred on washing in water.

Single strains of four species of Gram-negative bacteria were unaffected in their tinctorial properties by silicate.

The observations recorded in Table II show three patterns of behavior among the Gram-positive bacteria: (a) bacteria that become Gram-negative in silicate and retain this property after washing in water: pneumococci and several strains of *Streptococcus viridans* belong to this category; (b) bacteria that become Gram-negative in silicate but whose staining properties are restored by washing in water: the strains of *S. hemolyticus*, *M. leisodeikticus* and *Cl. welchii* are representatives of this category; (c) bacteria whose staining properties are not altered by silicate: *Streptococcus MG*, *S. salivarius* type 1, *Staphylococcus albus* and one strain of *Streptococcus viridans* belong in this group.

As will be described in the next section of this paper, certain of these properties are correlated with the lethal activity of silicates.

Effect of Silicate on Viability of Gram-Positive and Gram-Negative Bacteria.—Living pneumococci that had been treated with silicate, whether they were tested when in the Gram-negative state or upon restoration to Gram positivity on standing in weakly alkaline solution were found to be no longer bile-soluble. It seemed likely, therefore, that silicate causes death of pneumococci. The effect of silicate on viability of pneumococci and other Gram-positive and Gram-negative bacteria was tested as described under Methods. The results are listed in Table II. As shown in the right-hand column of Table II, silicate had a lethal effect on all eighteen strains of pneumococci studied. Of the various strains of Gram-positive bacteria tested, a lethal effect was observed only in those which were converted to Gram negativity in the presence of silicate and also required application of an alkaline solution to restore them to Gram positivity. Viability of Gram-positive bacteria that did not become Gram-negative in the presence of silicate was unaffected. Furthermore, organisms that became Gram-negative in presence of silicate but which were restored to positivity by simple washing in water, were not killed. No effect on viability

was observed among the single representatives of the four species of Gram-negative bacteria that were studied.

Investigations of the effects of time and temperature on the rate of killing of pneumococci are in a preliminary stage. It should be observed however, that the lethal effect is not immediate but occurs over the course of 45 minutes

TABLE II
Effect of Silicate on Gram Reaction and Viability of Various Bacteria

Bacterial species	Conversion to Gram negativity by silicate	Restoration to Gram positivity		Lethal effect of silicate*
		By washing in water	By suspension in alkaline solution	
Pneumococcus—18 strains (R and S)	Yes	No	Yes	+ to +++++
<i>Streptococcus viridans</i> (strain Mørch 1498/41)	No			—
“ “ (strain Mørch 2015)	Yes	Yes		—
“ “ (strain Kr. 13)	“	No	Yes	++
“ “ (strain NBS1)	“	“	“	+
“ “ (strain St. 16)	“	“	“	+
“ “ (strain Ad. 2)	“	“	“	++
“ “ (strain Dwyer R)	“	“	“	+++++
<i>Streptococcus hemolyticus</i> , Group A (C203S)	“	Yes		—
“ “ Group C (H47C)	“	“		—
“ “ Group D (Ro. 47)	“	“		—
<i>Streptococcus MG</i> (strain Horsfall No. 9)	No			—
<i>Streptococcus salivarius</i> (type 1, strain 78E)	“			—
<i>Streptococcus fecalis</i>	Yes	Yes		—
<i>Micrococcus leisodeikticus</i>	“	“		—
<i>Staphylococcus albus</i> (3895)	No			—
<i>Clostridium welchii</i>	Yes	Yes		—
<i>Escherichia coli</i>				—
<i>Shigella sonnei</i>				—
<i>Salmonella typhimurium</i>				—
<i>Hemophilus hemolyticus</i>				—

* + indicates decrease in the viable count by a factor of 10^1 ; +++++ indicates a decrease of 10^4 or greater. — indicates no observed effect upon viability.

to 2 hours at room temperature. Killing also occurs at 0°C . but is more rapid at higher temperatures. Among the 18 strains of pneumococci the viable count following exposure to silicate at room temperature decreased by a factor of 10^1 to 10^4 or more. A similar variation was observed among affected streptococci. The strains of streptococci in which a lethal effect was observed show heterogeneity in other respects. Under currently accepted criteria none of them fulfills the requirements for classification as pneumococcus.

Inhibition of Effects of Silicate upon Pneumococci.—When suspended in nutrient broth neither the staining properties nor the viability of pneumococci is affected by silicate when added in final concentration of 0.1 to 0.2 per cent. As noted previously, flocculation occurs rapidly when silicate is added to broth and brought to neutrality. Similarly, flocculation occurs when silicate is added to whole serum and the effects on pneumococci are nullified. On the other hand, addition of a solution of crystalline serum albumin in a final concentration of 0.4 per cent did not inhibit either the effect on Gram staining or killing of pneumococci by silicate.

Holt and Yates (4) have reported that silicic acid forms ethanol-soluble complexes with lecithin, choline, and cholesterol. It was of interest to determine, therefore, whether the effects of silicate on pneumococci are inhibited by these substances.

To 20 ml. 0.2 per cent Na_2SiF_6 at pH 7.0 was added 100 mg. choline chloride. After standing at room temperature for 15 minutes 5 ml. of the mixture was withdrawn and used to resuspend a flash-killed suspension of pneumococcus. The cell suspension was allowed to stand at room temperature for 30 minutes and a smear was then stained by Gram's method. The pneumococci were found to be Gram-negative, indicating that during this period of time an inactive complex of choline and silicate had not formed. However, upon standing for 4 hours at room temperature flocculation had occurred in the tube containing silicate and choline. This mixture when tested as above did not cause pneumococci to stain Gram negatively, nor did it cause death of the bacteria upon contact for 2 hours at room temperature. A solution of 0.2 per cent Na_2SiF_6 without added choline, under similar conditions of storage and use retained its activity.

Purified preparations of lecithin¹ behaved similarly to choline in inhibiting the effects of silicate on both the staining properties and viability of pneumococcus. A solution containing 20 mg. of lecithin in absolute alcohol was evaporated to dryness under reduced pressure and to it was added 2.5 ml. 0.1 per cent Na_2SiF_6 at pH 7.2. The mixture yielded a fine suspension on shaking and was placed in the icebox for 18 hours before application to pneumococci. Control solutions consisted of an aqueous suspension of lecithin and of 0.1 per cent Na_2SiF_6 at pH 7.2 similarly prepared and stored. Silicate alone converted all of the pneumococci to Gram negativity and caused a reduction in viable count by a factor of 10^5 after a period of contact of 2 hours at room temperature. The pneumococcal suspension to which the mixture of lecithin and silicate had been added showed a reduction in viable count by a factor of only 10^1 and most cells remained Gram-positive. Lecithin by itself had no demonstrable effect on either staining properties or viability.

¹ We are indebted to Dr. J. Murray Steele for preparations of purified lecithin and cholesterol and to Dr. Mary Pangborn for a sample of purified lecithin.

Repeated tests with mixtures of purified cholesterol and silicate did not reveal an inhibitory action of cholesterol on the effects of silicate on pneumococci.

Because of the inhibitory effect of lecithin and its choline moiety on the action of silicate, it was of interest to determine whether other substituted ammonium compounds have a similar inhibitory effect. This was found to be the case since addition of trimethylamine hydrochloride to a silicate solution nullified immediately its actions on pneumococci. Moreover, neutralization of a solution of Na_2SiF_6 by ammonium hydroxide instead of NaOH likewise results in immediate inactivation of the silicate solution. In this case no flocculation occurs because the ammonium ion prevents polymerization.

It would appear probable that the inhibitory action of whole serum is due in good part to its content of lecithin, although we have not carried out experiments that demonstrate this. The inhibitory action of nutrient broth can be explained in part by its known content of choline and possibly other compounds which react similarly with silicates.

Engel and Holzapfel (5) have reported that glycine is strongly adsorbed to quartz surfaces. Experiments in which glycine in final concentration of 0.4 per cent was added to 0.2 per cent sodium silicofluoride solution at pH 7.0 and allowed to interact overnight in the icebox showed no inhibition of the effect of silicate on Gram staining of pneumococci.

Engel and Holzapfel (5) have also reported that among the sugars tested by them more galactose than either glucose or lactose is bound to quartz surfaces and that adsorbed galactose is more difficult to remove by extraction with alkali than are the other two sugars. Accordingly, a mixture containing 0.4 per cent galactose and 0.2 per cent sodium silicofluoride pH 7.2 was allowed to stand overnight in the icebox before application to a suspension of flash-killed pneumococci. Under these conditions galactose did not inhibit the effect of silicate on Gram staining.

DISCUSSION

Two effects of silicate upon certain Gram-positive bacteria are described in the present paper: conversion to a state of Gram negativity and a lethal effect. The observations show that pneumococci and some streptococci that exhibit one or more characteristics similar to those of pneumococci physiologically behave in a more or less specific fashion in that silicate is firmly bound to them and they are killed by it. In other Gram-positive species silicate is not firmly bound, since they are promptly restored to Gram positivity by washing in water. Although temporarily converted to Gram negativity these bacteria are not killed. In a third class of Gram-positive bacteria neither the staining properties nor viability is affected by silicate. No effect was observed on the limited number of Gram-negative bacterial species that were studied.

In all cases in which an effect of silicate is observed it seems likely that silicate acts at the cell surface. The effect upon the Gram reaction does not, however, permit firm conclusions to be drawn as to the properties of cells that cause them to stain positively or negatively. If the Gram-positive reaction is due to properties of the intact cell wall which enable cells to retain the dye-iodine complex formed within them, it seems possible that silicate may alter permeability so that egress of the dye-iodine complex formed within the cell is readily permitted. Alternatively, crystal violet or iodine or possibly both substances may be prevented from entering the cells so that the complex formed remains on the surface and is therefore promptly removed by the mixture of acetone and alcohol used to decolorize. Although neither of these possibilities has been tested it seems probable that quantitative measurement of crystal violet uptake of normal and silicate-treated pneumococci as described by Kennedy and Barbaro (6) might indicate whether conversion to Gram negativity by silicate is due to a reduced permeability of the cell wall to crystal violet or iodine rather than to increased permeability to the dye-iodine complex formed within the cells. In the light of the experiments of Libenson and McIlroy (7) we are more inclined to the first of these possibilities, namely, that silicate prevents the crystal violet or iodine or perhaps both from traversing the cell wall and reaching the interior of the cell.

Other hypotheses can be advanced to explain the effect of silicate on the Gram staining of bacteria but in view of our lack of a precise explanation of the mechanism of the Gram reaction or the action of silicate, this does not seem profitable at the present time. It seems worth recording, nonetheless, that we have been unable to confirm the observations of Henry and Stacey (8) who reported conversion of Gram-positive bacteria to negativity by extraction with bile salts and subsequent restoration to Gram positivity by "replating" with magnesium ribonucleate. In our experience extraction with bile salts did not cause pneumococcus, staphylococcus, or *Cl. welchii* to become Gram-negative. Similarly, our efforts to convert Gram-positive bacteria to negativity by digestion with large amounts of crystalline pancreatic ribonuclease, as reported by Bartholomew and Umbreit (9) have been unsuccessful in repeated trials with several Gram-positive species. Our failure to reproduce the findings of these two groups of investigators has led us to the opinion that magnesium ribonucleate as such plays a minor part in the Gram reaction. It seems apparent that studies on the nature of the Gram reaction must take account of the effect of silicate which may contaminate the reagents used. This occurred in our experiments where silicate was present in the phosphate buffer solution used to suspend the bacteria that were exposed to digestion by ribonuclease.

The relatively specific effects of silicate on staining and viability of pneumococci and certain "*viridans*" streptococci are of importance from several as-

pects. In the first place, they add criteria that may be useful in the definition of pneumococcus as a species and the relationship of other species to it. Of possibly greater importance an understanding of the effects of silicate on these and other bacterial species may provide assistance in elucidating the nature of the toxic action of silicate upon the cells of higher animals.

As a working hypothesis we have considered that silicate may combine with lecithin or a related compound in the cell wall of pneumococci and some streptococci. This results in an alteration in permeability which leads on the one hand to a change from Gram positivity to negativity and on the other to death of the cells.

SUMMARY

Application of silicate solutions to living or heat-killed pneumococci and to certain "*viridans*" streptococci causes their conversion from a Gram-positive to a Gram-negative state. The original staining properties can be restored by suspending the silicate-treated bacteria in alkaline solutions of various salts but not by simple washing in water.

Living pneumococci and the strains of streptococci whose staining properties are similarly affected are killed when suspended in silicate solutions.

In other Gram-positive species silicate causes conversion to Gram negativity but restoration to positivity occurs upon washing in water. In a third group of Gram-positive organisms silicate has no effect on the Gram reaction. The viability of organisms in these two groups is unaffected by silicate under the conditions employed. No effect on staining or viability of Gram-negative bacteria has been observed.

The effects of silicate on staining and viability are inhibited by nutrient broth or whole serum but not by purified serum albumin. Lecithin, choline, and other substituted ammonium compounds also inhibit the effects of silicate on pneumococci.

We are indebted to Dr. Susan Hadley for determinations of silicon content of NaOH solutions and to Mrs. Patricia Charache for carrying out some of the experiments with cholesterol and silicate.

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