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Hemolytic Effect of Tyrothricin.*

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A bactericidal agent obtained from a spore-bearing soil bacillus was described by Dubos.[1] The crude extract obtained from the Dubos organism is alcohol-soluble, water-insoluble, and is called *tyrothricin.* From this substance it has been possible to isolate two fractions, *gramicidin* and *tyrocidine,*[2] the former appearing to possess considerably more bactericidal activity both *in vitro* and *in vivo.* Mice which receive intraperitoneal injections of pneumococci or streptococci[1] can be protected completely by small amounts of the crude extract. Tyrothricin, however, will produce definite toxic changes in the animal when injected intravenously[3] into local cavities or intradermally.[4] It has also been observed to cause hemolysis of rabbit's and sheep's erythrocytes *in vitro.*[5]

Heilman and Herrell[6] observed that tyrothricin in amounts as small as 0.001 mg caused hemolysis of rabbit's erythrocytes and that the addition of cholesterol did not neutralize this hemolytic effect. Later, the same authors[7] reported that the hemolytic effect of tyrothricin was due to the presence of *gramicidin.* Dubos and Hotchkiss[5] observed that 0.4 mg of gramicidin did not hemolyze rabbit's erythrocytes suspended in isotonic glucose solution during 24 hours' incubation while tyrocidine, on the other hand, caused some hemolysis in amounts as small as 0.020 mg. In buffer or saline solutions gramicidin was found to produce a slow hemolysis which could be prevented by the addition of small amounts of glucose.

In view of these somewhat conflicting reports it seemed important to study further the hemolytic action of tyrothricin, gramicidin,

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1 Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 311.

2 Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, 1940, **136**, 803.

3 MacLeod, C. M., Mirick, G. S., and Curnen, E. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 460.

4 Unpublished observations.

5 Dubos, R. J., and Hotchkiss, R. D., *J. Exp. Med.*, 1941, **73**, 629.

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

7 Herrell, W. E., and Heilman, D., *J. Clin. Invest.*, 1941, **20**, 433.

and tyrocidine, since certain undesirable toxic effects noted during the treatment of human infections may possibly be avoided by using pure gramicidin rather than tyrothricin.

Methods. The tyrothricin,† gramicidin,† and tyrocidine used in these experiments were stored in 95% alcohol in a concentration of 50 mg per cc. Varying amounts of these agents were added to a 5% suspension of rabbit's or human erythrocytes in (a) 0.85% saline, (b) 5 and 10% glucose in distilled water, and (c) 5 and 10% glucose in 0.85% saline, the total volume in each tube being 2 cc. Controls using similar dilutions of 95% alcohol were made in each instance. The tubes were then incubated at 37°C and readings for hemolysis made at 1, 3, 5, and 24 hours.

Results. Table I shows the results obtained in one experiment. In all of the experiments some hemolysis occurred within one hour in suspensions containing 0.375 mg of gramicidin. This was true even in the presence of glucose. In each experiment, however, tyrothricin and tyrocidine were found to cause a greater degree of hemolysis than gramicidin. Hemolysis was observed to occur especially during the first 5 hours of incubation and only slight increases were noted thereafter.

When the cells were suspended in 5% glucose in distilled water, hemolysis was usually somewhat more marked. It must be pointed out, however, that this solution is hypotonic and the controls showed some hemolysis after 24 hours' incubation. In 10% glucose in distilled water gramicidin caused the same degree of hemolysis as in saline alone. Tyrocidine, however, showed less laking of the red cells in 10% glucose than in the saline solution. Saline suspensions containing either 5 or 10% added glucose caused some slight inhibition of the hemolytic effect. Similar results were obtained when rabbit's erythrocytes were used, but hemolysis always occurred with smaller concentration of the 3 substances.

Comment. The results obtained in these studies agree with Dubos[5] in that the marked hemolytic effect of tyrothricin seems to be due chiefly to its tyrocidine content. In some experiments we have observed hemolysis of rabbit's erythrocytes with as little as 0.000005 mg of tyrocidine. Gramicidin, while not nearly as hemolytic as tyrothricin or tyrocidine, has been found to lake red cells. This hemolytic action is not inhibited in 5 or 10% glucose in distilled water, and is only slightly inhibited in 5 or 10% glucose in

† The tyrothricin was obtained from Lederle Laboratories, Inc., and Sharp and Dohme, Inc. Gramicidin was obtained from Sharp and Dohme, Inc., and was also made in our laboratories.

TABLE I.
Hemolytic Effect of Gramicidin on Human Red Cells.

	0.85% saline						5% glucose				5% glucose in saline			
	Tyrothricin		Gramicidin		Tyrocidine		Gramicidin		Tyrocidine		Gramicidin		Tyrocidine	
Mg	1°	24°	1°	24°	1°	24°	1°	24°	1°	24°	1°	24°	1°	24°
3.12			++	+++			++	+++			++	+++		
.75	++	+++	+	+++	++	+++	++	+++	+++	+++	+	++	++	+++
.185	++	+++	0	++	++	+++	+	+++	++	+++	0	++	++	+++
.09	+	+++	0	++	++	+++	+	+++	++	+++	0	+	++	+++
.045	+	+++	0	+	+	+++	0	+++	+	+++	0	0	+	+++
.022	+	+++	0	+	+	+++	0	++	+	+++	0	0	0	++
.011	0	++	0	+	+	++	0	++	+	+++	0	0	0	++
.005	0	++	0	0	0	++	0	++	0	+++	0	0	0	+
.0025	0	+	0	0	0	+	0	+	0	++	0	0	0	+
.0012	0	+	0	0	0	+	0	0	0	++	0	0	0	0
.0006	0	0	0	0	0	0	0	0	0	++	0	0	0	0
.0003	0	0	0	0	0	0	0	0	0	++	0	0	0	0
.00015	0	0	0	0	0	0	0	0	0	+	0	0	0	0
.00007	0	0	0	0	0	0	0	0	0	+	0	0	0	0

+++ Complete hemolysis.
++ Intermediate hemolysis.
+ Trace of hemolysis.

Addendum: The above experiments were repeated using gramicidin obtained through the courtesy of Drs. Dubos and Hotchkiss. Hemolysis was slight and delayed in all solutions; it was not completely prevented by the addition of glucose. The inhibition was most marked in 10% glucose in distilled water, the degree of inhibition being about 500-fold.

0.85% saline. Since the addition of either 5 or 10% glucose causes the same degree of inhibition, the amount of added glucose apparently is not an important factor.

Conclusions. Tyrothricin is markedly hemolytic for human and rabbit's erythrocytes. The hemolytic activity seems to reside primarily in the tyrocidine fraction, although gramicidin also exhibits a definite hemolytic effect. The addition of glucose to saline solution causes only slight inhibition of the hemolytic action.

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Peripheral Vascular Responses to General Anoxia.*

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In view of the current interest in the problem of general anoxia in man, it was considered important to study the local changes in blood flow in the extremities consequent to the inhalation of an oxygen-poor gas mixture.

The investigation was performed upon 9 normal, adult subjects, using the venous occlusion plethysmograph method. Blood flow determinations, expressed in cc per minute per 100 cc limb volume, were made upon the hand, leg, and forearm, according to the technic previously described.[1] Ten to 15 control readings were first obtained with an anesthesia mask firmly fitted to the face, but with the subject breathing air from the room. Then a gas mixture of approximately 10% oxygen and 90% nitrogen was allowed to enter the mask at atmospheric pressure for periods of 10 to 20 minutes; the exhaled air being automatically discharged into the room through an outlet tube and flutter valve. Blood flow determinations were made every 2 minutes during this interval. At the completion of the period of relative anoxia, another set of control readings was obtained with the mask still in place, but with the subject again breathing room air. The pulse rate, blood pressure, and respiration were recorded simultaneously with the blood flow readings.

The hand was examined in 4 subjects and in each instance there

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1 Abramson, D. I., Zazeela, H., and Marrus, J., *Am. Heart J.*, 1939, **17**, 194, 206; Ferris, E. B., Jr., and Abramson, D. I., *Am. Heart J.*, 1940, **19**, 233.