Acid Phosphatase Production by Mercuric Ion- or Penicillin-Resistant and -Sensitive *Staphylococcus aureus.** (28355)

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The production of acid phosphatase by *Staphylococcus aureus* is one of its biological properties, like production of coagulase and alpha-hemolysin, that has been closely associated with the virulence of the staphylococcus for a given host(1). During quantitative measurements of the increased production of acid phosphatase by coagulase positive strains of *S. aureus,* as compared to coagulase negative strains, it was observed that coagulase positive strains in phage group I produced considerably more acid phosphatase than strains in other phage groups(2). There have been conflicting reports regarding the relationship between acid phosphatase production and the penicillin resistance of *S. aureus* strains. Gillissen and Ruda(3) observed that strains of *S. aureus* that were penicillin-resistant when isolated, produced more than 2.5 times as much acid phosphatase as penicillin-sensitive strains did. On the other hand, 2 other groups of investigators found insignificant differences in the amounts of enzyme produced by penicillin-resistant and -sensitive strains(4-6). None of these studies, however, took into account either the phage type of the culture or the amounts of extracellular acid phosphatase produced, and both of these factors are involved in the total amount of acid phosphatase produced(2). Consequently, the present study was initiated to study the relationship between total acid phosphatase production and penicillin resistance of staphylococcal strains. The resistance of these strains to mercuric ions(7) was also investigated.

Materials and methods. A total of 145 strains of *S. aureus* derived from human sources were used in this study. Six strains were obtained from Henry Ford Hospital, Detroit, through the courtesy of Dr. J. Greer, 10 strains were provided by Dr. K. S. Read of Michigan Department of Health, and the remaining strains were provided by Mr. John Eisses of University Hospital, Ann Arbor. All strains were phage-typed at their source. There was no selection of strains. Because of the much higher incidence of penicillin-resistant strains, however, analysis of these strains was discontinued after a sufficient number had been analyzed. All available strains in phage group I without an 80 in the pattern were analyzed, regardless of their sensitivity to penicillin. Since we had previously noted the instability of acid phosphatase production, all except the Michigan Dept. of Health strains were quantitatively assayed for acid phosphatase activity, using the units and techniques previously described (2), within 14 days after the specimen was taken.

The sensitivity to penicillin of most cultures was determined by the laboratory providing the culture. However, all penicillin sensitivities were redetermined in this laboratory using a modification of a tube assay used at University Hospital(8). A 0.1 ml sample of an overnight trypticase-soy broth (B.B.L.) culture was used to inoculate tubes containing 1.28 and 12.8 units of benzylpenicillin per ml of trypticase-soy broth and the tubes were incubated for 6 hours at 37°C. If the culture did not grow in either tube containing penicillin it was considered to be sensitive to penicillin. If the culture grew in the tube containing 1.28 units but not in the tube containing 12.8 units of penicillin per ml, it was considered to be intermediate in penicillin resistance. If it grew in both tubes, it was considered to be resistant to penicillin. Since 5 units of penicillin per ml is the average attainable serum level of penicillin using a related penicillin assay(8),

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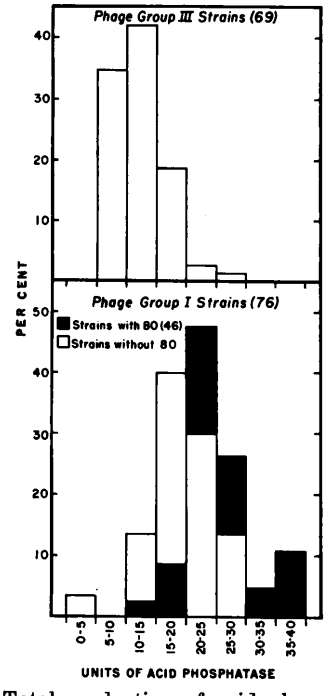

FIG. 1. Total production of acid phosphatase by 145 strains of *Staphylococcus aureus* in phage groups I and III.

our values conform reasonably well to clinical practice.

Resistance to mercuric ions was determined by a method modified slightly from the original(7). One loopful of an overnight culture of each strain in trypticase-soy broth was touched to a defined area on the surface of the medium containing trypticase-soy solidified with 1% agar, and another loopful to a second petri plate containing, in addition, a 1:27,500 dilution of mercuric chloride. The plates were scored for growth after incubation at 37°C for 12-15 hours.

Results. In a previous study, we noted that strains in phage group I lysed by phage 80 appeared to produce more acid phosphatase than other strains in this phage group without an 80 in the phage pattern(2). Since a comparison of phosphatase production by a population of strains differing only in their degree of penicillin resistance was to be made, it became necessary to establish with certainty whether there were 2 sub-groups in phage group I, as far as acid phosphatase production was concerned. Therefore, 76 strains in phage group I were analyzed, including 30 strains that did not contain an 80 in their phage pattern (Fig. 1, Table I). The mean units of phosphatase produced by strains with an 80 in their pattern, regardless of their penicillin resistance, was 25.5 with a standard deviation of $\pm$ 5.8, while the corresponding figures for all strains without an 80 was 19.1 $\pm$ 4.3. These data were submitted to an analysis of variance(9) and the results indicated a variance ratio of 26.24. The chance of obtaining a variance ratio this high if there were no true differences between the groups would be less than 1 in 1000. This being a highly improbable occurrence, the alternate hypothesis, that there is a true difference between the groups, was accepted. Consequently, the phage group I strains without an 80 were treated as a separate population as far as acid phosphatase production was concerned, and the remaining strains were grouped according to their sensitivity to penicillin.

A series of 69 strains in phage group III differing with regard to their penicillin sensitivities was also studied, the mean value for all strains being 12.0 with a standard deviation of $\pm$ 4.4 units (Fig. 1, Table II). After grouping these strains according to their penicillin resistance, an analysis of variance of the units of phosphatase produced by strains in the two populations selected was performed. The results indicated that there were no significant differences ($P>.05$) in phosphatase production among the 3 groupings of each population separated only on the basis of their sensitivity to penicillin.

Moore(7) reported that phage group I strains could be subdivided according to their sensitivity to Hg^{++}. He found that phage group I strains without an 80 were sensitive to Hg^{++} while 92% of phage group I strains lysed by phage 80 were resistant to Hg^{++}. Similar results were described by Smith(17) and Green(21) but not by Váczi *et al.*(16). Some of the variations in the percentages of Hg^{++}-resistant cultures found in different laboratories are probably due to the use of dissimilar media(17,21) and to varying percentages of antibiotic-resistant cultures. The

TABLE I. Acid Phosphatase Production and Mercuric Ion Resistance in Relation to Penicillin Resistance by 76 Strains of *Staphylococcus aureus* in Phage Group I.

	Strains lysed by phage 80			Strains not lysed by phage 80		
	Resistant to penicillin	Intermediate resistance to penicillin	Sensitive to penicillin	Resistant to penicillin	Intermediate resistance to penicillin	Sensitive to penicillin
Phage pattern*	80/81 (22); 52A/80/81 (6)	80/81 (4); 52A/80/81 (2); 80 (2)	80/81 (3); 52A/80/81 (2); others (5)			52A/79 (14); 29 (8); others (5)
Total strains	28	8	10	1	2	27
Avg units acid phosphatase	25.8	24.9	24.9	21.7	21.1	18.7
Range, units acid phosphatase	17.9–40.0	18.9–35.9	11.4–39.9		15.3–26.9	4.6–29.5
Hg^{++}-resistant strains	26	5	2	0	0	0

* Number in parentheses indicates No. of strains tested with that phage pattern. Only patterns occurring 2 or more times are listed.

sensitivity of all the strains used in the present study was determined, and the results are shown in Tables I and II.

All 30 strains in phage group I not containing an 80 in the pattern were found to be sensitive to Hg^{++}. The Hg^{++}-resistance of the remaining strains in this phage group and phage group III was observed to be generally related to the penicillin-resistance of the strains, confirming the findings of others(7, 14,16,21,22). The analysis of variance of the variability of acid phosphatase production by Hg^{++}-resistant and -sensitive strains in either phage group III or the phage group I subgroup lysed by phage 80 indicated that there were no true differences among the Hg^{++}-resistant and -sensitive strains in either group ($P>0.05$).

Discussion. The results of this study confirm and extend previous findings which indicated that the amount of acid phosphatase produced by coagulase-positive strains of *S. aureus* is correlated with the phage type of the strain(2). The previous suggestion that the susceptibility to phage 80 constituted a basis for the separation of phage group I into 2 sub-groups was found to be true, the strains not susceptible to phage 80 producing 25% less phosphatase than the other phage group I strains.

Statistical analysis of the results also led to the conclusion that there were no significant differences in the amounts of acid phosphatase produced by strains of phage group III or phage group I containing an 80 whether the strains were resistant or sensitive to penicillin (Table II). This finding supports the conclusions of Markov *et al.*(4, 5) and Akatov and Lebedeva(6) and is in disagreement with the findings of Gillissen and Ruda(3). Experiments in which phosphatase production was measured in mutants differing from the parent strain in penicillin resistance(3-6,10) were not performed in the present work because it is known that some properties of strains selected for penicillin-resistance *in vitro* differ from those of naturally occurring penicillin-resistant strains (11).

The methods used for the acid phosphatase

TABLE II. Acid Phosphatase Production and Mercuric Ion Resistance in Relation to Penicillin Resistance by 69 Strains of *Staphylococcus aureus* in Phage Group III.

	Resistant to penicillin	Intermediate resistance to penicillin	Sensitive to penicillin
Phage pattern*	77 (6); 47/53/54/83 (3); 6/47/53/54/75/77/83 (2); 47/53/54/75/77/83 (2); others (11)	7 (11); 77 (7); others (9)	7/77 (3); 6/47/53/54/75/77/83 (3); others (12)
Total strains	24	27	18
Avg units acid phosphatase	12.2	12.2	11.5
Range, units acid phosphatase	6.5–28.4	7.4–18.3	5.3–24.7
Hg^{++}-resistant strains	13	2	1

* Number in parentheses indicates No. of strains tested with that phage pattern. Only patterns occurring 2 or more times are listed.

assays in all of these studies were different. Still, it is unlikely that these differences alone could account for the findings of Gillissen and Ruda(3). Although the phage patterns and/or details of the clinical sources of the strains used in the various studies were not reported(3-6), increased phosphatase production might be realized if, for example, most of the penicillin-resistant strains were in phage group I with an 80 while most of the pencillin-sensitive strains were in phage group II or III(2). Different hospitals exhibit different patterns of penicillin-resistance and in some, phage group III strains predominate while in others phage group I strains predominate. Even in the same hospital, the pattern varies with time and source of strains(11). For example, the biochemical activity of strains isolated from pus and sputum are greater than those isolated from nasopharynx of normal individuals(12).

The practical significance of the Hg^{++}-resistance of "epidemic" strains of *S. aureus* is not known(13,15,16). It was probably only a coincidence that susceptibility to phage 80 differentiated phage group I strains into 2 sub-groups which were also relatively homogenous with regard to Hg^{++} resistance and phosphatase production. For example, many coagulase-negative strains tested were also Hg^{++}-resistant(17) although they produce, on the average, very little phosphatase (2).

The significance of the observations that different levels of acid phosphatase are produced by particular phage groups, with strains of the "80/81 Complex" producing the highest levels and coagulase-negative strains producing the lowest levels, remains to be ascertained. Although acid phosphatase can potentially exert a strong influence on the course of metabolic reactions in a cell(18), no major differences were found in the proportions of glucose metabolized aerobically *via* the Embden-Meyerhof and hexosemonophosphate pathways in staphylococci, regardless of their phage type, acid phosphatase production, or degree of penicillin resistance(19). It should be recalled, however, that "a predominating role in the production of staphylococcal disease cannot be assigned to any single virulence factor. The capacity of a strain to induce infection is derived from the sum total of the properties at its command"(20).

Summary. The average total amount of acid phosphatase produced by 46 strains of *S. aureus* in a sub-group of phage group I lysed by phage 80, was found to be 25% greater than the average of 19.1 units for 30 strains in the sub-group not lysed by phage 80. In turn, both of these phage group I sub-groups produced more enzyme than the 69 phage group III strains, for which the mean was 12.0 units. There were no significant differences in the amount of acid phosphatase produced by strains in phage group I without an 80 in their pattern or in phage group III when the strains within each of these 2 groups were segregated according to either penicillin-resistance or mercuric-ion resistance. The percentage of strains resist-

ant to mercuric ions was much greater among penicillin-resistant strains than in penicillin-sensitive strains.

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Localization of the Site of Action of Triamterene Diuretic.* (28356)

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The pteridine compound triamterene has been reported to cause a sodium diuresis in rats, dogs(1) and man(2,3,4). The drug not only antagonizes the sodium retaining effect of 9α fluoro hydrocortisone and dl aldosterone (2,3), but also produces a sodium diuresis in adrenalectomized rats and dogs(1,4), presumably exerting its effect directly on the renal tubule by interfering with sodium reabsorption. The following studies were done to localize the site of action in the kidney of this agent using the stop flow method(5, 6).

Materials and methods. Eleven dogs weighing between 11-20 kg, anesthetized with pentobarbital 30 mg/kg body wt., were infused into the jugular vein at a rate of 10 ml/min with the following: mannitol, 20 g/100 ml; NaCl, 0.4 g/100 ml; and creatinine, 0.12 g/100 ml, all dissolved in Ringer's solution. The left ureter was exposed through a flank incision and urine collected from a polyethylene catheter pushed into the renal pelvis and tied securely in place. Eight minute ureteral occlusions were used. Mean aortic pressure was measured with a mercury manometer. Triamterene† dosage consisted of a single i.v. injection of 3 mg/kg body wt. followed by a sustaining infusion of 3 mg/kg body wt/hr incorporated into the mannitol infusion.

In 4 dogs a control occlusion was done just before drug administration and a second occlusion was done 45 minutes after the triamterene infusion was initiated. In 7 dogs an initial occlusion was done after 30 minutes of drug administration and was followed

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