

methemoglobin by a trace of sodium nitrite. This produced methemoglobin without laking the blood and interfered with the decolorization reaction. Age of the red cells as indicated by reticulation was also eliminated as a factor since reticulated cells are present in greatest number at birth.

Since methylene blue is converted into its leuco-derivative by reduction, restoration of the color should be accomplished by oxidation. The clear supernatant layer, however, could not be oxidized chemically or by vigorous shaking to the original blue color. On the other hand, the dye was associated with the cellular layer for a blue color developed in the supernatant layer, after the addition of dye-free sodium citrate to the centrifuged cells at 37° C.

These studies show that the red blood cells of the young infant and animal possess the property of reacting with methylene blue which is modified during growth. Many of the phases of this reaction suggest a relationship to the adsorption phenomenon.

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Bacterial Variation in *Pneumococcus* and *Streptococcus* Hemolyticus.

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Arkwright¹ originally described 2 variant forms of members of the colon-typhoid-dysentery group which he designated by the terms S (smooth) and R (rough). Recently evidence has accumulated² to show that members of the colon-typhoid-dysentery group, as well as many other bacterial species, possess a third and distinct variant form, the M (mucoid). Organisms in the mucoid phase possess distinct capsules and form large mucoid colonies.

Griffith³ described 2 variant forms of pneumococcus and, adopting Arkwright's terminology, designated them as S (smooth) and R (rough). A new variant form of pneumococcus was recently described by the author.⁴ This new variant form was shown to be

¹ Arkwright, J. A., *J. Path. and Bact.*, 1921, **24**, 36.

² Hadley, P., personal communication.

³ Griffith, F., *Rep. Pub. Health and Med. Subj., Ministry of Health*, No. 18, 1923, 1.

⁴ Dawson, M. H., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 806.

strikingly different from the S and R forms described by Griffith.

A comparison of the characteristic features of the 3 chief variant forms of members of the colon-typhoid-dysentery, etc., group on the one hand, and of pneumococcus on the other hand, reveals a striking parallelism. Thus, (1) the M (mucoid) form of the colon-typhoid-dysentery, etc., group corresponds with the S (smooth) form of pneumococcus; (2) the S (smooth) form of the colon-typhoid-dysentery, etc., group corresponds with the R (rough) form of pneumococcus; and (3) the R (rough) form of the colon-typhoid-dysentery group corresponds with the newly described variant form of pneumococcus.

The striking inconsistency in terminology employed to describe corresponding variant forms of different bacterial species is obviously confusing and should be rectified if possible. Before proposing a radical change in the currently accepted terminology of pneumococcal variants a study of bacterial variation in *Streptococcus hemolyticus* was undertaken in order to determine whether similar variant forms could be demonstrated in this species.

Variant forms of *Streptococcus hemolyticus* have been described by various workers (Cowan,⁵ Todd,⁶ Eagles,⁷ Andrewes,⁸ Delves,⁹ Cooley,¹⁰ etc.) but a parallelism in the dissociative behavior of this organism and that of pneumococcus has not been demonstrated.

Isolated colonies of 7 strains of *Streptococcus hemolyticus* were allowed to age at 37° C. on blood-agar plates for periods of one to 2 weeks. At the end of this period the roughest marginal areas were picked and transferred to fresh blood-agar plates. This process was repeated several times and finally resulted in the development of extremely rough colonies which closely resembled those described as characteristic for the new variant of pneumococcus. The morphology of the individual organisms and their manner of growth in liquid media also corresponded closely with that of the newly described variant of pneumococcus. It therefore appears that *Streptococcus hemolyticus* produces a variant form which corresponds with the newly described variant form of pneumococcus.

If the phenomenon of bacterial variation in *Streptococcus hemo-*

⁵ Cowan, Mary L., *Brit. J. Exp. Path.*, 1922, **3**, 187; 1923, **4**, 241; 1924, **5**, 226.

⁶ Todd, E. W., *Brit. J. Exp. Path.*, 1927, **8**, 289; 1928, **9**, 1.

⁷ Eagles, G. H., *Brit. J. Exp. Path.*, 1928, **9**, 300.

⁸ Andrewes, F. W., and Christie, Ethel M., *Med. Res. Council*, 1931, Special Series, 169.

⁹ Delves, Edna, *J. Infect. Dis.*, 1932, **50**, 350.

¹⁰ Cooley, L. E., *J. Infect. Dis.*, 1932, **50**, 358.

lyticus corresponds with that exhibited by pneumococcus mucoid and capsulated phases of *Streptococcus hemolyticus* should also be demonstrable. Numerous references to the occurrence of capsulated and mucoid forms of *Streptococcus hemolyticus* occur in the literature. Observations recently made in this laboratory have shown that mucoid variants of *Streptococcus hemolyticus* occur much more frequently than had heretofore been supposed. It has been shown that freshly isolated strains of *Streptococcus hemolyticus* grown on neo-peptone blood agar plates frequently produce large mucoid colonies. The peritoneal exudates of mice injected with such cultures frequently reveal the presence of capsulated organisms. Over 50 such strains have recently been identified by the author. Furthermore, mucoid forms have been obtained from non-mucoid (smooth or "glossy") stock cultures by growth on neopeptone blood agar plates under suitable conditions. Further studies on the virulence and specificity of the mucoid form of *Streptococcus hemolyticus* and the relationship which it bears to the "matt" form of Todd will shortly be reported.

In conclusion evidence has been presented which suggests that *Streptococcus hemolyticus* possesses 3 chief variant forms, which correspond closely with the 3 chief variant forms of Pneumococcus. Furthermore, these 3 chief variant forms of *Streptococcus hemolyticus* and pneumococcus correspond closely with the 3 chief variant forms of a wide variety of other bacterial species. However, at the present time there exists a striking inconsistency in the terminology employed to describe 2 of the 3 chief variant forms of pneumococcus. The third chief variant form of pneumococcus has not yet received an appropriate designation.

Evidence is rapidly accumulating to show that the phenomenon of bacterial variation in a wide variety of bacterial species fits into a more or less orderly pattern. The adoption of a uniform terminology to describe the chief phases in that general pattern would assist in the further study of the many problems in bacterial variation that await solution.