SURFACE TENSION AND BACTERIAL GROWTH[1]

WILLIAM M. GIBBS, H. W. BATCHELOR, AND T. N. SICKELS

From the Department of Bacteriology, University of Idaho, and Moscow, Idaho, Agricultural Experiment Station

Received for publication May 15, 1925

HISTORICAL RÉSUMÉ

We have found only a few reports which bear directly on surface tension as a factor in bacterial growth.

Larson, Cantwell, and Hartzell (1919), studied the effect of lowered surface tension on the growth and characteristics of certain organisms, and concluded that the growth of bacteria in ordinary broth was greatly influenced by the surface tension of the medium. The surface tension was lowered by additions of castor oil soap and measured by the drop weight method. The standard broth which was employed had a surface tension of 59 dynes per square centimeter. *B. subtilis* did not form a pellicle when the surface tension was reduced to 45 dynes, but grew down in the body of the medium. They concluded that all pellicle formers ceased to grow at the surface when the surface tension was below 45 dynes per centimeter. *B. subtilis* was found to form spores more slowly in a medium of lowered surface tension.

Some anaerobes, particularly *B. tetani*, were found to grow aerobically in a medium of lowered surface tension. The authors suggest that the favorable action of the oil seal in anaerobiosis is probably due to the lowered surface tension. They are inclined to believe that the toxicity of the depressant is a factor of more or less importance. In a later report Larson (1921) found the surface tension of ordinary broth to be about

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as scientific paper No. 38, College of Agriculture and Agricultural Experiment Station, Moscow, Idaho.

59 dynes, and of inorganic media with glycerol as a source of carbon, 73 dynes. The surface tension was depressed by additions of castor oil soap, giving a range from 32 to 73 dynes per square centimeter. The growth of pneumococcus and streptococcus was depressed at a surface tension of 50 dynes and lower. The limit of *B. anthracis* was sharply defined at 46 dynes. The intestinal bacteria grew well at lowered surface tension. The ability of this group to develop in media of low surface tension was thought to be related to the resistance of the group to bile, which has a low surface tension. The concentration of the colon-typhoid group of bacteria at the surface of the medium was attributed to the concentration of the surface tension depressants at the surface. Pellicle formers were found to grow throughout the body of the medium when the surface tension was reduced, while non-pellicle formers were found to develop pellicles after long cultivation in media of high surface tension. Symbiosis and antibiosis, were, in some cases, attributed to surface tension.

Ayers, Rupp, and Johnson (1923) studied the effect of surface tension on the streptococci. Castor oil soap, sodium glycocholate, iso-amyl alcohol, and sodium oleate were used as depressants. The basic medium employed had a surface tension of 59.6 dynes, and was depressed as low as 40 dynes. These authors conclude that some species of streptococci are retarded in growth when the surface tension of the medium is lowered to 53 dynes, and suppressed at 45 dynes, while others are retarded at 43 dynes. Still other species are depressed at 40 to 41 dynes. In general 40 dynes prevented growth. *Streptococcus pyogenes* was the most susceptible to lowered surface tension, and *Strept. lactis* least affected. Some toxicity of the depressants was noted.

Mellon, Hastings, and Anastasia (1924) studied the cohesive power responsible for the spontaneous agglutinability of certain bacteria. They found that this power could be diminished or entirely eliminated by varying the surface tension of the solution. Strains which immediately agglutinated, or failed to produce emulsions, were found to emulsify readily in a solution contain-

ing sodium oleate. This was attributed to the interfacial tension between organism and solution.

EXPERIMENTAL METHODS

Preparation of broth. The stock broth used in the experiments was prepared as follows: Parke Davis and Company, pepton 10 grams, beef extract 3 grams, distilled water 1000 grams. These ingredients were cooked together in the steamer one and one-half hours, the reaction adjusted to pH 7.0 and steamed for another half hour, then cooled and filtered under pressure through macerated filter paper. Broth prepared in this manner had a surface tension of 48 to 50 dynes per square centimeter.

Surface tension depressants. Soaps were prepared from castor oil, cocoanut oil, olive oil, and palmitic acid. The oils were saponified by adding an alcoholic solution of KOH in excess and heating under a reflux condenser for several hours, then allowing to stand overnight. The solution was neutralized to phenolphthalein by a solution of HCL in absolute alcohol and filtered hot through filter paper to remove chlorides. It was then concentrated to small volume and poured into an excess of saturated solution of sodium chloride in water. The precipitated glycerides were collected on filters and washed with saturated sodium chloride solution, then dried at 28°C. and finally dried at 60°C. They were then dissolved in absolute alcohol, filtered, again evaporated and dried, and again taken up in absolute alcohol and filtered. They were then dried at 28°C. and finally at 60°C. and stored for use.

Stock solutions of the four soaps were prepared by dissolving 2 grams in 100 cc. of distilled water. These 2 per cent solutions were then cooled to just above the freezing point and filtered under pressure through macerated filter paper. This cooling and filtering process was repeated until the solutions were perfectly clear and gave little or no precipitate when added to the stock broth. These 2 per cent solutions were used as bases and were added to broth or other media by pipette. A broth referred to as containing 5 per cent soap contains 5 cc. of this 2 per cent soap solution per 100 cc. of medium.

The four soaps mentioned were used throughout the work, in order that any phenomena observed, in reality due to the nature of the depressant, would not be ascribed to surface tension. This precaution was justified in nearly every experiment.

The surface tension depressing efficiency of these soaps varied considerably, not only in the actual depression possible, but in the proportion of soap necessary to produce it. With each soap there was a maximum concentration, beyond which further additions had little effect on the surface tension. With castor soap this was 1 per cent, with olive soap about 2 per cent, with palmitic soap 6 per cent, and with cocoanut soap as high as 10 per cent. From the basis of the amount of soap necessary to produce a given depression, cocoanut soap was the most efficient, 0.1 per cent giving a depression of 7 to 8 dynes and 1 per cent giving a depression of 18 dynes. Olive soap was only slightly less efficient. Palmitic soap was used in concentrations as high as 5 per cent without securing any more depression of the surface tension than with olive or castor soap at 1 per cent. Cocoanut produced the lowest surface tension of any of the soaps but was less satisfactory, due to the fact that it sometimes produced turbidity or precipitates. Castor and palmitic soaps gave perfectly clear solutions in all concentrations used and olive soap slightly opalescent solutions but without precipitates.

The possible toxicity of depressants is an important factor. These four soaps varied considerably in this respect to different organisms. Some organisms were very susceptible to a given soap, others apparently not at all.

Surface tension measurement. The surface tension was determined by the film method as outlined by Fahrenwald (1922). In one experiment, the drop weight method was also employed for the sake of comparison. The film method has the advantage that several determinations can be made in a relatively short time, not at the expense of accuracy. It is also possible by this method to distribute the surface tension depressants, which accumulate at the surface, throughout the body of the medium, thus enabling one to check on separate determinations. It will appear that the surface tension varies slightly with each measure-

ment of the same solution, and an equilibrium cannot be obtained. This may be avoided by gently stirring the depressants into the body of the medium, then quickly measuring the tension. A second measurement will then be found to check the first. Results obtained by the method of Fahrenwald are lower than those obtained by the drop weight method.

EXPERIMENTAL DATA

Escherichia coli and surface tension

Beef extract broth containing the various depressants was inoculated with a twenty-four-hour culture of *Escherichia coli* and incubated at 28°C. The flasks were so arranged that the surface tension could be checked at intervals to determine the effect of the metabolism of the organism on the surface tension of the medium. The pH of the broth was 7.0. Table 1 shows the arrangement of the cultures, the surface tension, and the hydrogen ion concentration. Each soap had a series not inoculated in order to trace changes due to factors other than bacterial development. The surface tension was determined by the method of Fahrenwald and by the drop weight method. The results are shown in table 1 and chart 1.

It is seen from the data in the table and chart that the depressants gave a range in surface tension, as determined by the film method from 41.2 to 51.2 dynes per cubic centimeter. The same broths as determined by the drop weight method gave a range of 54.2 to 63.2 dynes per cubic centimeter. The latter method in this case gives, in general, results about 12 dynes higher than the film method. It will be noted that the difference in the two methods remains somewhat constant throughout. In discussing the results the data obtained by the film method will be taken.

The surface tension in the uninoculated control broth increased 1.5 dynes per cubic centimeter during the ten-day period. The hydrogen ion concentration remained constant. The uninoculated broths which received depressants gave a general small increase in surface tension during the period, an average increase

TABLE 1

Escherichia coli and surface tension

TREATMENT 2 PER CENT SOLUTION PER 100 CC. BROTH	TIME OF INCUBATION	pH	SURFACE TENSION PER CENTIMETER			
			Inoculated.		Not inoculated	
			Film method	Drop weight method	Film method	Drop weight method
	days		*dynes*	*dynes*	*dynes*	*dynes*
None	0	7.0	51.2	63.2	51.2	63.2
	1	7.1	54.3	64.4	53.4	63.9
	2	7.8	52.5	61.9	51.4	61.4
	3	8.0	51.8	62.2	52.4	63.7
	5	8.3	51.9	65.6	52.3	63.5
	7	8.3	52.6	65.6	52.0	64.8
	10	8.3	54.8	70.0	52.7	64.3
Castor oil soap, 0.1 cc	0	7.0	44.9	58.3	44.9	58.3
	1	7.3	48.5	60.8	47.8	58.3
	2	7.7	49.4	59.3	46.6	56.6
	3	8.1	50.0	61.5	47.3	58.7
	5	8.2	51.4	64.1	47.1	59.7
	7	8.4	52.8	65.6	47.2	57.7
	10	8.4	53.4	69.1	47.5	63.5
Olive oil soap, 0.2 cc	0	7.0	46.8	59.6	46.8	59.6
	1	7.0	49.7	62.9	49.1	62.2
	2	7.6	50.3	60.7	49.7	61.4
	3	8.1	49.6	61.5	49.0	60.8
	5	8.2	50.4	64.1	48.7	62.5
	7	8.3	51.4	64.6	49.1	60.3
	10	8.3	51.0	68.2	48.5	63.5
Cocoanut oil soap, 1 cc	0	7.0	41.2	54.2	41.2	54.2
	1	7.2	45.1	56.5	44.3	55.2
	2	7.6	49.1	61.4	43.5	53.8
	3	8.1	49.6	60.8	44.4	54.5
	5	8.3	51.8	64.1	43.2	54.8
	7	8.4	51.6	64.0	44.9	53.6
	10	8.4	52.0	69.1	43.8	58.7
Palmitic soap, 3 cc	0	7.0	43.6	57.7	43.6	57.7
	1	7.1	47.1	60.1	48.3	62.2
	2	7.8	48.1	61.4	47.2	61.4
	3	7.9	47.5	59.4	46.1	57.5
	5	8.1	49.2	62.5	46.1	56.5
	7	8.2	50.1	63.2	46.4	59.0
	10	8.3	53.0	63.5	46.8	61.4

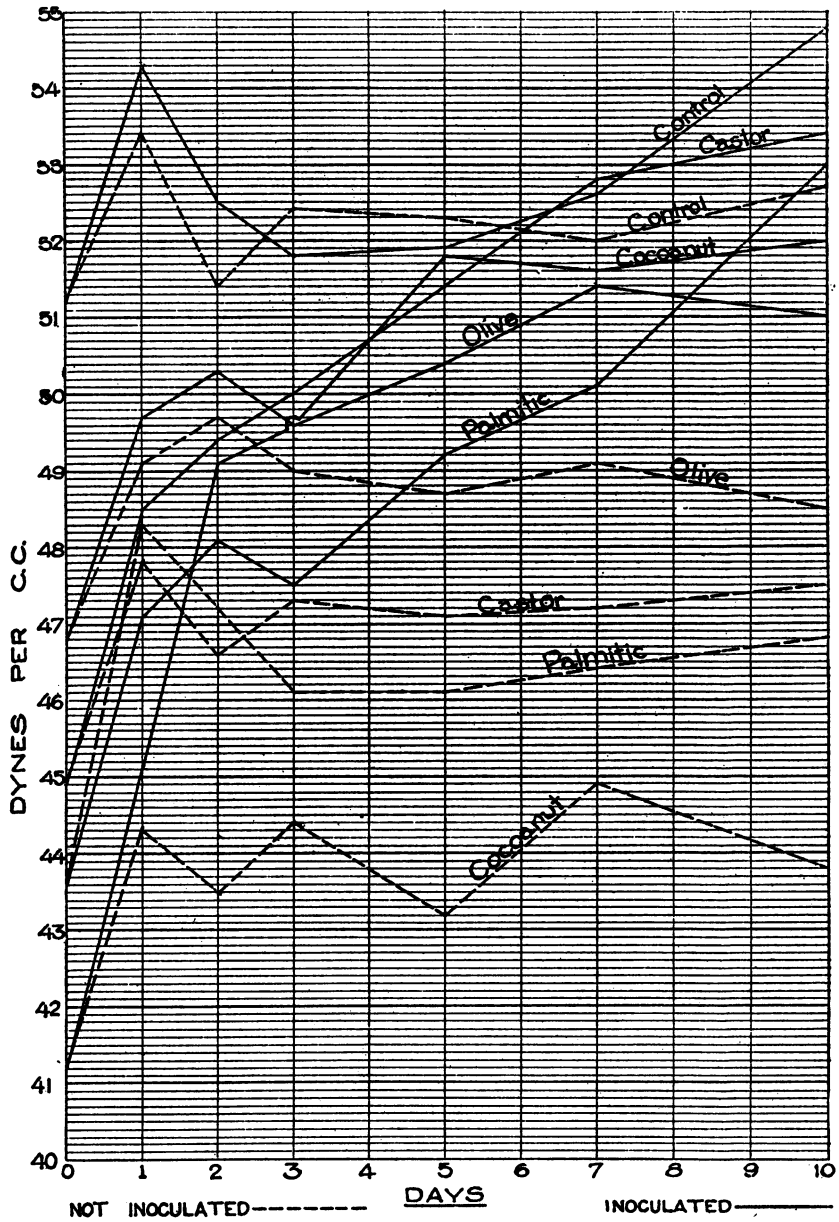

CHART 1. ESCHERICHIA COLI AND SURFACE TENSION

of 2 to 3 dynes, while the hydrogen ion concentration remained constant. The inoculated flasks, with the exception of the untreated control, gave an increase in surface tension varying from 4.2 to 10.8 dynes per cubic centimeter. The control inoculated flask increased but 3.6 dynes per cubic centimeter in the ten days. The maximum increase in surface tension occurred in the cocoanut treated series which also had the greatest depression at the start.

It will be noted that the increase in surface tension during the ten-day period is inversely proportional to the surface tension at the beginning of the experiment. In nearly all cases in the inoculated series the maximum increase in surface tension occurred within the first forty-eight hours. There was then in general a steady climb until the end of the experiment. The data show a tendency for the surface tension in the inoculated broths containing depressants to approximate that found in the uninoculated broth with no depressant, within seven to ten days. This shows that growth characteristics after several days' incubation cannot be attributed to a surface tension found at the onset of the experiment. This is brought out strikingly in chart 1.

Even in the absence of inoculation the surface tension of all broths containing depressants showed a decided increase within twenty-four hours. There was then some slight fluctuation throughout the period, but in no case did the surface tension drop to the level found at the onset, or increase to the point found in the control broth without depressant.

There was a steady increase in pH in all inoculated tubes until they reached 8.3 to 8.4 at the tenth day. Thus we find the surface tension and hydrogen ion concentration approaching a constant as the result of the metabolism of *Escherichia coli* regardless of the initial surface tension.

Growth of aerobes in broth of varying surface tension

The four soaps were added to stock broth in varying concentrations and hydrogen ion concentration and surface tension determined. Each experiment was so arranged that the pH of

all the tubes was approximately the same at the start. Tubes of each concentration were then inoculated with a twenty-four-hour culture of each of the following organisms: *B. subtilis, B. anthracis, Serratia marcesens, Pseudomonas aeruginosa, Eberthella typhi,* and *Staphylococcus aureus.* The tubes were then incubated at 28°C. and observations made after twenty-four and forty-eight hours and again at the end of one week.

The surface tension and soap additions are shown in table 2.

All the soaps except palmitic seemed to be somewhat toxic to both *B. subtilis* and *B. anthracis.* Palmitic proved to be stimulating to *B. subtilis* in forty-eight-hour incubation, and the organism produced a heavier pellicle with this soap at 33 dynes

TABLE 2

Surface tension of broth containing depressants

	CASTOR SOAP			OLIVE SOAP			PALMITIC SOAP			COCOANUT SOAP		
Cubic centimeter of 2 per cent solution per 100 cc. broth.....	0.1	0.5	1.0	1.0	3.0	5.0	1.0	3.0	5.0	1.0	3.0	5.0
Surface tension, dynes per square centimeter.	43	35	33	33	32	31	37	34	33	44	38	30

Surface tension of control broth, 49 dynes per square centimeter.

than in the control of 49 dynes. At the end of a week this difference was not noted, the growth being about the same as that in the control. *B. anthracis* failed to develop well in any of the tubes containing soap.

Results were somewhat striking with *Pseudomonas aeruginosa* and *Serratia marcesens,* particularly the former. Both organisms showed stimulated pigment formation, heavier pellicles, and less growth in the body of the media, in all tubes which contained soap. Since castor oil soap was not toxic to *Pseudomonas aeruginosa* it was later employed in concentrations of 1, 3, and 5 per cent. These higher concentrations of the soap did not produce any further depression of the surface tension, yet the stimulation of pigment formation and pellicle, and diminution of growth throughout the body of the medium was more marked than in the previous concentrations. The observed stimulation

was then due to the nature of the depressant rather than to the lowered surface tension. This shows the importance of using several depressants in order not to ascribe observed phenomena to the surface tension rather than the nature of the depressant.

Eberthella typhi was apparently unaffected by any of the soaps, growth in the treated tubes differing in no way from the control. The soaps were toxic to *Staphylococcus aureus* in the higher concentrations, and no differences could be ascribed to surface tension.

These results do not agree with those of Larson who states that all pellicle formers cease to grow at the surface when the surface tension is below 45 dynes. The above experiments were repeated many times and in every case, especially with the two pigment formers, the pellicle formation was stimulated in the presence of the soap. This stimulation is not ascribed to surface tension. It must be borne in mind that the lowest surface tension obtained in the above experiment, 31 dynes per square centimeter, would be about 42 to 43 dynes as determined by the drop weight method.

Growth in Uschinsky's medium. The same organisms used in the above experiment were used in a similar experiment employing Uschinsky's medium instead of the stock broth. The surface tension varied from 26 to 71.6 dynes per square centimeter. Control flasks showed that the surface tension of the medium remained approximately the same after sterilization as before.

Pseudomonas aeurginosa and *Serratia marcesens* developed very intense pigments and grew well. The latter organism produced very little pigment in the medium containing cocoanut soap, but the pigment was intense in the other tubes. The differences noted could in no way be ascribed to the lowered surface tension.

Growth of anaerobes

Soaps were added to the stock broth containing 1 per cent glucose. The surface tension is shown in table 3.

The surface tension of the control broth was 53 dynes. Tubes of each concentration of each soap were then inoculated with the following anaerobes: *C. histolyticum, C. welchii, C. sporogenes, C. bifermentans, B. bellonensis, C. oedematis-maligni, C. oedematiens,* and two strains of *C. botulinum.* Controls consisted of an aerobic control, an anaerobic control, and a toxicity control for each organism. The aerobic control consisted of a tube of the glucose broth inoculated with the organism in the usual manner. The anaerobic control consisted of a tube of the glucose broth boiled for ten minutes, plunged in cold water, inoculated, and then sealed with a layer of sterile paraffin oil. The toxicity control consisted of tubes of the glucose broth containing the various soaps, boiled, and after cooling and inoculating, sealed with paraffin oil.

TABLE 3

Surface tenstion of glucose broth containing depressants

	CASTOR SOAP			OLIVE SOAP			PALMITIC SOAP		
Cubic centimeter of 2 per cent solution per 100 cc. broth	0.1	0.4	0.8	0.1	0.4	0.8	1.0	3.0	5.0
Surface tension, dynes per cubic centimeter	52	43	39	51	42	38	48	44	40

In no case did any of the anaerobes show any development in the tubes not sealed with paraffin oil. All showed excellent growth in the tubes treated with the paraffin seal. The toxicity controls in general gave good growth, some showing less growth than the controls. *C. botulinum* was completely inhibited by both palmitic and castor soaps, but grew well in the olive soap. This would indicate that lowered surface tension within the limits of this experiment, does not bring about the development of anaerobes under aerobic conditions.

Surface tension and nitrogen fixation by Azotobacter chroococcum

Ashby's medium received various concentrations of soap, as indicated in table 4, and was then inoculated with a soil sus-

TABLE 4

Surface tension and nitrogen fixation by Azotobacter chroococcum

NUMBER	TREATMENT	SURFACE TENSION PER CUBIC CENTIMETER	NITROGEN FIXED PER 100 CC.	
				Average
	per cent	*dynes*	*mgm.*	*mgm.*
1–2	Castor 0.1	54.5	9.1 9.3	9.2
3–4	Castor 0.5	43.0	8.3 9.1	8.67
5–6	Castor 1.0	40.3	11.8 10.4	11.1
7–8	Cocoanut 1.0	54.5	10.3 11.5	10.9
9–10	Cocoanut 3.0	40.3	10.1 12.0	11.05
11–12	Cocoanut 5.0	34.8	10.6 9.7	10.15
13–14	Palmitic 1.0	48.0	10.2 10.1	10.15
15–16	Palmitic 3.0	45.8	10.7 10.9	10.8
17–18	Palmitic 5.0	38.2	11.2 11.6	11.4
19–20	Olive 0.1	48.0	10.4 9.7	10.05
21–22	Olive 0.5	39.8	9.4 10.2	9.8
23–24	Olive 1.0	38.2	11.7 9.4	10.55
25–26	Controls	74.2	10.8 11.7	11.25

pension containing Azotobacter. The flasks were then incubated at 25°C. for two weeks and the total nitrogen determined.

The Azotobacter film was uniform, pigmented, and heavy on all flasks, little or no difference being noted. Table 4 shows the surface tension of the media and the amount of nitrogen fixed.

It is obvious that neither the lowered surface tension nor the presence of the depressants have materially affected nitrogen fixation by *Azotobacter chroococcum.*

Oil seals

Interesting results were obtained with various oil seals sometimes employed in the cultivation of anaerobes in broth. The surface tension of ordinary paraffin oil was found to be about 28 dynes, olive oil 34 dynes, and castor oil 35 dynes. By using these oils as seals over ordinary broth the difference in tension of the phases is lessened and consequently the tension is lowered. No change was noted in the development of an ordinary pellicle forming organism under such a seal, but *Eb. typhi* formed a well-developed pellicle at the two phases, particularly in the case of olive oil. This did not develop in the case of paraffin oil. Azotobacter developed a splendid film under a half-inch layer of paraffin oil, and fixed as much N. as in the control flasks. Other data with reference to interfacial tension and bacterial growth will be presented at a later date.

SUMMARY

The foregoing experiments show that one of the most important factors in work on the relation of surface tension to bacteriological activities is the nature of the depressants employed. Soaps were found to be very satisfactory depressants because a very small amount produced a material depression in the surface tension. There is a considerable difference in various soaps, not only in their ability to depress surface tension, but in the effect on the organisms. Error in observations can only be avoided by using several soaps in concentrations such that each produces similar depression in the surface tension. If an observed phenomenon does not occur with several depressants, at approximately the same surface tension, it cannot be cor-

rectly ascribed to surface tension, but must be due to the nature of the depressant. Keeping this in mind, and using four depressants, no case has been found in the preceding experiments in which variation from control might better be attributed to surface tension than to the influence of the depressants themselves.

No observation has been made which would lead to the conclusion that pellicle formation is suppressed at lowered surface tension. Organisms produced pellicles at a surface tension as low as 32 degrees per square centimeter as determined by the method of Fahrenwald. This would correspond to a surface tension of 42 to 44 dynes as determined by the drop weight method.

Anaerobic bacteria failed to develop under aerobic conditions even when the surface tension was greatly lowered.

REFERENCES

AYERS, S. HENRY, RUPP, PHILIP, AND JOHNSON, WILLIAM T. JR. 1923 The influence of surface tension depressants on the growth of streptococci. Jour. Infect. Dis., **33**, 202.

FAHRENWALD, A. W. 1922 The film method of measuring surface tension and interfacial tension. Jour. Opt. Soc. and Rev. Sci. Instr., **6**, 722.

LARSON, W. P., CANTWELL, W. F., AND HARTZELL, J. B. 1919 The influence of surface tension of the culture medium on the growth of bacteria. Jour. Infect. Dis., **25**, 41.

LARSON, W. P. 1921. The effect of surface tension of the culture medium on the growth of bacteria. Absts. Bact., **5**, 2.

MELLON, RALPH R., HASTINGS, W. S., AND ANASTASIA, C. 1924 On the nature of the "cohesive factor" in spontaneous agglutination of bacteria. Jour. Immunol., **9**, 365.