

gives the pH values required by the different indicators for transformation to the alkaline form.

Add 0.5 cc. of the dilute indicator solution to 10 cc. of the unknown, place in left cup and set at 10 mm. The right cup containing the standard is adjusted until the fields match. The reading of the standard multiplied by 10 gives the per cent alkaline form of the indicator in the unknown. pH values are readily obtained from a dissociation curve of the indicator. The standard solutions should be made up fresh every few determinations since the strong colorimeter light seems to affect the intensity of color.

The method is sensitive, under favorable conditions, to changes of ± 0.02 pH. However the absolute accuracy of the procedure depends upon a correct evaluation of pK' in the solution being investigated. Also it is essential to check the pK' for each new lot of indicator used. The value obtained is constant over the main pH range of the indicator, which is approximately 1.1 pH units for the dyes studied. A wider range can be utilized if the changing values for pK' are employed in plotting the extremities of the dissociation curve.

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Note on the Susceptibility of Certain Strains of Hemolytic Streptococcus to a Streptococcus Bacteriophage.

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A bacteriophage active against certain mucoid strains of hemolytic streptococci, originally isolated by Clark and Clark¹, was used in tests with a series of 119 strains of hemolytic streptococci which had been obtained from a variety of sources, some pathological and some normal. Of these, 37 strains were isolated from human sources, including 5 throat cultures from normal persons; 42 strains were recovered from mastitis in cows and from milk and cheese; 18 from lymphadenitis in guinea pigs; 7 from spontaneous rabbit infections with hemolytic streptococcus; 7 from pleuro-pneumonia, chronic endometritis and distemper in horses; 2 from pneumonia in

¹ Clark, P. F., and Clark, A. S., *J. Bact.*, 1926, **11**, 89; *Proc. Soc. Exp. Biol. and Med.*, 1927, **24**, 635.

foxes; 3 from streptococcus infections in swine; and 3 from a disease known as "slipped tendon" in chickens.

TABLE I.
Susceptibility of Strains of Hemolytic Streptococcus to Streptococcus Bacteriophage (Clark).

Source of strains	Susceptible		Insusceptible		Total
	No.	Serological group	No.	Serological group	
Human	6*	A	31	A, B	37
Bovine	14	C, E	20	A, B	34
Cheese			8	D	8
Guinea pig	18	C			18
Rabbit	5	C	2	A, B	7
Horse	6	C	1	C	7
Fox	2	C			2
Swine	2	C	1	C	3
Chicken	3	C			3
Total	56		63		119

The tests were performed by adding 1 cc. of a rapidly growing 2-hour broth culture to 1 cc. of bacteriophage. The phage was either the Berkefeld filtrate of a lysed culture or the unfiltered, crystal clear, lysed culture. Controls of culture plus broth were also included. The tubes were incubated for 1 to 18 hours at 37°C.; but lysis was usually complete in one hour if it occurred at all. Overgrowth of resistant forms frequently occurred if the tubes were incubated over night.

* The strains of human origin were only slightly susceptible to bacteriophage.

The table shows the number of strains of hemolytic streptococcus which were susceptible to the Clark bacteriophage and the number insusceptible, as well as the source of the cultures. All susceptible strains, except the 6 of human origin, were strikingly affected by the action of this phage, and were further characterized by the formation on blood agar plates of mucoid colonies with exceptionally large zones of hemolysis. The susceptible human strains, on the contrary, did not form mucoid colonies, and were only slightly sensitive to the action of the phage and with much less regularity than the strains of animal origin.

The addition of bacteriophage to susceptible cultures did not lead to the formation of plaques or "moth-eaten" colonies. This observation is in accord with the findings of others who have worked with bacteriophage active against hemolytic streptococci isolated from guinea pigs, and have rarely or never found such colonies.^{1, 2, 3}

Without the addition of bacteriophage, however, one of the strains in the present series occasionally showed "moth-eaten" colonies. Some further suggestion of the association of a lytic

² Cunningham, J. S., *J. Inf. Dis.*, 1929, **45**, 474.

³ Colvin, M. G., *J. Inf. Dis.*, 1932, **51**, 17.

agent with the susceptible cultures, except those of human origin, was also indicated by the formation of transparent colonies as the cultures aged, although the complete disappearance of the colonies from plates was not observed. The presence of a lytic principle in these cultures was also suggested by the fact that, after a few rapid 2-hour subcultures in broth, several strains underwent spontaneous lysis, which was transmissible in series and caused lysis of broth cultures of other strains without the addition of bacteriophage from any other source. These cultures were of guinea pig origin, but no systematic investigation was undertaken to determine the possible association of spontaneous lytic properties with strains isolated from other animals.

The bacteriophage, used in the tests recorded here, was obtained by Clark and Clark from sewage, and was highly active against 2 mucoid strains of hemolytic streptococcus isolated by them from spontaneous lung infections in a rabbit and a guinea pig. Because of an unpublished observation of Dr. A. G. Kuttner, made in 1926, when the Clark phage was brought into this laboratory, that all strains of hemolytic streptococci isolated by her from spontaneous guinea pig infections were lysed by this bacteriophage, the cultures in the present series were tested for susceptibility to the same lytic agent, which was recovered from a Berkefeld filtrate kept sealed during the intervening years.

Of 119 strains of hemolytic streptococci isolated from various sources, 56 were found susceptible to lysis by bacteriophage. Fifty of these strains were especially susceptible to phage and were all derived from lower animals. These observations were made in connection with a study dealing with a serological differentiation of human and other groups of hemolytic streptococci. While no definite relationship was found to exist between the serological specificity and the susceptibility of these organisms to bacteriophage, it is, nevertheless, interesting that, of the 56 strains susceptible to phage, 50 fell into 2 sharply defined serological groups: 47 in group C and 3 in group E. All 50 of these were of animal origin. The letters in the table indicate the serological groups to which the strains belonged, and the basis of that grouping will be the subject of a separate paper.