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Effect of Serum on Hemolysis by Gramicidin and Tyrocidine.

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Since two of us (Heilman and Herrell) reported on the hemolytic activity of tyrothricin[1] and its fractions, gramicidin and tyrocidine,[2] some conflicting views have appeared in the literature regarding the relative hemolytic activity of the two fractions. Experimental data from different sources are not in disagreement for the most part but differences of technic and interpretation have led to divergent conclusions.

When gramicidin or tyrocidine in minimal effective amounts is added to suspensions of washed sheep erythrocytes, the rate of hemolysis is much more rapid with tyrocidine than with gramicidin but the latter, although slower in action, ultimately causes a greater degree of hemolysis than the former. If incubation is permitted to proceed for more than 4 hr, the amount of hemolysis caused by gramicidin is greater, in our experience, than that caused by tyrocidine. It can be seen that if similar tests are done and the final readings are made after a short period of incubation, tyrocidine would appear to be much more hemolytic than gramicidin. About the same length of time is required for gramicidin to lyse erythrocytes as to destroy pneumococci.[3]

The results of studies on hemolysis by gramicidin and tyrocidine vary greatly with the kind of medium in which the tests are done. Many agents known to be hemolytic in saline solutions are ineffective in whole blood. Since hemolysis in the animal body takes place in the presence of blood serum, we have found it convenient to study the effect of various agents on rabbit erythrocytes in a tissue culture clot consisting of rabbit's plasma and serum containing a small amount of chick embryo extract. With this kind of preparation we have found that hemolysis by gramicidin takes place within an hour if relatively large amounts of the drug are used, and that smaller amounts of gramicidin require a longer period.[2] Tyrocidine, even in large amounts (0.1 mg per cubic centimeter), did not cause any hemolysis in this kind of preparation after 24 hr of incubation.

The subject appeared to be of sufficient importance to present the results of the following experiments. Sheep erythrocytes were washed 4 times with 0.85% solution of sodium chloride and were used to make 1% suspensions of erythrocytes in 0.85% solution of sodium chloride and in saline solution containing varying amounts of horse serum. The horse serum in these experiments had been kept under sterile conditions at 4°C for several weeks and had come to equilibrium with the air. The gramicidin used was prepared by Osterberg from tyrothricin according to the method of Hotchkiss and Dubos.[4] The purified tyrocidine hydrochloride, as well as the tyrothricin used in the preparation of gramicidin, was furnished through the courtesy of Sharp and Dohme. Stock solutions were prepared containing 2% of gramicidin or tyrocidine hydrochloride in 95% alcohol. Dilutions of each drug were made by the addition of varying amounts of the stock solutions to 0.85% solution of sodium chloride. To small test tubes were added 1.8 cc of the suspension of erythrocytes and 0.2 cc of a dilution of the drug being tested. The tubes were shaken and placed immediately in a water bath at 37°C. Observations on the degree of hemolysis were made after one, 2, 4, 6 and 18 hr of incuba-

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

2 Herrell, W. E., and Heilman, Dorothy, *J. Clin. Invest.*, 1941, **20**, 583.

3 Heilman, Dorothy H., and Herrell, W. E., *Proc. Staff Meet., Mayo Clin.*, 1942, **17**, 321.

4 Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, 1941, **141**, 155.

TABLE I.
Effect of Serum on Hemolysis by Gramicidin and Tyrocidine.
1% Sheep Erythrocytes in 0.85% Solution of Sodium Chloride.

Gramicidin, mg per cc	No serum (pH 6.9) Hrs incubation					1% serum (pH 7.1) Hrs incubation					5% serum (pH 7.7) Hrs incubation					50% serum (pH 8.25) Hrs incubation				
	1	2	4	6	18	1	2	4	6	18	1	2	4	6	18	1	2	4	6	18
.04	1*	2	4	4	4	1	1	4	4	4	0	0	3	4	4	0	0	4	4	4
.02	0	1	3	4	4	0	0	3	4	4	0	0	2	3	4	0	0	4	4	4
.004	0	0	3	4	4	0	0	1	2	4	0	0	0	1	4	0	0	0	0	4
.002	0	0	2	4	4	0	0	0	1	4	0	0	0	0	1	0	0	0	0	1
.001	0	0	1	3	4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
.0005	0	0	0	2	4	0	0	0	0	0										
Tyrocidine																				
.04	4	4	4	4	4	3	3	4	4	4	0	0	0	0	0	0	0	0	0	0
.02	2	3	4	4	4	1	1	2	2	3	0	0	0	0	0	0	0	0	0	0
.004	1	1	1	1	1	0	0	0	0	0	—	—	—	—	—	—	—	—	—	—
.002	0	0	0	0	0	0	0	0	0	0	—	—	—	—	—	—	—	—	—	—

*Degree of hemolysis, graded on basis of 1 to 4.
0—No hemolysis.

tion. Results taken from a typical experiment are presented in Table I.

The addition of only 1% of horse serum to the saline solution decreased the hemolytic activity of both gramicidin and tyrocidine. Five and 10% of horse serum caused further inhibition of the hemolytic activity of gramicidin and completely prevented hemolysis by tyrocidine. Increasing the ratio of horse serum to 20 and 50% did not result in a further decrease of the hemolytic activity of gramicidin beyond the inhibition caused by 5% of serum.

Dubos has shown that 5% dextrose will prevent hemolysis by as much as 0.4 mg per cc of gramicidin but will not prevent hemolysis by tyrocidine. Two of us (Herrell and Heilman) have also found that 5% dextrose will prevent hemolysis by relatively large amounts of gramicidin (0.1 mg per cc).[5] The data presented in similar studies by Rammelkamp and Weinstein[6] are not in agreement with the data published by Dubos or with our experimental results. They found that hemolysis of human or rabbit erythrocytes by gramicidin was not prevented or even decreased by the presence of 5% dextrose. It is well known that dextrose in a concentration of 5% protects erythrocytes from hemolysis by a number of agents. For instance, we have found that 10 μg per cc of aerosol OT (dioctyl ester of sulfonated succinic acid) will cause hemolysis within a few minutes of 1% sheep erythrocytes in 0.85% solution of sodium chloride containing 0.4% glucose, whereas increasing the amount of glucose to 2.0% delays hemolysis for 4 hr. We have shown, however, that hemolysis by gramicidin is not prevented by any concentration of glucose that would normally be present in the blood.[5]

That the inhibition of hemolysis obtained by the addition of serum is not due to the glucose present in the serum is also evident from the fact that the activity of tyrocidine is affected as well as that of gramicidin. The addition of 0.04 mg per cc of tyrocidine to the suspensions of erythrocytes used in these experiments caused a slight shift toward acidity of not more than 0.15 pH units. The same amount of gramicidin did not alter the pH of the solutions used. Both gramicidin and tyrocidine were found to be as active in causing hemolysis in Tyrode's solution at pH 8.0 as they were in 0.85% solution of sodium chloride. Therefore, the hydrogen ion concentration of the medium did not appear to be a factor in these experiments.

Summary. When gramicidin or tyrocidine in minimal effective amounts is added to suspensions of washed sheep erythrocytes, the rate of hemolysis is much more rapid with

5 Herrell, W. E., and Heilman, Dorothy, (Abstr.) *J. A. M. A.*, 1942, **118**, 1401.

6 Rammelkamp, C. H., and Weinstein, Louis, PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 211.

tyrocidine than with gramicidin but the latter, although slower in action, ultimately causes a greater degree of hemolysis than the former.

Comparative experiments have been done to determine the influence of horse serum on the rate and amount of hemolysis caused by gramicidin and tyrocidine. The hemolytic activity of both substances is decreased in the presence of only 1% of horse serum. Five percent of serum causes further inhibition of the hemolytic activity of gramicidin and completely prevents hemolysis by tyrocidine. Increasing the amount of serum beyond 5% did not result in a further decrease of the hemolytic activity of gramicidin.

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Influence of Vitamins and Coliform Bacteria on Sulfaguanidine Tolerance by Young Chickens.*

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Sulfaguanidine is well established as an effective bacteriostatic agent for treatment of enteric disorders, but prolonged feeding of this drug in large amounts is known to cause a variety of pathological changes, including loss of weight or failure to maintain normal growth in experimental animals such as rabbits,[1] rats,[2] and chickens.[3] Black *et al.*[4,5] reported that a basic cause of growth inhibition by sulfaguanidine is its interference with intestinal bacteria which normally synthesize growth-promoting substances used by the animal body. At least some of the harmful effects of the drug can be counteracted with liver extract,[4,6] p-aminobenzoic acid,[4] vitamin K,[5] or biotin concentrate[6] but not with nicotinic acid.[7] Presumably these agents act by supplying vitamins ordinarily procured from intestinal bacteria, or by preventing the interference of sulfaguanidine with essential metabolic processes. To our knowledge no substances which increase the harmful effects of sulfaguanidine have been reported.

The wide implications and apparent acceptance of this concept of interference with synthesis by the intestinal flora led us to undertake a further investigation of the relation of vitamins and intestinal bacteria to the action of sulfaguanidine. The results obtained with young growing chickens are presented here to indicate the complexity of the relationship; they are regarded as neither an evaluation of the phenomena involved nor a criticism of previous work.

Experimental methods. Single-comb, white Leghorn chicks of uniform size and vigor were individually fed 3 times daily on an adequate ration designated as Nebraska 8-S.[8] Controlled feeding was begun 24 hr after hatching and individual records of food intake and body weight were recorded throughout the experimental period of 3 to 4 weeks.

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1 Corwin, W. C., *Bull. Johns Hopkins Hosp.*, 1941, **69**, 39.

2 Spicer, S. S., Daft, F. S., Sebrell, W. H., and Ashburn, L. L., *Pub. Health Rep.*, 1942, **57**, 1559.

3 Farr, M. M., and Allen, R. W., *J. Am. Vet. Med. Assn.*, 1942, **100**, 47.

4 Black, S., McKibbin, J. M., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 308.

5 Black, S., Overman, R. S., Elvehjem, C. A., and Link, K. P., *J. Biol. Chem.*, 1942, **145**, 137.

6 Daft, F. S., Ashburn, L. L., and Sebrell, W. H., *Science*, 1942, **96**, 321.

7 Dann, W. J., *J. Biol. Chem.*, 1941, **141**, 803.

8 Ackerson, C. W., Ham, W. E., and Mussehl, F. E., 1940, *U. of Nebr. Agric. Exp. Sta. Res. Bull.*, 120.