glucose for every millimole of potassium that enters the cell.

1. Raker, J. W., Taylor, I. M., Weller, J. M., and Hastings, A. B., *J. Gen. Physiol.*, 1950, v33, 607.
2. Geiman, Q. M., Anfinsen, C. B., McKee, R. W., Ormsbee, R. A., and Ball, E. G., *J. Exp. Med.*, 1946, v84, 583.
3. Mullins, L. J., Fenn, W. O., Noonan, T. R., and Haege, L., *Am. J. Physiol.*, 1941, v135, 93.

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The Hemolytic Action of Gramicidin and Tyrocidin. (19217)

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Tyrothricin, an antibiotic complex isolated by Dubos(1) from the soil organism *Bacillus brevis*, consists of at least 2 hemolytic substances in the following approximate proportions: tyrocidin, 85%, and gramicidin, 15%. The hemolytic activities of these 2 substances as well as that of tyrothricin have been reported by various research workers(2-6). Quantitative data on the rates of reaction of the pure substances as well as mixtures are lacking, however. This paper presents comparative data on the hemolytic activities of gramicidin and tyrocidin and various mixtures. The analyses were made under the conditions developed by the author(7) for quantitative determination of tyrothricin.

Materials and methods. Stock alcohol solutions of the 2 purified substances† were made to contain 20 μg per ml of gramicidin or tyrocidin. Working solutions and mixtures were prepared by dilution with 95% alcohol.

One-half ml aliquots of the diluted solutions were added to 10 ml of a 0.25% twice-washed rat erythrocyte suspension in 0.87% saline, and the course of hemolysis was determined with a Klett-Summerson photoelectric colorimeter. A 660-millimicron filter was employed. Scale readings are proportional to optical density.

Action of gramicidin and tyrocidin. The course of hemolysis is markedly different for these two agents (Fig. 1). The hemolytic effect of gramicidin is represented by a sigmoidal curve with a pronounced lag period. The length of this period is dependent upon the concentration of gramicidin and the temperature. Tyrocidin, on the other hand, causes very rapid hemolysis, which is inhibited after about 10 minutes. The extent of hemolysis is dependent upon the concentration of the tyrocidin. Experiments discussed below indicate that the tyrocidin combines with some component of the cell stromata and becomes inactivated.

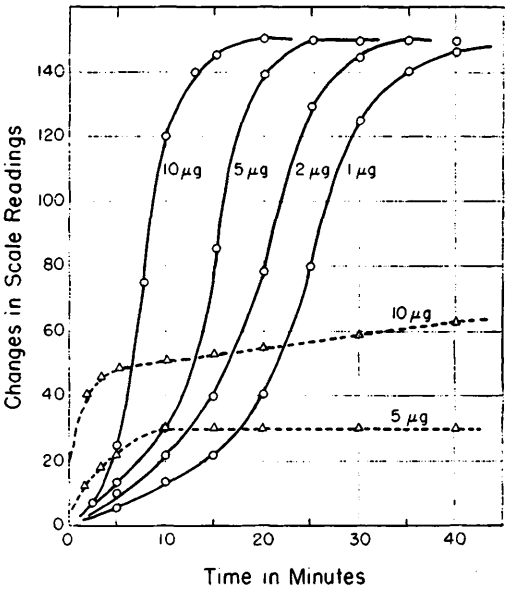

FIG. 1. Hemolysis curves for gramicidin and tyrocidin. Solid lines, gramicidin; broken lines, tyrocidin.

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† Several times recrystallized gramicidin and tyrocidin hydrochloride were prepared from crude tyrothricin according to methods of Hotchkiss and Dubos(4).

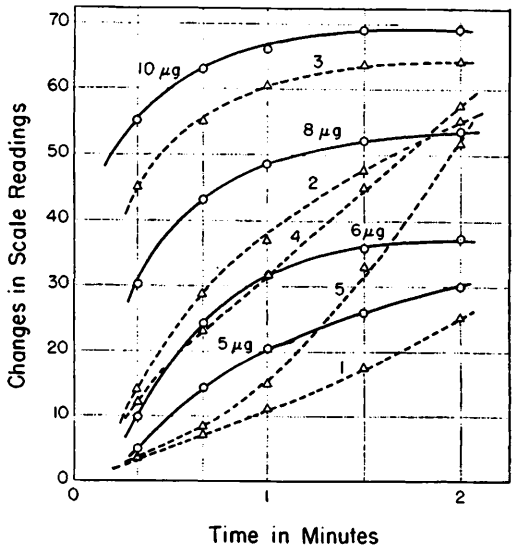

FIG. 2. Hemolysis curves for tyrothricin and for various mixtures of gramicidin and tyrocidin. Solid lines, natural tyrothricin.

Curve	% grami'	% tyro'	μg total.
1—	.0	100	8
2—	4.8	95.2	8.4
3—16		84	9.6
4—23		77	7.8
5—33.3		66.6	7.5

The hemolysis curves obtained with mixtures were found to be characteristic of the composition of the mixture in the early part of the reaction (dotted curves, Fig. 2). The solid curves represent the rate of hemolysis produced by various concentrations of a sample of tyrothricin which was isolated in this laboratory. The data for this sample agreed very well with those for other preparations made here, for samples obtained from commercial sources, and for a mixture of 16% gramicidin and 84% tyrocidin (see curve 3, Fig. 2). Hotchkiss and Dubos(4) have found, by chemical isolation, that tyrothricin is composed of approximately this same proportion of gramicidin and tyrocidin.

Although critical experiments have not been made, it should be possible to estimate the relative proportion of these 2 substances by determining the type of curve produced. It is easy to determine either of these compounds by the hemolytic procedure when the other is absent. Such measurements were made on chemically modified gramicidin in an attempt to correlate loss of hemolytic activity with loss of toxicity(11-13).

Tyrocidin inactivation. Tyrocidin appears to combine with some component of the red blood cell to inactivate its hemolytic activity. Several experiments were designed to investigate further the inactivating effect of the blood cells on tyrocidin and gramicidin.

Three or 4 times the amount of tyrocidin necessary to completely hemolyze was added to a sample of washed erythrocytes (1000 μg in 1 ml of cells) and allowed to stand for one hour. Alcohol was added to the completely hemolyzed solution to 90% concentration in order to extract the tyrocidin. Aliquots of this extract were mixed with a fresh aliquot of red cells, and no trace of hemolysis was observed, although an excess of tyrocidin had been added to the original erythrocyte suspension.

To test this possibility further, aliquots of a 90% alcoholic extract of carefully washed erythrocytes were added to an alcoholic solution of tyrocidin. No hemolytic action was obtained when the mixture was tested on fresh red blood cells. In order to determine whether this inactivating agent was present in the cell wall or in the water-soluble part of the cell, a sample of erythrocytes was laked with distilled water and the cell walls (ghost cells) were centrifuged off and washed twice with distilled water. An alcoholic extract of the cell walls was found to contain approximately all of the inactivating agent, while the water-soluble components of the cell had no inhibitory effect. A benzene extract of dried erythrocytes was found also to inhibit the hemolytic activity of tyrocidin.

Several investigators(8-10) have found that phospholipids will inhibit the bactericidal action of tyrothricin on bacterial cells, while Heilman and Herrell(2) have found that cholesterol does not neutralize the hemolytic effect of gramicidin. The inhibitory agent or agents obtained from blood cells is similar to phospholipids with regard to solubility characteristics. Similar experiments with gramicidin showed that it was inhibited, but to a much lesser extent, than tyrocidin. Inhibition of gramicidin and tyrocidin with regard to their bactericidal action was not studied.

Summary. (1) The courses of hemolysis for gramicidin, tyrocidin, and mixtures of the

two have been determined. Tyrocidin causes a rapid hemolysis, the extent being proportional to the concentration of the lysin. Gramicidin causes a slower initial rate of hemolysis but this initial lag is followed by rapid destruction of red blood cells at a rate at least equal to the initial rate due to tyrocidin. Mixtures of the two components gave hemolysis patterns characteristic of the proportions of gramicidin and tyrocidin. (2) An alcohol-soluble substance or substances present in the wall of the red blood cell of the rat, possibly phospholipid in nature, combines with tyrocidin, and to a lesser extent with gramicidin, causing them to lose their hemolytic activity.

1. Dubos, R. J., and Hotchkiss, R. D., *J. Exp. Med.*, 1941, v73, 629.

2. Heilman, D., and Herrell, W. E., PROC. SOC. EXP. BIOL. AND MED., 1941, v46, 182.

3. Herrell, W. E., and Heilman, D., *J. Clin. Invest.*, 1941, v20, 583.

4. Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, 1941, v141, 155.

5. Rammelkamp, C. H., and Weinstein, L., PROC. SOC. EXP. BIOL. AND MED., 1941, v48, 211.

6. Mann, F. C., Heilman, D., and Herrell, W. E., PROC. SOC. EXP. BIOL. AND MED., 1943, v52, 31.

7. Dimick, K. P., *J. Biol. Chem.*, 1943, v149, 387.

8. Baker, Z., Harrison, R. W., and Miller, B. F., *J. Exp. Med.*, 1941, v74, 621.

9. Dubos, R. J., *J. Lancet*, 1941, v41, 405.

10. Miller, B. F., Abrams, R., Dorfman, A., Klein, M., *Science*, 1942, v96, 428.

11. Lewis, J. C., Dimick, K. P., Feustel, I. C., Fevold, H. L., Olcott, H. S., and Fraenkel-Conrat, H., *Science*, 1945, v102, 274.

12. Fraenkel-Conrat, H., Lewis, J. C., Dimick, K. P., Edwards, B., Reitz, H. C., Ferrel, R. E., Brandon, B. A., and Olcott, H. S., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 302.

13. Olcott, H. S., Lewis, J. C., Dimick, K. P., Fevold, H. L., and Fraenkel-Conrat, H., *Arch. Biochem.*, 1946, v10, 553.

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Serum Cholinesterase Activity of Dogs in Shock or after Hepatectomy.* (19218)

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Since the cholinesterase of the serum is probably derived in large part from the liver (1-3), its serum concentration may reflect hepatic function(4). Therefore, serum cholinesterase levels were measured as a part of a study of hepatic dysfunction in experimental hemorrhagic shock. For comparison these data were also obtained in hepatectomized dogs.

Methods. Hemorrhagic shock was produced in unanesthetized dogs, using the elevated reservoir bleeding technic previously described (5). Serum from arterial blood was obtained (a) before hemorrhage, (b) during hypotension before transfusion, (c) when hypotension recurred after blood replacement, and (d) just before death (Table I). Total hepatectomy was performed in 3 dogs by a one-stage method, preserving the integrity of the inferior vena cava(6). In one other dog the hepatic artery and portal vein were ligated and a portal-caval anastomosis was carried out without removal of the liver. In another, the hepatic artery alone was ligated. Serum from arterial blood was obtained before these procedures and at intervals thereafter. Serum cholinesterase and total esterase were determined colorimetrically, employing beta-carbonaphthoxy-choline iodide and beta-naphthyl acetate as the respective substrates

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