Preliminary Observations on the Effect of Sodium Alginate on Selected Nonsporing Organisms[1,2]

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The sodium salt of alginic acid, commonly called "algin" or "alginate," has been used as a stabilizer in foods for over a quarter of a century. In experiments conducted in this laboratory in which sodium alginate was a component part of the suspending medium for selected nonsporing organisms, under varying conditions, it appeared that there may be a difference in response to the presence of alginate among genera. This observation was incidental to the original purpose and design of most of the experiments and is reported here briefly both because the results appear to offer interesting possibilities for further studies and because there may be possible practical applications of the findings.

In 1956 a series of experiments was underway which sought to investigate the relationship between viscosity of the medium in which the cells were heated and the heat resistance of *Salmonella typhimurium* strain no. 84 (Woodburn, 1956). Sodium alginate[5] was selected as the agent by which to achieve variation in viscosity of physiological saline solution. This product formed a homogeneous suspension neither coagulated by heating nor gelled by cooling. No observable scum formed during its use as described. Preliminary experiments were conducted to determine the effect of the sodium alginate on the test organism. In so doing, a known number of cells of *S. typhimurium* 84 were added to 1 ml of sterile sodium alginate suspension, adjusted to pH 7. A suitable dilution was plated at once with tryptone glucose extract (TGE) agar. Following an incubation period of 1 hr at room temperature, a second plating was made, similarly. After 24 hr incubation of the plates at 37 C, colony counts were made in both cases.

In the original inoculum, the average number of salmonella cells per ml of a 4 per cent sodium alginate suspension for 7 trials was 1600. After 1 hr incubation, for a similar number of trials, the average value was found to be 2000 cells per ml. Thus under conditions of this study, the results indicated that at a concentration of 4 per cent the alginate in suspension had no significant adverse effect on the inoculated cells and furthermore only a slight effect was noted in similar experiments using a 5 per cent concentration. The medium did not markedly encourage multiplication of the salmonella cells.

In the experiments dealing with viscosity, the concentrations of sodium alginate in physiological saline solution used for the suspending media were: very thin, 1 per cent; thin, 2 per cent; medium, 4 per cent; and medium thick, 5 per cent by weight. The weighed alginate was added to 225 g of the physiological saline solution with rapid agitation until a uniform suspension was formed. (After the pH had been adjusted as described below, sterile saline was added to give a total of 250 g.) The suspensions were made up the day prior to the test series, covered with foil, and refrigerated.

On the day of the thermal resistance determinations, the sodium alginate suspensions were sterilized by autoclaving for 15 min at 15 lb pressure. After cooling to 24 C in a water bath, the pH of the suspension was determined using a potentiometric pH meter with glass electrode.[6] The pH was adjusted to 7 ($\pm$0.05) with the addition of 0.01 N NaOH or 0.01 N HCl.

The viscosity of the sodium alginate suspension at 24 C was checked with a falling ball type viscosimeter.[7]

Table 1 presents an example of the type of results obtained in these studies, using sodium alginate and with *S. typhimurium* 84 as the test organism.

Sodium alginate was next employed in our laboratory as a representative colloid in experiments designed to test the effect on bacterial cells of single substances added to a simplified medium in combination with freezing, frozen storage, and thawing (Woodburn, 1959; Woodburn and Strong, 1960). The organisms at test were *S. typhimurium* 84, *Staphylococcus aureus* strain no. 196 and *Streptococcus faecalis* strain no. R26. Among the data obtained were those presented in table 2.

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[5] Kelgin XL. Kelco Company, Chicago, Illinois.

[6] Coleman model 18A pH meter, Coleman Instruments Inc., Maywood, Illinois.

[7] Hoeppler precision viscosimeter, Fish Schurman Corporation, New Rochelle, New York.

These data were of such nature as to suggest that *S. aureus* 196 was more highly sensitive to the presence of 4 per cent sodium alginate suspended in 0.0003 M phosphate buffer than were the other two organisms. In three trials, viable cells of *S. aureus* 196 were recovered after 24 hr of freezer storage in only one instance and then at a very low level. Both *S. typhimurium* and *S. faecalis* persisted in greater numbers and for longer periods of time at all freezing temperatures.

The observations made in the experiments dealing with freezing just outlined suggested the possibility that the addition of sodium alginate to food might serve as a means of controlling or elimination of food poisoning staphylococci. Experiments were devised which sought to test the effect of sodium alginate on *S. aureus* 196 and *S. typhimurium* 84, when present in amounts which perhaps might reasonably be expected to be permitted in foods. The procedure included adding 10 ml of standardized cell suspension to 90-ml quantities of each of the sodium alginate dispersions, thus producing dispersions of approximately 10,000 cells per ml. In this series, the range of concentration of sodium alginate in the dispersions tested included 0.125 g, 0.250 g, 0.500 g, and 2.5 g suspended in 100 ml of 0.0003 M potassium phosphate buffer. Each of the inoculated dispersions was incubated at a constant temperature of 37 C. Platings were made with TGE agar at 0 hr and after intervals of 2, 4, 6, 8, and 24 hr. Just prior to plating, the cell suspensions were placed on a wrist-action shaker for 15 min. Subsequent to dilution, the bottles were shaken for 2 min by hand in an attempt to disperse the cells. Duplicate plates were made at all times, and incubated at 37 C for 24 hr, at which time colony counts were made and recorded.

The findings for these experiments are presented in figures 1 and 2.

Since the results of these latter studies were somewhat at variance with the implication of the work on frozen suspending media, a series of tests using the lesser amounts of sodium alginate and storage at a freezing temperature was undertaken.

The same method of addition of the cell suspension to the sodium alginate dispersions in phosphate buffer was followed as just described. However, only two concentrations of sodium alginate were included, namely, 0.25 g per 100 ml and 2.5 g per 100 ml. Dilutions

TABLE 1

*Survival of Salmonella typhimurium 84 following heat treatment in suspending media of varying viscosities as expressed in concentration of sodium alginate**

(Original inoculum approximately 10,000 cells per ml)

Temperature of Heating	Concentration of Sodium Alginate	Avg Cell Count per ml of Suspension (Heating Period in min:)				
		2	3	5	10	20
C	%					
50	1			3,800	50	<10
	2			3,000	230	<10
	4			4,700	1,000	25
60	2	750	<10	<10	<10	<10
	4	1,400	10	<10	<10	<10
	5	2,700	<10	<10	<10	<10

* Each figure represents a minimum of 6, and usually 12 thermal death time tubes.

TABLE 2

Survival of three organisms in physiological saline, 0.0003 M phosphate buffer or 4 per cent sodium alginate when held at three freezing temperatures

(Each figure is an average of 12 platings representing 6 trials)

Suspending Medium	Temperature of Frozen Storage	Time				
		0	24 hr	1 week	4 weeks	10 weeks
	C	After Thawing Viable cells of *Staphylococcus aureus* 196, per ml				
Saline (0.85%)	−11	13,000	25	1	1	0
	−21	13,000	28	11	0	0
	−30	13,000	11	8	4	4
Phosphate buffer (0.0003 M)	−11	13,000	170	26	4	0
	−21	13,000	400	120	48	19
	−30	13,000	620	560	240	120
Sodium alginate (4%) in PO_4 buffer	−11	19,000*	0*	0*	0*	0*
	−21	19,000*	0*	0*	0*	0*
	−30	19,000*	1*	1*	0*	0*
		After Thawing Viable cells of *Salmonella typhimurium* 84, per ml				
Saline (0.85%)	−11	14,000	44	4	0	0
	−21	14,000	52	3	0	0
	−30	14,000	24	1	0	0
Phosphate buffer (0.0003 M)	−11	18,000	192	28	1	1
	−21	18,000	540	95	41	10
	−30	18,000	7,000	310	130	47
Sodium alginate (4%) in PO_4 buffer	−11	18,000	250	0	0	0
	−21	18,000	6,700	6,600	900	1
	−30	18,000	9,100	11,000	10,000	7,400
		After Thawing Viable cells *Streptococcus faecalis* R-26, per ml				
Saline (0.85%)	−11	14,000	2,900	1,100	130	2
	−21	14,000	510	400	28	8
	−30	14,000	97†	20	11	3
Phosphate buffer (0.0003 M)	−11	11,000	1,000	140	24	3
	−21	11,000	930	320	83	30
	−30	11,000	1,800	310	180	94
Sodium alginate (4%) in PO_4 buffer	−11	18,000	310	3	1	0
	−21	18,000	2,700	200	1	0
	−30	18,000	7,600	2,400	1,300	1,100

* Average of three trials.

† Average of five trials.

of each dispersion were individually plated with TGE agar to obtain a 0 hr cell count. Following this procedure, 1-ml quantities of each dispersion were placed in individual sterile thermal-death-time tubes which were then cotton-plugged and sealed with Parafilm. The tubes containing the cell suspension were placed in an ice bath. After all the tubes were filled, they were labeled and placed in frozen storage at −21 C.

At the end of 24 hr and again after 1 week of storage, tubes representing the buffer control, and the respective concentrations of the sodium alginate dispersions were removed from frozen storage. These tubes were immediately placed in a constant temperature water bath held at 37 C to thaw the contents of the tubes. After 3 min of holding time in the water bath, the tubes were removed and held in an ice bath until the contents were plated. Portions representing 0.1 ml of the tube contents were individually plated with TGE agar (*S. aureus*) or trypticase soy (TS) agar (*S. typhimurium*). The plates were incubated at 37 C for 24 hr, at which time colony counts were made and recorded.

The findings for those experiments in which lesser concentrations of sodium alginate than were employed in the first studies on freezing are shown in table 3.

With the techniques applied in these latter experiments the 24-hr plate counts were substantially higher than the 0-hr counts in some instances. This phenomenon was not observed when larger amounts of sodium alginate were employed. It may possibly be explained by the breaking up of cell clumps by ice crystals which may have been more numerous or larger when lesser amounts of the colloidal alginate was present. It should be noted, however, that relatively fewer cells of *S. aureus*, than *S. typhimurium* remained viable in the presence of sodium alginate. When the level of sodium alginate reached 2.5 g per 100 ml of buffer, there was a definite trend toward lowering the percentage of surviving cells of *S. aureus* 196. Thus the combination of low temperature and sodium alginate in the bacterial environment seems to offer some stress for *S. aureus* not encountered by *S. typhimurium*.

One further series of observations was made directed

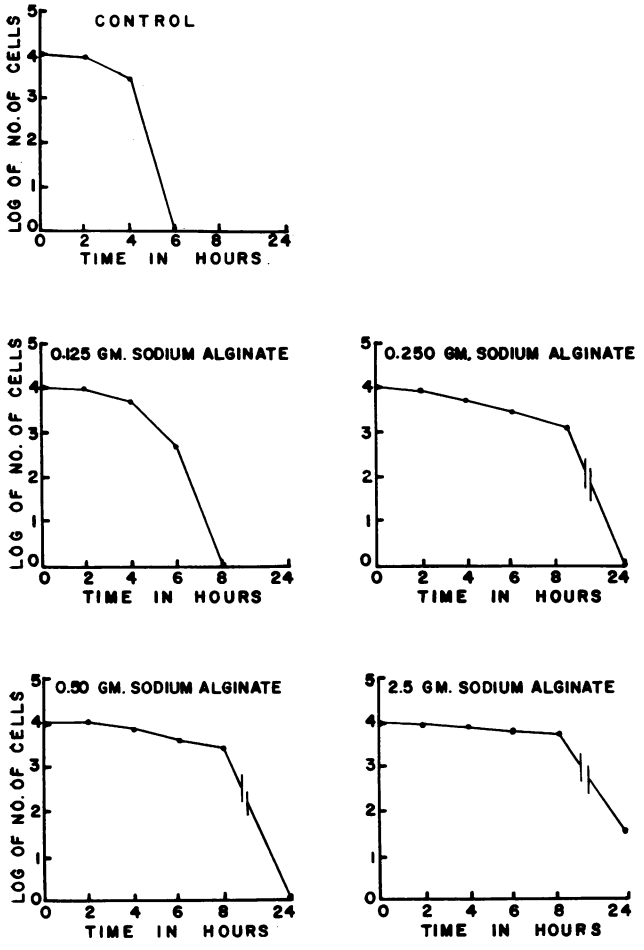

Figure 1. Effect of sodium alginate dispersed in phosphate buffer upon *Staphylococcus aureus* 196. (Each curve represents an average of five trial series; quantity of sodium alginate expressed as amount suspended in 100 ml of buffer.)

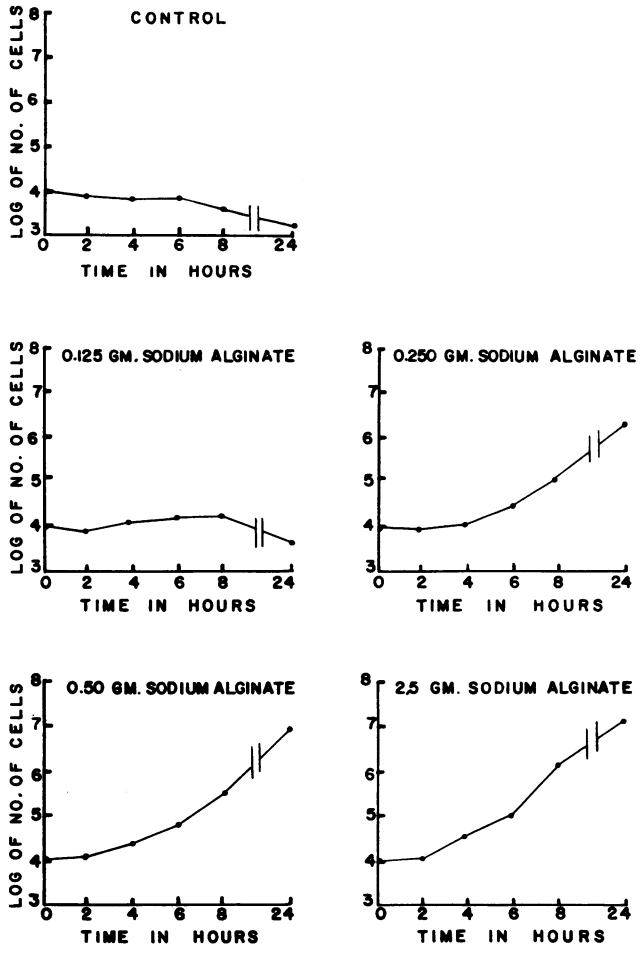

Figure 2. Effect of sodium alginate dispersed in phosphate buffer upon *Salmonella typhimurium* 184. (Each curve represents an average of four or five trial series; quantity of sodium alginate expressed as amount suspended on 100 ml of buffer.)

toward a possible practical application of the use of sodium alginate for reducing numbers of staphylococci in food. Milk was chosen as an example of a food system first because it was easy to manipulate in the laboratory and second because sodium alginate has been used as a stabilizing agent in some cases in the past in the preparation of chocolate milk and some other dairy products. For this fifth portion of the investigation, one quart of raw milk was obtained from the University of Wisconsin dairy on each morning on which a test series was to be prepared. To determine the number of viable organisms present in the raw milk, dilutions of the raw milk were plated with TGE agar and TS agar. Dilutions of the raw milk were also plated upon Staphylococcus medium no. 110 (SM-110)[8] (Difco) for the purpose of estimating the number of staphylococcus present, and upon Salmonella-Shigella (SS) agar (Difco) to detect possible salmonella contamination.

The first step of the pasteurization process was the aseptic measurement of the milk into sterile Erlenmeyer flasks which were then cotton-plugged. A sterile thermometer was placed into each of the flasks of milk, and they were lowered into a constant temperature water bath set at 63 C. When the milk reached the temperature of 63 C, a water dispersion of sodium alginate was quickly swirled into the appropriate quantity of milk.

In adding the sodium alginate to the milk, a larger quantity of water (20 ml) was required for the 2.5-g sample as compared to the 0.25-g sample (10 ml). Early tests were made in which the sodium alginate in dry form was added to the heated milk. A granular and less desirable dispersion was formed by this method. The process of dispersing the sodium alginate in water before addition to the heated milk was established and continued throughout the tests. After the addition of the sodium alginate dispersion to the milk, the heating was continued using the holding method of pasteurization. At the end of the heating period, the flasks were plunged into ice water and cooled to 10 C. A total volume of 100 ml finally resulted in each case when the standardized cell suspension was added to the milk and the sodium alginate dispersion subsequent to the completion of pasteurization (10 ml volume). Measured samples appropriately diluted were plated immediately to establish the number of organisms present. Tryptone glucose yeast agar and trypticase soy agar were used for enumeration of the total number of viable cells in the milk-rich media. SM-110 was used to detect the possible staphylococci in the milk, and SS agar was the plating medium used for testing for salmonella organisms in the milk. After incubation of 37 C and in preparation for plating, the milk-containing suspension was shaken by a wrist action shaker for 15 min to disperse the cell clumps. Dilutions of portions of the suspension were then prepared and the dilution bottles were shaken for 2 min by hand. Platings were made at 24, 48, and 72 hr. Plates were incubated at 37 C for 24 hr before colony counts were made.

Table 4 presents certain of the data obtained from this last series.

S. typhimurium 84 and *S. aureus* 196 reacted differently when dispersed in a milk-rich medium containing sodium alginate. Staphylococcus organisms lived for 72 hr in the milk-rich control sample. However, at all periods tested (24, 48, and 72 hr), staphylococcus organisms were greater in number in the milk-rich controls than in milk-rich medium containing 0.25 g or 2.5 g sodium alginate.

In contrast to staphylococcus, an initial increase in *S. typhimurium* was noted after 24 hr of incubation in a milk-rich medium containing 0.25 g sodium alginate. The control had 23,350,000 cells per ml, whereas the comparable sample containing sodium alginate showed 68,400,000 cells per ml. After this initial increase, both the control and sodium alginate sample exhibited a decrease in population. The increase in viable organisms in the milk-rich medium containing 2.5 g sodium alginate did not appear until after 48 hr of incubation. At this time, viable organisms in the milk-rich sodium alginate medium numbered approximately 46,300,000 cells per ml as compared to 41,700,000 cells per ml in the control. Because of such a slight variation and allowing for experimental error, such results cannot be considered conclusive.

It is obvious that the results obtained in this series of experiments dealing with the effect of sodium alginate on one strain of staphylococcus and one strain of salmonella are not clear-cut nor definitive. Yet they are suggestive and permit tantalizing speculation. Over-all for the strain of salmonella tested it appeared that cells maintained themselves in the presence of 4

[8] Difco Laboratories, Inc., Detroit, Michigan.

TABLE 3

*Survival of Staphylococcus aureus 196 and Salmonella typhimurium 84 held in frozen storage (−21 C) in media containing varying concentrations of sodium alginate**

Concentration of Sodium Albinate (Grams Added to 00 ml Buffer)	Cells Suspended in Sodium Alginate		
	0 hr	24 hr	168 hr
	cells/ml	*cells/ml*	*cells/ml*
S. aureus 196:			
0 (*control*)	10,500	22,500	16,500
0.25	10,500	14,500	10,500
2.5	12,000	8,500	4,400
S. typhimurium 84:			
0 (*control*)	9,100	14,000	9,300
0.25	8,800	44,500	32,000
2.5	7,800	43,000	26,000

* Each figure represents an average of five trial series.

per cent sodium alginate, that this substance offered some degree of protection when the cells were submitted to moderate heat, and the picture with regard to freezing was not consistent. The viability of *S. typhimurium* 84 was prolonged at both −21 and −30 C in the presence of 4 per cent sodium alginate. This was not true when the cells were held at −11 C. With lesser amounts of sodium alginate and at a temperature of −21 C, the cells recovered after 7 days of frozen storage exceeded in number the original inoculum. It may at least be concluded that large number of cells were not killed by the latter treatment.

For the *S. aureus* 196, the effect of 4 per cent sodium alginate at a series of freezing temperatures seemed to be that of creating a most unfavorable environment for the organism and brought about rapid death. With lesser amounts of the sodium alginate and at −21 C, the staphylococci decreased in numbers, but viable cells remained after 7 days of storage. When the strain of staphylococci at test was incubated in phosphate buffer containing sodium alginate, more cells survived for a longer period of time in the presence of the salt than in the buffer alone. Furthermore, the length of the survival period seemed to be proportional to the amount of sodium alginate present. For the staphylococci, however, there was no evidence of growth as was shown under similar conditions by the salmonella. This may indicate a difference in metabolic processes of the two organisms for sodium alginate or it may be a reflection only of the greater stress placed on the staphylococci by the use of phosphate buffer as a suspending medium. Steiner and McNeely (1954) reviewed studies which indicated a difference among species in the production of alginolytic enzyme activity. It is hoped that the experiment can be repeated using peptone water or some other fluid more favorable to the staphylococci as a carrier for the sodium alginate. The role played by the phosphate may possibly prove to be more important than the alginate in explaining the observations reported here.

Chemically, sodium alginate is a salt of anhydro-D-mannuronic acid of high molecular weight (Steiner and McNeely, 1954). It is used commercially as a stabilizing agent and to give increased viscosity to such items as ice creams and pie fillings but in lesser concentrations than many of those chosen for this study (Gibson and Rothe, 1955). Work reviewed by Steiner and McNeely (1954) indicated that sodium alginate is utilized metabolically by only a small number of species of microorganisms and by none of the gram positive or pathogenic strains tested. Sodium alginate, of course, enters into colloidal dispersion in water and the effects observed on the microorganisms may center around this property. Hodge and Metcalfe (1958) reported sodium alginate at a level of 0.15 per cent to be an effective agent in flocculating bacteria from suspension. They found, further, that this property was lost upon standard autoclaving. Media used in the experiments in this laboratory were autoclaved so, presumably, flocculation by the alginate did not enter into the reaction observed here. A limited number of tests were made in this laboratory using the Hodge and Metcalfe technique for sterilization of sodium alginate without destroying the ability to flocculate microorganisms. In these tests it was not found possible to attain sterility. For this reason the autoclaving of the sodium alginate was continued. It may have

TABLE 4

*Survival of Staphylococcus aureus 196 and Salmonella typhimurium 84 in a milk medium containing varying concentrations of sodium alginate**

(Plated on SM-110 and SS agar, respectively; incubated at 37 C)

Concentration of Sodium Alginate (Grams Added to 100 ml of Medium)	Concentration of Milk (Percentage of Milk in Medium)	Incubation Time for Cells Suspended in Milk Medium			
		0 hr	24 hr	48 hr	72 hr
		cells/ml	*cells/ml*	*cells/ml*	*cells/ml*
S. aureus 196:					
0 control	90	9,200	180,000,000	184,100,000	7,800,000
0.25	80	9,400	90,300,000	84,100,000	650,000
0 control	90	8,900	169,900,000	187,600,000	20,800,000
2.5	70	7,800	89,900,000	59,100,000	11,000,000
S. typhimurium 84:					
0 control	90	5,100	23,400,000	52,700,000	360,000
0.25	80	4,700	68,400,000	4,800,000	43,000
0 control	90	2,800	68,700,000	41,700,000	300,000
2.5	70	2,800	42,300,000	46,300,000	170,000

* Each figure represents an average of 10 plates, 2 from each of five trial series.

been that the sodium alginate in our laboratory had a higher original bacterial load.

Since sodium alginate is a colloid, it might be of interest to note the effect of various colloidal substances upon the growth of microorganisms. An early investigator, Beckhold (1919), reported a moderate inhibition of growth of *S. aureus* by colloidal silver, but no destruction of cells was apparent in this medium. He noted that the size of the particles in the hydrosol was of great importance. Dispersions of finely granular particles were more active in producing microorganism inhibition than the solution of coarser particles. Beckhold also reported that colloidal mercury in a dilution of 1:132,000 inhibits the development of staphylococcus. Later, Woiwod (1954) reported that colloidal copper sulfide was inhibitory to staphylococcus and many other gram-positive organisms but had little effect on gram-negative organisms. The authors inferred, however, that both the colloidal properties and the metallic properties of these substances mentioned above must be considered as to their effect upon microbial growth.

Kaminato (1955) studied the effect of antibacterial substances extracted from seaweeds on the growth of several pathogenic organisms. Extractions were taken from 12 species of seaweeds. Of these 12 species, *Undaria pinnatifida* and *Sargassum* seemed most closely related to the purified sodium alginate, according to descriptions presented by Chapman (1952). Extracts from each of the two species mentioned permitted poor to moderate growth of staphylococcus and moderate to good growth of salmonella. The results obtained indicate a possible detrimental effect of these types of seaweed on growth of staphylococci.

The observations of Allen and Dawson (1960) concerning the selective inhibition of bacteria representing various genera by algae and extracts of algae, is most pertinent to the experiments here reported. These investigators stated that among the marine algae there is a wide distribution of materials which inhibit the growth of gram-positive bacteria, whereas the gram-negative bacteria are unaffected by the algal extracts.

SUMMARY

A consideration of data from a number of types of experiments suggests that one strain of *Staphylococcus aureus* may be inhibited by the presence of sodium alginate in the suspending medium. One strain of salmonella did not show a similar degree of inhibition under like conditions. The possibility that sodium alginate if incorporated into food, especially frozen food, might serve to assist in the control of food poisoning staphylococci is tentatively suggested. Obviously, to recommend such a procedure, more evidence than offered in this paper must be accumulated. Also, the legal aspects of the problem must be considered as well as the quality of any final product so prepared.

REFERENCES

ALLEN, M. B. AND DAWSON, E. Y. 1960 Production of antibacterial substances by benthic tropical marine algae. J. Bacteriol., **79**, 459–460.

BECKHOLD, H. 1919 *Colloids in biology and medicine*, pp. 367–369. D. Van Nostrand Co., New York, New York.

CHAPMAN, V. J. 1952 *Seaweeds and their uses*. Pitman Publishing Corp., New York, New York.

GIBSON, K. F. AND ROTHE, L. B. 1955 Algin-versatile food improver. Food Eng., **27**, 87–89.

HODGE, H. M. AND METCALFE, N. S. 1958 Flocculation of bacteria by hydrophilic colloids. J. Bacteriol., **75**, 258–264.

KAMINATO, K. 1955 Studies on the antibacterial substances extracted from seaweeds; on the effect of the extracts from seaweeds against the growth of some pathogenic organisms. Japan. J. Bacteriol., **10**, 897–902.

STEINER, A. B. AND MCNEELY, W. H. 1954 *Natural plant hydrocolloids; algin in review*, pp. 68–79. American Chemical Society, Washington, D. C.

WOIWOD, A. J. 1954 The inhibition of bacterial growth by colloidal heavy-metal sulphides and by colloidal sulphur. J. Gen. Microbiol., **10**, 509–520.

WOODBURN, M. 1956 The influence of the viscosity of the suspending medium upon the thermal resistance of *Salmonella typhimurium*. M.S. thesis, University of Wisconsin.

WOODBURN, M. J. 1959 Studies on the survival of bacteria in frozen foods. I. Home frozen; II. Simplified substrates. Ph.D. thesis, University of Wisconsin.

WOODBURN, M. J. AND STRONG, J. H. 1960 Survival of *Salmonella typhimurium*, *Staphylococcus aureus*, and *Streptococcus faecalis* frozen in simplified food substrates. Appl. Microbiol., **8**, 109–113.