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Acid Agglutination Zones of Typical and Variant Pneumococci.

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The present basis for differentiation between typical and variant forms of pneumococci involves a subjective opinion of colony morphology. It became desirable, in the course of our studies, to develop a method for this differentiation depending entirely on some objective physical measurement. Such a method has been found to be available by utilization of the phenomena of agglutination of organisms in solutions of varying acidity. This method has been of service in recognition of variants of other bacterial species.[1, 2] Gillespie[3] has studied the acid agglutination zones of several types of typical pneumococci and found them to be similar.

The organisms to be tested were grown in pneumococcus broth, the period of incubation being kept as short as consistent with adequate growth, usually about 8 hours. The organisms were packed by centrifuging and washed 3 times with saline. After the final centrifugation, they were restored to original volume with saline. 0.5 cc. quantities of this suspension were added to a similar volume of standard buffer solution of the desired acidity, incubated for 3 hours at 56°, and then recorded. The buffer system used was that of Northrup and deKruif.[4] Results obtained with various strains are shown in the accompanying table.

These results indicate that the zones of acid agglutination for variant strains of pneumococci, either occurring spontaneously or derived through laboratory manipulation, are widely separated from those of typical strains. The strains possessing the wide zone of complete agglutination possess also the characteristics accompanying the "rough" colony form.

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1 Webster, L. T., and Burn, C. G., *J. Exp. Med.*, 1926, xliv, 343.

2 Hughes, T. P., *J. Exp. Med.*, 1930, li, 225.

3 Gillespie, L. J., *J. Exp. Med.*, 1914, xix, 28.

4 Northrup, J. H., and de Kruif, P. H., *J. Gen. Physiol.*, 1922, iv, 639.

TABLE I.

Acid Agglutination Zones of Pneumococci.

Strain	pH										Description
	2.1	2.3	2.7	3.0	3.6	4.1	4.7	5.1	5.6	6.3	
I	0	0	0	0	0	0	0	0	A	A	Type I, virulent, smooth colony
II	0	0	0	0	0	0	0	0	A	A	Type II, " " "
III	0	0	1	1	1	0	0	0	0	A	Type III, " " "
736	1	0	0	0	0	0	0	0	0	A	Group IV, " " "
750	0	0	0	0	0	0	0	0	0	A	Group IV, " " "
735	0	0	0	0	0	0	0	0	0	A	Group IV, " " "
R 1	0	0	0	0	4	4	4	0	0	0	Rough, avirulent, no serum specificity
R 2	0	0	0	0	4	4	4	0	0	0	Rough, avirulent, no serum specificity
I R	0	0	0	0	4	4	4	0	0	0	Rough, avirulent, no serum specificity, derived from Type I
II R	0	0	0	0	4	4	4	0	0	0	Rough, avirulent, no serum specificity, derived from Type II
III R	0	0	0	2	4	4	4	4	A	A	Rough, avirulent, no serum specificity, derived from Type III
78	0	0	0	0	0	0	0	0	A	A	Type I, avirulent, smooth colony
78 R	0	0	0	0	4	4	4	4	0	0	Rough, avirulent, no serum specificity. Strain 78 after 6 passages in Type I antiserum
734	0	2	4	4	4	4	4	2	2	2	Rough, avirulent, no serum specificity. Spontaneously occurring

0 = No agglutination or autolysis.
1 = Trace of agglutination.
2 = Partial agglutination.
4 = Complete agglutination.
A = Marked autolysis.