STUDIES OF THE RELATIONSHIP BETWEEN THE PRODUCTION OF BACTERIOCINES BY GROUP A STREPTOCOCCI AND ACUTE GLOMERULONEPHRITIS*

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Reeves (1) has reviewed the various theories concerning the actions and functions of the bacteriocines, particularly those of the Gram-negative organisms. Recently, several investigators (2–5) have examined the possible biologic roles of bacteriocines produced by Group A streptococci and enterococci. In 1966 Kuttner (2) studied strains of Group A streptococci recovered from patients with various clinical syndromes, and presented data suggesting that bacteriocine production related to nephritogenicity. Using the 18-Westinghouse strain as an indicator lawn, she tested 94 strains and demonstrated production of bacteriocines by 25% of strains from cases of rheumatic fever, 50% of strains from acute pharyngitis, and 100% of nephritis-associated strains. In addition, she found that approximately 50% of 45 strains from 11 other groups of streptococci produced bacteriocines.

The present study was undertaken to examine further Kuttner's observation that nephritogenicity among Group A streptococci is associated with bacteriocine production. The methods employed were therefore as close as possible to those of her study.

Materials and Methods

Strains of Streptococci.—17 strains were obtained from local clinical infections. These strains included four nephritis-associated strains, 1 from a patient with rheumatic fever, and the remainder from cases of impetigo, pharyngitis, wounds, and infected lacerations. 9 strains were obtained from Dr. Hugh Dillon (Birmingham, Alabama) including four from patients with nephritis and five impetigo strains. Dr. Elia Ayoub of the University of Minnesota provided 31 strains; these were isolated from cases of nephritis and from various other sources. Knowledge of the specific diseases associated with the strains obtained from Dr. Ayoub was withheld until testing was completed. These strains included 25 from cases of nephritis or familial recurrent hematuria and one from a child with the nephrotic syndrome. An additional 16 strains were provided by the late Dr. Alan Siegal of Chicago.

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18-Westinghouse Strain.—The Type 18-Westinghouse strain was supplied by Dr. Max Moody of the National Communicable Disease Center, Atlanta, Georgia. This strain was also supplied by Dr. Moody for Dr. Kuttner's original study.

Media.—Cultures of the lawn strains and test strains were grown in brain-heart infusion broth with 10% horse serum and 0.5% yeast extract. Solid media were made by the addition of 3.0% agar to the above nutrients. Cultures were transferred twice daily in broth with a 5% inoculum and were checked for purity on sheep-blood agar plates.

Techniques.—Overnight cultures of the test strains and the 18-Westinghouse (lawn strain)

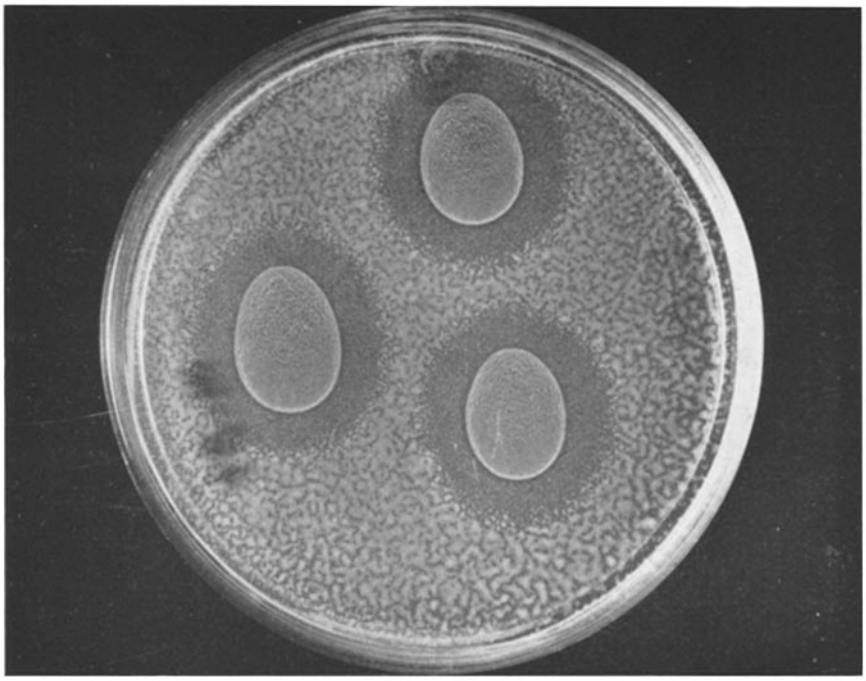

FIG. 1. Bacteriocine lysis of lawn strain by test strain of Group A streptococci (positive test).

streptococcus were transferred to fresh broth tubes and incubated at 37°C for 4–5 hr. After incubation, the 18-Westinghouse strain was diluted 100-fold in broth. 1 ml of this dilution was placed on the agar plates which were gently swirled until the entire surface of the plate was covered evenly. The Petri dish was tipped and excess fluid discarded. The lawn plates were then dried in the incubator for 35–40 min. After drying, one drop of the strain to be tested for bacteriocine production was placed on the surface of the lawn with a Pasteur pipette. Plates were incubated at 37°C overnight (12–16 hr) before being read for bacteriocine production.

Results were interpreted as positive if a clear zone of inhibition appeared around the test strain colony (Figs. 1 and 2). A weakly positive result was defined as a diffuse zone of decreased lawn density or a zone less than 2 mm in diameter. Because variation in inhibition by individual test strains occurred, all strains were tested on at least 5 consecutive days and, in instances of variable results with a single strain, positive results on 50% of trials were considered to indicate bacteriocine production and therefore a positive result.

RESULTS

The results of the testing of 73 strains of streptococci are shown in Tables I and II, and summarized in Table III. All strains were Group A and beta hemolytic with the exception of two Group G strains isolated from patients with nephritis. Six additional strains were tested which were excluded from the results due to difficulties with repeated contamination. Of the 73 strains tested, 39 (53%) produced bacteriocine-like activity at least 50% of the time. There

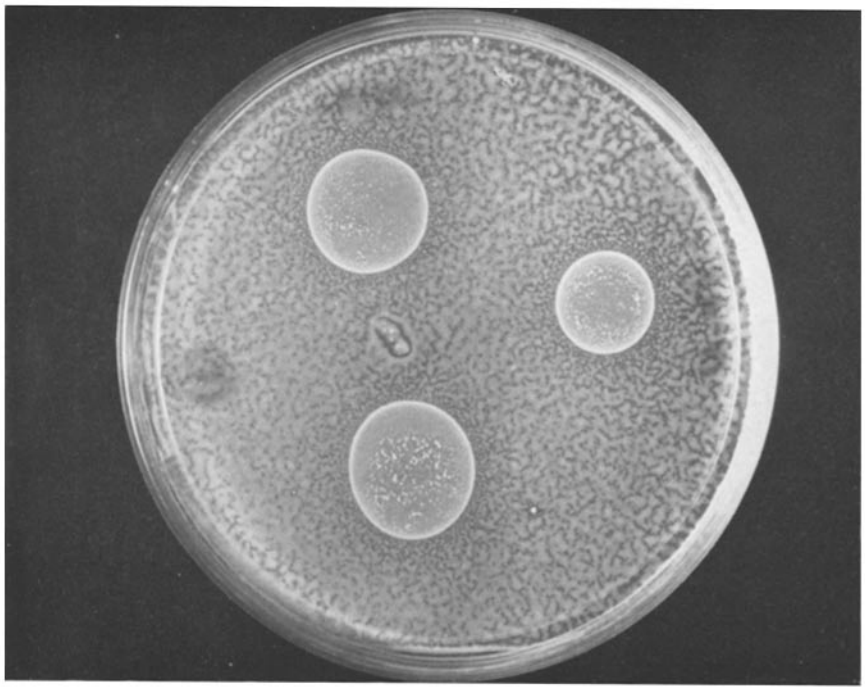

FIG. 2. Lack of bacteriocine lysis of lawn strain by test strain of Group A streptococci (negative test).

were 42 strains of streptococci isolated from patients with the clinical findings of acute glomerulonephritis. 25 (62%) of these 42 strains produced bacteriocines at least 50% of the time. If the additional 12 strains which yielded a positive result on at least one occasion are included, 88% of nephritis-associated strains produced bacteriocines. Among the 24 strains isolated from sources other than cases of nephritis, 54 and 75% produced bacteriocine activity on at least half of the tests and on one trial respectively. The number of rheumatic fever strains was very small. Only one of seven strains displayed bacteriocine production on more than half the tests. An additional three strains produced bacteriocines on one occasion.

TABLE I

Bacteriocine Production by Nephritis-Associated Strains of Streptococci

Strain	Source	Type	Bacteriocine Production	
			No. Positive/ No. of Tests	Result*
A 2	Unknown	12	5/6	+
A 3	Unknown	12	1/6	0
A 4	Pharynx‡	12	0/6	0
A 5	Pharynx	12	0/6	0
A 6	Pharynx	12	2/6	0
A 7	Pharynx‡	12	5/6	+
A 8	Unknown‡	1	3/6	+
A 11	Pharynx	T25/Imp 19	6/6	+
A 12	Pharynx	12	0/6	0
A 13	Unknown	12	0/6	0
A 14	Unknown	49	6/6	+
A 15	Pharynx	12	1/6	0
A 16	Pharynx	12	3/6	+
A 17	Unknown	12	4/6	+
A 20	Unknown	49	4/6	+
A 21	Pharynx	12	3/6	+
A 22	Pharynx	12	1/6	0
A 23	Unknown	12	4/6	+
A 24	Pharynx	12	4/6	+
A 27	Pharynx	U§	6/6	+
A 28	Pharynx	12	6/6	+
A 29	Unknown	12	4/6	+
A 30	Unknown	12	6/6	+
A 31	Pharynx‡	12	2/6	0
D 1	Pharynx	49	1/6	0
D 2	Skin	49	6/6	+
D 3	Skin	2	3/6	+
D 4	Skin	2	3/6	+
S 1	Ear	U	1/6	0
S 3	Skin	U	1/6	0
S 7	Unknown	1	2/6	0
S 9	Skin	49	6/6	+
S 11	Unknown	U	2/6	0
S 13	Skin	U	5/6	+
S 15	Pharynx	Grp G	6/6	+
S 17	Pharynx	Grp G	1/6	0
S 19	Unknown	4	1/6	0
S 21	Unknown	5	0/6	0
M 105	Unknown	U	17/17	+
M 89	Unknown	U	11/11	+
J 12	Skin	31	7/7	+
Z 6	Skin	31	9/9	+

* Designated + if at least half of tests were positive.

‡ Recovered from family contact of patient.

§ U = Unknown or untypable.

TABLE II

Bacteriocine Production by Nonnephritis-Associated Strains of Streptococci

Strain	Source	Type	Disease	Bacteriocine Production	
				No. Positive/ No. of Tests	Results*
A 1	Pharynx	12	Nephrosis	0/6	0
A 10	Pharynx	9	Recurrent Hematuria	0/6	0
A 18	Unknown	12	None	2/6	0
A 19	Unknown	3	None	0/6	0
A 25	Unknown	49	None	6/6	+
A 26	Unknown	24	None	6/6	+
A 32	Unknown	T14/51	None	2/6	0
D 5	Pharynx	6	Impetigo	6/6	+
D 6	Skin	33	Impetigo	6/6	+
D 7	Skin	33	Impetigo	6/6	+
D 8	Skin	11	Impetigo	4/6	+
D 9	Pharynx	4	Impetigo	6/6	+
S 2	Unknown	U‡	Rheumatic Fever	1/6	0
S 8	Unknown	29	Rheumatic Fever	4/6	+
S 12	Unknown	3	Rheumatic Fever	0/6	0
S 16	Unknown	5	Rheumatic Fever	0/6	0
S 18	Unknown	5	Rheumatic Fever	2/6	0
S 20	Unknown	U	Rheumatic Fever	1/6	0
M 422	Unknown	U	Rheumatic Fever	0/13	0
M 14	Pharynx	22	Pharyngitis	8/11	+
M 15	Skin	41	Impetigo	0/10	0
M 39	Wound	31	Wound Infection	17/17	+
M 54	Ear	2	Impetigo	5/5	+
M 152	Pharynx	1	Pharyngitis	1/11	0
M 188	Ear	12	Impetigo	1/12	0
M 83	Sputum	5	Carrier	6/6	+
M 109	Laceration	12	Wound Infection	10/12	+
M 123	Ear	5	Impetigo	0/10	0
J 1	Skin	31	Impetigo	0/6	0
M 23	Skin	31	Impetigo	5/5	+
M 21	Skin	31	Impetigo	0/5	0

* Designated positive if at least half of tests were positive.

‡ U = Unknown or untypable.

TABLE III

Relationship of Bacteriocine Production by Group A Streptococci and Associated Disease Syndromes

Strains tested		Strains producing bacteriocines on repeated testing	
Disease	Number	50% or more tests	At least one test
		%	%
Nephritis	42	60	88
Rheumatic Fever	7	14	57
Miscellaneous	24	54	75
Total	73	53	81

Bacteriocine production appeared to be independent of the type of the organism. Among those strains prefixed by "A" (Tables I and II), 12 of the 19 Type 12 strains produced bacteriocines; 17 of these 19 were isolated either from patients with nephritis or family members. Among these 17 strains 11, (65%) were positive on at least 50% of tests. Examples among Types 49, 2, 5, and 31 also demonstrated that the property of bacteriocine production appears to be independent of type and clinical source.

DISCUSSION

The results of this study suggest that there is no significant difference in bacteriocine production by nephritis-associated (62%) and nonnephritis-associated (54%) Group A streptococci. Subjectively, nephritogenic strains displayed a tendency to produce larger and clearer zones of inhibition. This latter observation may be of some significance in view of the fact that lawn dilutions in the present study were 10^{-2}, whereas Kuttner used lawns diluted to 10^{-4}. Inasmuch as extreme sensitivity of strains to lawn density had been noted from day to day, the results could have been altered by this factor. However, a random group of positive and negative strains was tested at dilutions of 10^{-3} and 10^{-4} and the results were identical to the 10^{-2} lawns.

The experimental technique used in these studies of Group A streptococcal bacteriocines was originally designed in an attempt to reproduce the results of Kuttner's original study. The protocol varied little with that of Kuttner, except that ultraviolet light was never used for decreasing the lawn density and the lawn inoculum was more concentrated. In addition, incubation in 10% CO_2 was not employed because preliminary studies indicated this was not necessary.

The problem of day-to-day variability in bacteriocine production by isolated strains was not discussed by Kuttner, although she did stress the extreme susceptibility of bacteriocine production to multiple growth factors of both the lawns and test strains. She also noted variation depending on the time during the disease at which the organism was isolated. In addition, with regard to Kuttner's use of ultraviolet light, it is known that in some strains of *Escherichia coli*, ultraviolet light is necessary for bacteriocine production. However, evidence for a similar phenomenon in relation to the streptococcus has not been described.

Many theories have been advanced of possible physiological roles played by bacteriocines. It has been suggested (6–8) that the bacteriocines of Gram-negative bacteria may serve as fertility factors by fostering cell-to-cell contact in some enteric bacteria. However, this possible action of bacteriocines would not apply to Gram-positive cocci because of their different modes of reproduction.

Brock and Davie (3) studied strains of *S. zymogenes* which produce a bacteriocine active against lactic acid bacteria and most other Gram-positive bacteria. Variants of these strains have been shown to lose their hemolytic characteristic simultaneously with the ability to produce bacteriocines. A bacteriocine-resis-

tant strain which was nonhemolytic and nonbacteriocinogenic yielded two hemolytic strains following ultraviolet irradiation. These variants were also bacteriocinogenic. Thus, both activities were gained and lost with mutation. In addition, both activities are destroyed by chloroform vapors and antagonized by lecithin, and both are produced and disappear concomitantly. Thus, the relationship of bacteriocine production to certain physiologic or pathogenetic features of a given organism may simply indicate genetic association with other factors and not direct action of the bacteriocine. Brock and Davie (3) also suggested host invasion as a possible role for bacteriocines in the pathogenesis of disease and, working with mixed cultures in vitro, have shown that some bacteriocinogenic strains are able to replace bacteriocine-sensitive strains, even when the latter were originally present at 100 times the concentration of the former. Pohunek (9) suggested that streptococci which produce bacteriocines that are active against vaginal lactobacilli may be able because of this property to invade the vagina and replace the resident bacteria.

In the light of these experiments, it is of interest that Kuttner found variation in Group A streptococcal strains isolated at different times from the same child during uncomplicated pharyngitis treated with penicillin. In some cases the initial culture did not display bacteriocine production. However, the same organism, recovered 10 days later, sometimes showed bacteriocine production. It might be speculated that bacteriocinogenic ability in a fraction of the original invading organism represents a selective process for survival.

In contrast to Kuttner's results, the present study does not suggest a relationship between bacteriocine production among Group A streptococci and the capacity to produce acute glomerulonephritis. The potential for bacteriocine production was uniform throughout, except for the few rheumatic fever strains. Whatever the role of Group A streptococcal bacteriocines may be in infection, it is unlikely that they relate to acute glomerulonephritis.

SUMMARY

The previously reported relationship between nephritogenicity and bacteriocine production among Group A streptococci has been examined. Using techniques comparable to the earlier study, the present study did not reveal any such relationship. Among 73 strains tested, 53% produced bacteriocines; no significant difference was noted between bacteriocines from strains isolated from cases of nephritis and those from a group of strains recovered from patients with other conditions. In addition, no correlation between type and bacteriocine production was observed.

BIBLIOGRAPHY

1. Reeves, Peter. 1965. The Bacteriocines. *Bacteriol. Rev.* **29**:24.
2. Kuttner, Ann G. 1966. Production of Group A streptococcal bacteriocines with special reference to nephritogenic types. *J. Exp. Med.* **124**:279.

3. Brock, T. D., and J. M. Davie. 1963. Probable identity of a group D hemolysin with a bacteriocine. *J. Bacteriol.* **86:**708.
4. Brock, T. D., B. Preacher, and D. Pierson. 1963. Survey of the Bacteriocines of Enterococci. *J. Bacteriol.* **86:**702.
5. Stark, J. M. 1960. Antibiotic Activity of Hemolytic Enterococci. *Lancet.* **1:**733.
6. Hamon, Y., and Y. Peron. 1963. Quelques remarques sur les bacteriocines produites par les microbes gram-positifs. *C. R. H. Acad. Sci. Ser. D.* **257:**1191
7. Hamon, Y., and Y. Peron. 1963. Individualisation de quelques nouvelles familles D'enterobactériocines. *C. R. H. Acad. Sci. Ser. D.* **257:**309.
8. Ozaki, H., and S. Howarth. 1961. Colicine factors as fertility factors in bacteria. *Nature (London).* **190:**986.
9. Pohunek, M. 1961. Streptococci Antagonizing the Vaginal Lactobacillus. *J. Hyg. Epidemiol. microbiol. Immunol. (Prague).* **5:**267.