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Mode of Action of Gramicidin.

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Gramicidin has been found to be bactericidal in small amounts for Gram-positive bacteria growing in tissue culture media.[1] The relative sensitivity of the bacterial species studied coincides with the results of bactericidal tests reported by Dubos.[2] Gramicidin is also hemolytic in bactericidal concentrations;[3, 4] however, similar amounts of the substance do not appear to be toxic for granulocytes, fibroblasts or cells of the lymphoid series growing in tissue culture.[5] These qualities, (1) bactericidal activity, (2) hemolytic activity and (3) low toxicity for tissues, are also characteristic of some substances generally classified as detergents. Moreover, gramicidin shows a definite specificity of bactericidal action. The growth of pneumococci and hemolytic streptococci is inhibited by small amounts of the substance. Larger amounts are necessary to inhibit other kinds of streptococci and relatively large amounts must be used to inhibit staphylococci. Gram-negative bacteria in general are not affected. A similar type of bactericidal selectivity has been shown for soaps of the unsaturated fatty acids by Lamar,[6] Avery,[7] Walker,[8] Eggerth,[9] and Bayliss and Halvorson,[10] and for sodium lauryl sulfate by Birkeland and Steinhaus.[11] Baker, Harrison and Miller[12] recently showed that anionic synthetic detergents display the same kind of specificity in their inhibitory effect on the metabolism of various bacterial species.

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3 Dubos, R. J., *Ann. Int. Med.*, 1940, **13**, 2025.

4 Heilman, Dorothy, and Herrell, W. E., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 182.

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6 Lamar, R. V., *J. Exp. Med.*, 1911, **13**, 380.

7 Avery, O. T., *J. A. M. A.*, 1917, **71**, 2050.

8 Walker, J. E., *J. Infect. Dis.*, 1924, **35**, 557.

9 Eggerth, A. H., *J. Exp. Med.*, 1929, **50**, 299.

10 Bayliss, Milward, and Halvorson, H. O., *J. Bact.*, 1935, **29**, 9.

11 Birkeland, J. M., and Steinhaus, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 86.

12 Baker, Zelma, Harrison, R. W., and Miller, B. F., *J. Exp. Med.*, 1941, **72**, 249.

As a rule the hemolytic activity of a detergent coincides with its bactericidal activity. Lamar found that soaps which were most bactericidal against the pneumococcus were also most active in causing hemolysis. Eggerth,[13] in a study of alpha-brom soaps of saturated fatty acids, concluded that the bactericidal activity paralleled the hemolytic activity. Katz and Lipsitz,[14] reporting on a synthetic detergent derived from naphthalene sulfonic acid, stated that a concentration of this substance which would inhibit the growth of *Mycobacterium smegmatis* also caused hemolysis.

From cultures of the soil bacillus which yields gramicidin, Hotchkiss and Dubos[15] have isolated a related substance, tyrocidine, which is less bactericidal for Gram-positive organisms than gramicidin, but which is effective against Gram-negative as well as Gram-positive bacteria. It has been pointed out previously that tyrocidine resembles the cationic detergents in its bactericidal activity. The cationic detergents have been shown by Baker, Harrison and Miller to inhibit the metabolism of Gram-positive and Gram-negative organisms to the same degree.

In view of some points of similarity between gramicidin and tyrocidine and certain detergents a study was made of the ability of gramicidin and tyrocidine to depress the surface tension of aqueous solutions. The samples of tyrocidine and gramicidin used in this study were prepared by Osterberg from tyrothricin, the crude bactericidal substance, by the method of Hotchkiss and Dubos. The tyrothricin was supplied by Sharp and Dohme. Tyrocidine is partially soluble in water; however, gramicidin is quite insoluble in this medium. As a result, 2% solutions of these substances in 95% alcohol were added to the solutions to be tested. The resulting suspensions and solutions used as controls contained 0.475% of alcohol. The surface tensions of these suspensions were determined with a precision type DuNoüy tensiometer within an hour of their preparation. It was observed that suspensions of gramicidin showed a rapid decrease of surface tension within the first 10 minutes after being placed in the watch glass for study with the tensiometer; hence readings were made at 3 intervals, the first immediately after the material was placed in the watch glass, and the second and third at 10 and 30 minutes thereafter. This initial drop of surface tension with time occurred regardless of how long the suspension had been prepared before the observations on surface tension were made.

[13] Eggerth, A. H., *J. Exp. Med.*, 1929, **49**, 53.

[14] Katz, Joseph, and Lipsitz, Aaron, *J. Bact.*, 1935, **30**, 419.

[15] Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, 1940, **136**, 803.

The rapid decrease of surface tension with time has been described by DuNoüy[16] in the case of certain colloidal solutions. The solutions prepared with 0.9% solution of sodium chloride were adjusted to pH 7.2 and the preparations made with Tyrode's solution had a pH between 8.0 and 8.2. Similar amounts of sodium oleate and a synthetic detergent, dioctyl ester of sulfonated succinic acid (aerosol OT) were included in some of the tests for comparison. The results of these tests are shown in Fig. 1.

It may be seen that tyrocidine was effective in lowering the surface tension regardless of the kind of solution used. Gramicidin is less active in water or saline solutions but when its solubility is in-

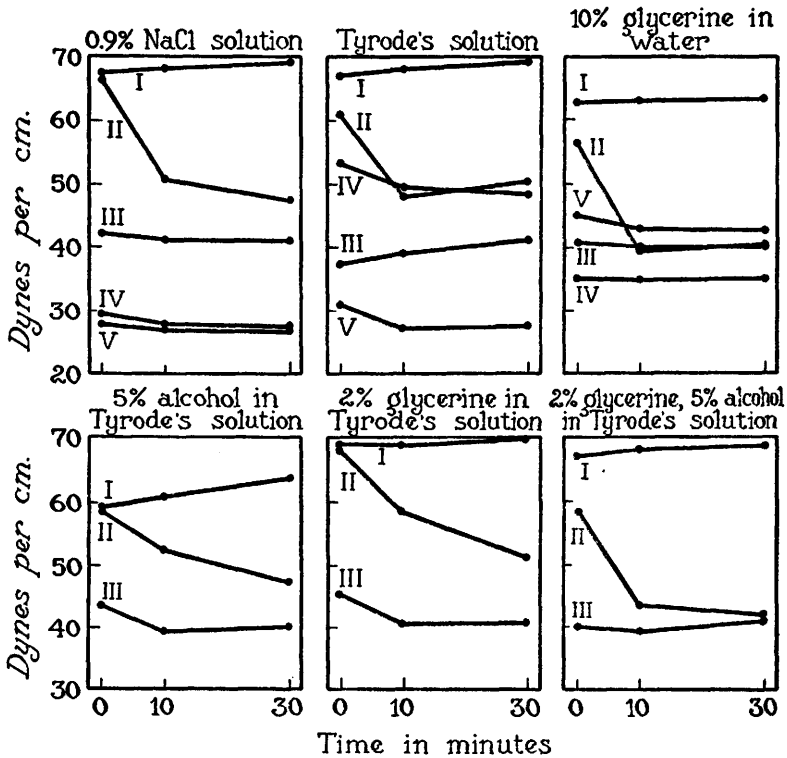

FIG. 1.

Effect of gramicidin, tyrocidine, sodium oleate and aerosol OT on surface tension of various solutions at 25°C. Key to curves (type of solution concerned in each chart is given in its caption) is as follows: I, solution with 0.5% alcohol added; II, solution same as I, gramicidin added 1 to 10,000; III, solution same as I, tyrocidine added 1 to 10,000; IV, solution same as I, sodium oleate added 1 to 10,000; V, solution same as I, aerosol added 1 to 10,000.

[16] Du Noüy, P. L., *Surface Equilibria of Biological and Organic Colloids*, New York, The Chemical Catalog Company, 1926, 212 pp.

creased by the addition of 10% of glycerin or 2% glycerin and 5% alcohol, its ability to lower the surface tension after 30 minutes of observation approaches that of tyrocidine. It may be seen that sodium oleate is less active in Tyrode's solution, presumably because of the presence of calcium and magnesium ions.

A study was made of the effect of pH on the activity of tyrocidine and gramicidin. Concentrations of 1:5000 of these substances were prepared in fifteenth-molar phosphate buffers (Sörensen). There was no significant change in the activity of either substance over a pH range of 5.3 to 8.0. Likewise, heating suspensions of gramicidin in water or Tyrode's solution to 90°C for 10 minutes did not appear to inhibit their surface tension depressing ability when these suspensions subsequently were added to 2% glycerin in water or 10% rabbit's serum in 0.9% solution of sodium chloride.

The inhibitory effect of serum on the bactericidal action of soaps has been pointed out by Eggerth,[17] Walker,[8] and others. The antagonistic effect of serum on the surface tension depressing activity of sodium oleate is discussed by DuNoüy.[15] Table I records the effect of increasing amounts of rabbit's serum in inhibiting the surface tension depressing activity of gramicidin, tyrocidine, tyrothricin (the crude bactericidal substance containing about 85% of tyrocidine and 15% of gramicidin), sodium oleate and aerosol OT. Tyrocidine shows a remarkable degree of activity in the presence of large amounts of serum. The crude bactericidal substance appears to be somewhat

TABLE I.

Effect of Rabbit's Serum on Depression of Surface Tension of Saline Solutions by 1:5,000 Concentrations of Gramicidin, Tyrocidine, Tyrothricin, Sodium Oleate and Aerosol OT.

	Gramicidin	Tyrocidine	Tyrothricin	Sodium oleate	Aerosol OT
0.9% NaCl solution	18*	26	26	40	41
1% serum in .9% NaCl sol.	14	20	21	31	31
5% serum in same	10	19	18	18	24
10% " " "	8	17	17	10	17
20% " " "	4	14	16	4	10
40% " " "	3	18	17	1	6
Tyrode's solution	20	28	27	19	40
1% serum in Tyrode's sol.	12	19	21	13	32
5% serum in same	6	17	19	9	24
10% " " "	4	15	16	8	16
20% " " "	4	15	15	4	10

*Values indicate the difference in dynes between the surface tension of the original solution and that of the same solution to which the various substances have been added. Determinations were made at 10 minutes. Temperature 25°C. All solutions were adjusted to pH 7.6.

17 Eggerth, A. H., *J. Exp. Med.*, 1927, **46**, 671.

more active than would be expected from its content of gramicidin and tyrocidine. The inhibitory effect of increasing amounts of serum appears to be proportionately about the same for gramicidin and aerosol OT, but it is much greater in the case of sodium oleate, either in the presence of 0.9% solution of sodium chloride or in Tyrode's solution.

It is evident that the relative ability of gramicidin and tyrocidine to depress the air-liquid interfacial tension of the solutions studied is not proportional to their respective bactericidal activities against Gram-positive bacteria or to their hemolytic activity. The effectiveness of gramicidin appears to depend on its partial solubility. It is possible that the activity of gramicidin might be altered by traces of organic solvents or by enzyme action at the cell surface. However, the fact that tyrocidine is more active in depressing surface tension under all conditions studied might indicate that the greater bactericidal action of gramicidin on Gram-positive organisms depends on a more specific activity of this substance. Such a possibility seems more likely in view of the fact that heating destroys the bactericidal and hemolytic properties of gramicidin but does not alter its ability to depress surface tension. It would seem that the tendency of this substance to concentrate at an interface might enhance any such specific metabolic activity greatly.

Conclusions. 1. Gramicidin and tyrocidine depress the surface tension of aqueous solutions. Tyrocidine is more active in this regard than is gramicidin. 2. The ability of gramicidin to depress surface tension is improved by the addition of organic solvents such as glycerin which increases the solubility of gramicidin. 3. Serum decreases the activity of tyrocidine less than it does the activity of gramicidin, sodium oleate and aerosol OT. 4. The bactericidal and hemolytic effects of gramicidin are destroyed by heat but its property of altering surface tension is heat stable.