

Production of Staphylococcal Enterotoxin in Chemically Defined Media.*

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Production of staphylococcal enterotoxin in synthetic media has not been previously reported, although Favorite and Hammon¹ and Hammon² used successfully a casein hydrolysate medium. The present is a report of enterotoxin production in synthetic media modified from those used by Gladstone³ in studies of the hemolysins.

Four enterotoxigenic strains (Nos. 8, 147, 161 and 196) and one non-enterotoxigenic strain (No. 184) of staphylococci were used in these experiments. All strains are alpha-hemolytic and strains No. 184 and 196 produce potent beta-lysin.

The compositions of 3 basic media used in these experiments are given in Table I. Media were adjusted to pH 7.6, dispensed into nursing bottles or flasks and sterilized in the

autoclave. Inoculations were made with 0.1 ml of a 1:1000 dilution (in phosphate buffer) of culture in synthetic medium.

Cultures were grown at 37°C in an atmosphere of 20% CO₂. During incubation they were rotated continuously on a vertical wheel. Centrifugates of 3 to 7 day cultures were tested for enterotoxin and hemolysins.

Enterotoxin was assayed by the monkey (*Macacca mulatta*) feeding and intravenous cat tests.^{4,5} Untreated centrifugates were fed to monkeys; preparations heated for 30-35 minutes in a boiling water bath were injected into cats. Vomiting was used to indicate a positive reaction. Alpha-hemolysin titers are expressed in terms of the M.H.D. (minimal hemolytic dose) of toxin.⁶ Beta-lysin tests were made against sheep erythrocytes and

TABLE I.
Composition of Basic Media.

Amino acids	Concentration	Medium 1	Medium 2	Medium 3
<i>l</i> (+) arginine HCl	M/110	+	+	+
<i>l</i> -cystine	M/10,000	+	+	+
<i>dl</i> -alanine	M/1500	+	+	
glycine	M/198	+	+	
<i>dl</i> -phenylalanine	M/4000	+	+	
<i>dl</i> -valine	M/1500	+	+	
<i>l</i> -aspartic acid	M/1500	+		
<i>d</i> -glutamic acid	M/1500	+		
<i>l</i> -histidine 2HCl	M/4000	+		
<i>l</i> -hydroxyproline	M/1500	+		
<i>l</i> -leucine	M/1500	+		
<i>d</i> -lysine 2HCl	M/4000	+		
<i>dl</i> -methionine	M/4000	+		
<i>l</i> -tryptophane	M/20,000	+		
<i>l</i> -tyrosine	M/4000	+		

The media also contained M/6000 MgSO₄; M/20,000 FeSO₄·(NH₄)₂SO₄·6H₂O; M/30 KH₂PO₄; M/37 NaOH; M/80 glucose; 1.23 µg/ml nicotinic acid; 0.0337 µg/ml thiamine HCl; 1.0 µg/ml calcium pantothenate (omitted from Media No. 1 and No. 2 in early tests).

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¹ Favorite, G. O., and Hammon, W. McD., *J. Bact.*, 1941, **41**, 305.

² Hammon, W. McD., *Am. J. Public Health*, 1941, **31**, 1191.

³ Gladstone, G. P., *Brit. J. Exp. Path.*, 1938,

19, 208.

⁴ Davison, E., Dack, G. M., and Cary, W. E., *J. Infect. Dis.*, 1938, **62**, 219.

⁵ Dack, G. M., 1943, *Food Poisoning*, University of Chicago Press, Chicago.

⁶ Dolman, C. E., and Kitching, J. S., *J. Path. and Bact.*, 1935, **41**, 137.

TABLE II.
Production of Staphylococcal Enterotoxin and Alpha Hemolysin in Chemically Defined Media.

Medium Basic* Modification		Staphylococcal enterotoxin						Alpha hemolysin M.H.D. per ml
		Monkey feeding test			Intravenous cat test			
		Dosage (ml per monkey)	Reaction pos. neg.		Dosage (ml per kg body wt)	Reaction pos. neg.		
No. 1		50	0	5	1.0-2.0	10	3	4-16
	+ proline M/690	50	0	4	1.0	2	2	64-128
	+ " M/280	50	6	3	1.0	6	3	32-64
	+ " M/280							
	— aspartic acid							
	glutamic "	50	1	0				4-16
	histidine							
No. 2		40-100	2	2	2.0	1	2	32
	+ proline M/690	50	0	2	2.0	2	2	16-32
	+ glutamic acid							
	M/280	40-100	1	2	2.0	2	1	64
	+ hydroxyproline							
	M/280	40-100	1	3	2.0	2	1	16-32
No. 3		55-100	0	3	2.0	0	4	<4
	+ (NH ₄) ₂ SO ₄ M/250	50-100	0	3	2.0	1	3	4-16
	+ glycine M/198	40-100	0	3	2.0-3.0	3	1	8

* Numbers correspond to Table I.

were read after incubation for one hour at 37°C followed by one hour in an ice water bath.

Results of tests for enterotoxin and alpha-lysin are shown in Table II. Alpha hemolysin production and vomiting reactions in cats were obtained with cultures grown in all media except Medium No. 3. In the latter medium negative results may be correlated with nitrogen depletion, since addition of (NH₄)₂SO₄ or glycine increased growth hemolysin titers and enterotoxigenic reactions. Four cats failed to react to centrifugates of the nonenterotoxigenic strain grown in Medium No. 1. Monkeys reacted irregularly to centrifugates of cultures grown in the more

complex media. The positive tests are significant since they demonstrate toxicity following feeding. Beta-lysin was detected in centrifugates of beta-positive strains in titers ranging from 8 to 256 M.H.D. (for sheep r.b.c.) per ml.

In general growth and hemolysin production decreased with simplification of the media. Qualitative tests suggest a similar decrease in enterotoxin production.

Summary. Production of staphylococcal enterotoxin in chemically defined media is reported. These experiments have not demonstrated specific requirements for enterotoxin production.