fluid media. They were found in every case to satisfy these criteria. What is more, the acid agglutination point is distinctly different to that of the D variety. It is in the nature of a physical constant for each type, and is an important differential criterion. All of these characters persist throughout many passages in undiluted serum, a medium markedly antagonistic to the original change. It cannot be said that the presence of the peptone causes the mutation D → G, since the change occasionally occurs, though very rarely and in small amount, in undiluted rabbit serum. On the other hand, the presence of peptone in suitable concentration greatly accelerates a reaction toward which a tendency already exists. It is of interest to note that four pure-line strains, kept on ice in undiluted rabbit serum for three months without passage, showed no evidence of the appearance of G colonies.

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Dissociation of microbic species.

III. Differentiation of microbes D and G by acid agglutination.

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Granular sedimenting growth in liquid medium is one of the principal characters differentiating microbe G (bacillus of rabbit septicemia) from its parent D form. Type G exhibits the granular appearance not only in plain broth, but in serum broth and in undiluted serum as well. This fact led to the examination of the comparative acid flocculation points of the two types. The method used was that of Michaelis, later described in full by Beniasch.

The suspensions of types G and D were prepared by washing the sediments from 5 per cent. serum broth cultures in large volumes of distilled water. After this procedure had been repeated four times, the final suspensions were carefully brought to equal turbidity. Prepared in this way, the G type suspension shows a stability equal to that of D.

The tests for acid agglutinability were carried out with mix-

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.

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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