tures of Na lactate-lactic acid, range $P_H = 4.7$ to $P_H = 2.4$, and with Na acetate-acetic acid, range $P_H = 5.6$ to $P_H = 3.2$. The mixtures of these buffer series with the microbic suspensions were incubated at 43° C. for 16 hours. Readings were taken at the end of this time. A distinct difference in acid agglutination optimum for the two types was observed. The optimum for type G in general occurs at a range between $P_H = 4.7$ and $P_H = 4.0$. Type D, on the other hand, shows complete sedimentation between $P_H = 3.5$ and $P_H = 3.0$. Many strains of the two types have been examined with invariably the same result. This observation furnishes an important differential criterion for the two varieties. The constancy of the acid agglutination optimum for type D is very strict. That for type G is slightly less so, but the variation is never so great as to cause it to be confused with D.

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Dissociation of microbic species.

IV. Factors influencing the acid agglutination optimum of types D and G.

By PAUL H. DE KRUIF.

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It is generally supposed that the acid flocculation optimum of bacteria is referable only to the C_{H+} and is not influenced by the character of the buffer salts or the anion of the acid. This interpretation is questionable in the light of the following facts. Microbes D and G were tested against a glycocol-HCl buffer series, range $P_H = 3.0$ to $P_H = 1.2$. The same suspensions were tested simultaneously with the Na lactate-lactic acid and the Na acetate-acetic acid series employed in the experiments described in the preceding paper. The results are presented in the following table.

This experiment indicates that other factors besides the C_{H+} are important in the interpretation of the acid agglutination point of the organisms in question. For example, complete flocculation of type G occurs at $P_H = 3.0$ in the glycocol HCl series, while no

flocculation whatever was observed at the same P_H in the Na lactate-lactic acid series. It would appear that either the Cl^- of the acid, or the glycocol possessed the property of broadening the optimum zone or of shifting it toward a higher C_{H+}.

ACID AGGLUTINATION OF TYPES G AND D IN VARIOUS BUFFER SERIES.

Buffer Series.	PH Range.	Complete Flocculation.	
		G.	D.
Glycocol HCl.....	3.0 to 1.2	3.0, ++ at 2.8	3.0 to 2.4
Na lactate-lactic acid.....	4.7 to 2.4	4.7 to 4.1	3.5 to 3.3[1]
Na acetate-acetic acid.....	5.6 to 3.2	4.7 to 3.8	3.5 to 3.2

This and other considerations led to experiments which suggest an explanation for the granular growth of microbe G in plain broth. Washed suspensions of this organism are strongly agglutinated by beef infusion between $P_H = 7.4$ and 6.8. This range represents the C_{H+} occurring during the growth of type G in broth. On the other hand, peptone (Fairchild) and Na_2HPO_4, the other constituents of broth, agglutinate type G very little or not at all in this acidity.

Types G and D were next subjected to tests with varying amounts of beef infusion, which were adjusted to varying acidities, from $P_H = 7.5$ to $P_H = 2.0$. The dilutions of beef infusion were varied from 1–2 to 1–40. Each dilution was tested over the range of acidity just mentioned. Incubation at 43° C. for 16 hours. It was found for type G that as the acidity increases, down to $P_H = 4.5$, the amount of beef infusion necessary to cause complete agglutination becomes less. At $P_H = 4.5$ to $P_H = 4.0$, complete flocculation occurs with traces of beef infusion or with none at all. But as the acidity is increased beyond this point, that is, at $P_H = 4.0$, *increasing* amounts of the beef infusion are necessary to produce this result. The same phenomenon is observed for type D, the only difference being that the complete flocculation of this variety by a given concentration of beef infusion demands a higher C_{H+} than in the case of type G. For type D, the optimum zone lies between $P_H = 4.0$ and $P_H = 3.0$. Beyond this, in the direction of greater acidity, more and more beef infusion is necessary to produce complete flocculation.

[1] No flocculation at 2.7 and 2.4.

It will be observed that the range of C_{H+} at which the smallest amount of beef infusion is required is for each type precisely the zone of the acid agglutination recorded in the preceding paper. This experiment indicates that the beef infusion, *per se*, does not cause the agglutination. It merely widens the acid agglutination zone. This would seem to throw light upon the mechanism of the granular growth character of type G in plain broth.

Suspensions of types D and G were similarly tested against decreasing concentrations of peptone at varying C_{H+}. In these experiments the results were of a different nature, as might have been expected from the failure of peptone to agglutinate type G at $P_H = 7.5$ to $P_H = 6.8$. In the case of peptone, the optimum for type G lies at a range between $P_H = 3.0$ and $P_H = 2.5$. That for D, at $P_H = 2.5$. Peptone, therefore, seems to shift the optimum zone in the direction of a higher C_{H+}, an effect analogous to that observed in the glycocol-HCl buffer mixtures. In the case of microbe D, strong concentrations of peptone (1–2 and 1–4) actually suppress flocculation completely at $P_H = 3.0$. This effect is analogous to the pre-zone phenomenon in immune reactions, since for the higher dilutions of peptone at this C_{H+}, complete agglutination readily occurs.

It would appear from the foregoing that while the flocculation in all cases under consideration is due to H-ions, at the same time other factors, such as glycocol, peptone or beef infusion, either shift or broaden the acid agglutination optimum.

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V. Further considerations in regard to the virulence of microbes D and G.

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The wide variations in virulence between microbes D and G, bacillus of rabbit septicemia, has been demonstrated in the first paper of this series.[1] Microbe D, the type found in natural in-

[1] *Jour. Exper. Med.*, 1921, xxxiii, 773.