trinsic MSH activity as shown recently.

1. Sulman, F. G., and Eviator, A., *Acta Endocrinol.,* 1956, v23, 120.
2. Bell, P. H., *J. Am. Chem. Soc.,* 1954, v76, 5565.
3. Dixon, H. B. F., *Biochim. et Bioph. Acta,* 1956, v19, 392.
4. Harris, J. I., and Roos, P., *Nature* (Lond.) 1956, v90, 178.
5. Geschwind, I. I., Li, C. H., and Barnafi, L., *J. Am. Chem. Soc.,* 1956, v78, 4494.
6. Lee, T. H., and Lerner, A. B., *J. Biol. Chem.,* 1956, v221, 943.
7. Landgrebe, F. W., Ketterer, B., and Waring, H., in *The Hormones,* G. Pincus and K. V. Thiman, ed., Academic Press, N. Y., vIII, chp. ix, 1955.
8. Lerner, A. B., and Lee, T. H., *J. Am. Chem. Soc.,* 1955, v77, 1066.
9. Porath, J., Roos, P., Landgrebe, F. W., and Mitchell, G. M., *Biochim. et Bioph. Acta,* 1955, v17, 598.
10. Raben, M. S., Rosenberg, I. N., and Astwood, E. B., *Fed. Proc.,* 1952, v11, 126.
11. Deutsch, S., Angelakos, E. T., and Loew, E. R., *Endocrinology,* 1956, v58, 33.
12. Emmens, C. W., *Principles of Biological Assay,* Lond., Chapman and Hall, 1948.
13. Astwood, E. B., Raben, M. S., Payne, R. W., and Grady, A. B., *J. Am. Chem. Soc.,* 1951, v73, 2969.
14. Sulman, F. G., *J. Clin. Endocrinol. and Metab.,* 1956, v16, 755.
15. Lerner, A. B., Shizume, K., and Bunding, I., *ibid.,* 1954, v14, 1463.

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Correlation of Pigmentation and Bacteriophage Sensitivity in Coagulase Positive Staphylococci. (23578)

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In the past decade several investigators have demonstrated correlations between bacteriophage type of staphylococci and antibiotic resistance, habitat or clinical disease. Sensitivity to bacteriophage seems prevalent in the coagulase positive staphylococci, although Rountree(1) and Rippon(2) have reported phages that attack coagulase negative strains. The typing phages are generally limited in range to the coagulase positive staphylococci, with 60-70% of strains typable by test dilutions of phage and the majority of the remaining strains showing some degree of sensitivity to the undiluted phages. Attempts to correlate phage sensitivity with properties other than those mentioned above have generally been unsuccessful. The present report describes the association between pigmentation and phage sensitivity first observed during phage typing of staphylococci isolated from clinical specimens.

Methods. Two series of staphylococcal cultures* freshly isolated from routine clinical specimens were streaked, as received, on plates of Staphylococcus Medium No. 110(3) and incubated at 30°C for 2 days. Typical isolated colonies were subcultured on trypticase soy agar slants for further study. The original Medium No. 110 plates were scored for pigment production and incubated an additional 2 days at room temperature (25°C). In scoring, "pigmented" included the range from deep orange to pale orange or yellow, and "white" included the various shades of white and off-white. The additional incubation period enabled us to observe a number of mixtures in which slight differences in pigmentation emerged on the third or fourth day. The 2 components of such mixtures were treated as separate strains rather than color variants if they also differed in phage pattern or other properties than pigment. Tests for coagulase production were performed by the hospital laboratory and their findings were rechecked by the slide method with diagnostic plasma (Warner-Chilcott). Mannitol fermentation and proteolytic activity on Staphylococcus Medium No.

* Obtained from Parkland Memorial Hospital and Baylor University Hospital, Dallas.

TABLE I. Phage Sensitivity of 143 Strains of Staphylococci (Selected Series).

		Total	Phage sensitive			Phage insensitive
			Typable	Nontypable	Total	
Pigmented	coagulase +	72	40	26	66	6
"	" −	7	0	0	0	7
White	" +	10	1	1	2	8
"	" −	54	0	0	0	54

110 were observed in the first series of strains and hemolysis of rabbit blood agar plates was evaluated in the second series. The phage typing methods of Blair and Carr(4) were employed throughout this study, with a series of 25 typing phages kindly supplied by Dr. John E. Blair. In the first series, all strains that were not typable with the routine test dilutions of phage were retested with undiluted typing phage. This procedure was simplified in studying the second series of strains by applying a mixture of undiluted phage at the same time that the routine test dilutions were spotted. The mixture used was composed of equal volumes of 3 different typing phages (79, 3B and 54) representing the 3 major groups. Phage sensitivity, in addition to typability, includes any visible reduction in bacterial growth produced by undiluted phage suspensions, which may or may not represent true lysis (Williams and Rippon(5)).

Results. The initial series of 143 recently isolated strains was selected to include all combinations of pigment and coagulase production together with any unusual or aberrent strains. Results are summarized in Table I. A correlation between pigmentation, coagulase production and sensitivity to bacteriophage seems apparent since 66 of 72, or 93%, of the pigmented coagulase positive strains were sensitive to phage but only two of the ten white coagulase positive strains reacted to the typing phages. Coagulase negative strains were insensitive to the phages. Gelatin liquefaction and mannitol fermentation did not correlate with phage sensitivity as did pigmentation.

Laboratory stock strains of staphylococci, cultivated and stored for two years or longer, were next studied. These included the propagating strains for the typing phages plus a series of eight strains from the departmental collection. The correlation of phage sensitivity with pigment was not as marked as with the freshly isolated strains since several of the laboratory strains, originally coagulase positive and pigmented, had become altered in either or both of these properties during maintenance and storage without loss of sensitivity to phage. No attempts were made to enhance these properties by cultural methods and they were tested in the same manner as the freshly isolated clinical strains. One propagating strain, P.S. 73, was originally isolated as a phage sensitive coagulase negative component of a mixture of coagulase positive and negative cells (Rippon(2)).

These results with the laboratory strains suggested that phage sensitivity is a relatively stable property of staphylococci. A number of hospital strains of the first series were therefore retested after they had been stored 5 to 8 months. Occasional changes in pigmentation and coagulase production were observed, but no alteration in phage sensitivity, although two typable strains were modified in their lytic pattern. The most striking change occurred in the group of 8 white coagulase positive strains that were insensitive to phage. Seven of these were coagulase negative when retested, suggesting that strains with this set of characteristics represent a markedly unstable group.

A second, and larger series of strains from clinical sources was next examined for a more critical confirmation of the observed relationship between phage sensitivity and pigmentation. This series also provided an opportunity to evaluate a simpler and more economical test for phage sensitivity. The strains in this series were unselected, except for coagulase production and all coagulase positive strains were tested as received from the hospital. Undoubtedly there is some duplication due to the prevalence of a number of "hospi-

TABLE II. Phage Sensitivity of 265 Strains of Coagulase Positive Staphylococci (Unselected Series).

	Total	Phage sensitive			Phage insensitive
		Typable	Nontypable	Total	
Pigmented	247	133	111	244	3
White	18	3	2	5	13

tal strains", but close to 40 different lytic patterns are represented among the typable strains, and the highest number of strains with the same pattern was a total of 19 sensitive to phage 44A, which was found to possess a broad host range.

The test for sensitivity to phage was simplified by using a pool of 3 undiluted typing phages, applied in a single drop at the same time as the routine test dilutions of typing phage. Hood(6) reported the successful use of pools of phage for typing, and we observed in preliminary tests that undiluted suspensions of phages 79, 3B and 54 when pooled together did not show evidence of antagonism, although they represent different major groups (I, II and III). The results, summarized in Table II, would even indicate a synergistic action of the pooled phages, in view of the high percentage of phage sensitivity observed. In this unselected series over 98% of the pigmented coagulase positive strains were phage sensitive while only 5 of 18 white strains were sensitive.

Phage sensitivity is not as highly correlated with hemolysin production as it is with pigmentation and coagulase production. Sixteen strains in this series were nonhemolytic on rabbit blood agar, including 14 in the pigmented phage sensitive group and 2 in the white phage insensitive group.

Additional studies on the mode of action of the high titer phage pool have been undertaken. One group of strains undoubtedly is sensitive to infection and lysis, either by the typing phage or by contaminants derived from lysogenic propagating strains (Rippon(2)). Another group is "inhibited" by concentrated phage which do not multiply on the cells (Williams and Rippon(5)). Studies of this "inhibition phenomenon" indicate appreciable reduction in the number of viable cells. The exact mechanism of the killing is not yet understood but probably involves neither bacteriocin-like particles nor a phage-enzyme interaction as observed by Ralston *et al.*(7).

Discussion. Several reasons may explain the failure of previous workers to find correlation between phage sensitivity and pigmentation. One factor might have been the use of laboratory stock strains of staphylococci in earlier studies, since the highest correlations were obtained with strains freshly isolated from clinical specimens. Another might have been inclusion of strains from animal sources, since the typing phages have been developed primarily for the study of strains of human origin. Levy, Rippon and Williams(8), for example, have observed that 30% of coagulase positive staphylococci from animal sources were not sensitive to typing phages, and 40% of the animal strains were white or off-white. No correlation between phage sensitivity and pigmentation was observed in their study. Another factor involves the method of testing for coagulase production. The present studies and those of Finkelstein and Sulkin† indicate that the few strains which are coagulase negative by the slide test and positive by the tube test are not sensitive to phage. In the strict sense the correlation is between pigmentation, phage sensitivity and "bound" coagulase(9).

Some taxonomic considerations are involved in this study. The work of Chapman and his associates(10) would imply a continuous gradation of staphylococci from the deeply pigmented, hemolytic, mannitol and coagulase positive pathogenic strains to the white, usually non-hemolytic, mannitol and coagulase negative strains of feeble pathogenicity. The results reported in this study indicate that sensitivity to the typing phages is characteristic of the first extreme, is absent in the other extreme and occurs with limited frequency among the intermediate forms. Sensitivity to

† *J. Bact.*, in press.

typing phages is a relatively stable characteristic. It would also seem that white, phage insensitive strains are relatively unstable and tend to lose the ability to produce coagulase.

No attempt was made to correlate phage sensitivity with potential pathogenicity, although from the relationship to pigmentation and coagulase production some degree of association does exist. Further evaluation would be required before phage sensitivity could replace the commonly accepted criteria for pathogenicity.

A phage sensitivity test, employing a drop of pooled undiluted phages would be a simple, economical and expedient procedure for routine testing of staphylococci, but at present seems limited to use as an adjunct to phage typing procedures. By itself it may serve as an index of typability since all typable strains were sensitive to the pooled phage with but one exception (lytic pattern 52 44A 81). The combination of phages in the pool was selected empirically; other combinations may serve equally well.

Summary. 1) A high correlation was observed between pigmentation, bound coagulase and sensitivity to typing phages in staphylococcal strains freshly isolated from routine clinical hospital specimens. 2) A simple method for determining phage sensitivity using a pool of 3 undiluted typing phages is described and possible applications are discussed.

1. Rountree, P. M., *J. Gen. Microbiol.*, 1949, v3, 164.
2. Rippon, J. E., *J. Hygiene*, 1956, v54, 213.
3. Chapman, G. H., *J. Bact.*, 1946, v51, 409.
4. Blair, J. E., and Carr, M., *J. Inf. Dis.*, 1953, v93, 1.
5. Williams, R. E. O., and Rippon, J., *J. Hygiene*, 1952, v50, 320.
6. Hood, A. M., *ibid.*, 1953, v51, 1.
7. Ralston, D. J., Baer, B. S., Lieberman, M., and Krueger, A. P., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 502.
8. Levy, E., Rippon, J. E., and Williams, R. E. O., *J. Gen. Microbiol.*, 1953, v9, 97.
9. Duthie, W. S., *J. Gen. Microbiol.*, 1954, v10, 427.
10. Chapman, G. H., Berens, C., Nilson, E. L., and Curcio, L. G., *J. Bact.*, 1938, v35, 311.

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Isolation and Characterization of Two Hemoglobins Found in the Turtle, *Pseudemys scripta elegans.* (23579)

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Evidence has accumulated which indicates that a correlation exists between the taxonomic relationships of animals and the chemical and physiological properties of their hemoglobins(1). With such evidence as a background, an attempt was made to correlate paper electrophoretic migration of hemoglobins with phylogeny in Amphibia and Reptilia(2). Of the reptilian hemoglobins studied those of turtles were found to be especially interesting. Hemolysates of turtles included in families Chelydridae and Kinosternidae have one hemoglobin; those included in Emydidae, Trionychidae and Testudinidae have at least 2 hemoglobins. When the 2 hemoglobins of such turtles were fractionated by means of paper electrophoresis a trail of pigment marked the area on the paper strip between the slow and fast migrating hemoglobins. This trailing suggested that an interaction was occurring between the 2 hemoglobins. Because of these interesting properties, we decided to investigate somewhat more thoroughly the hemoglobins of one of the species in which we found 2 hemoglobins, the red eared slider turtle, *Pseudemys scripta elegans.*

Materials and methods. Turtles used in this study were captured in the New Orleans area. In captivity they were kept in con-