

together all the peoples of the earth. I believe that the art which was founded by Alexander Graham Bell, our first pioneer, will provide the means for transmitting throughout the earth a great voice proclaiming the dawn of a new era in which will be realized that grandest of all our earthly aspirations—the brotherhood of man.

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THE AGGLUTINATION OF BACTERIA¹

ONE of the characteristic properties of suspensions of finely divided matter in general and of bacteria in particular is the fact that under certain conditions the particles remain discrete, whereas, under other conditions, they collect into larger aggregates, *i. e.*, they agglutinate. This aggregation or agglutination is caused by certain concentrations of salts or acids and in the case of suspensions of bacteria by proteins and especially by the homologous immune serum. It is evident, therefore, that there must be a force which tends to keep the individual particles apart from each other, and another force which tends to hold them in contact. It has long been known that such particles carry an electric charge with reference to the surrounding liquid and it was early suggested that it was the repulsion due to the charge carried by the organisms which prevented them from coming into contact; the attractive force has usually been assumed to be surface tension. Many experiments have been performed to test this relation of the charge on the particles to the stability of the suspension, but nearly all have failed to show any definite relationship between the two phenomena. An exception is the work of Powis² on the coagulation of oil emulsions. Powis found that coagulation occurred whenever the potential between

the drops and the surrounding liquid was reduced below a critical value of about 30 millivolts. The stability of these oil emulsions therefore is determined solely by the potential of the droplets. In the case of bacteria, however, it has been found repeatedly that agglutination, especially by immune serum, may occur without any change in the potential. Some qualitative relation has been found between the acid agglutination point and the potential inasmuch as the potential passes through a minimum and changes its sign at the acid agglutination point.

It will be evident from the result of the experiments reported here that the difficulty with the bacteria suspensions is due to the fact that electrolytes affect not only the potential and hence the repellent force but also the cohesive or attractive force. In order to account for the observations it is therefore necessary to measure *both*.

The experiments performed by the writers were carried out with thoroughly washed suspensions of *B. typhosus* and the bacillus of rabbit septicemia. The potential was calculated from the rate of migration of the organisms in an electric field.

AGGLUTINATION BY SALTS OR ACIDS

It was found that the experiments could be divided at once into two groups; first, those in which agglutination was caused by very low concentrations (less than 0.01 N), and second, those in which high concentrations of electrolytes were needed. In the first group it was found that agglutination occurred whenever the potential between the organisms and the solution was reduced below about 13 millivolts (either positive or negative). In this case apparently the stability is determined solely by the potential. In the second group, however, there was no critical potential and agglutination with many salts did not occur even though the potential was reduced to a value too small to measure. This distinction between the two groups is not due to a specific effect of the salt, since the presence of a high concentration of sodium chloride for instance, which does not cause agglutination in any concentration, completely prevents agglutination by acids.³

¹ A detailed account of the work described in this paper has been published in the *J. Gen. Physiol.*, July and November, 1922. References to other work will also be found in these papers and have been omitted from the present summary.

² Powis, F.: *Z. physik. Chem.*, 1914-15, lxxxix, 186.

³ It follows from this experiment that in determining the acid agglutination point of bacteria

It is evident from the above results that the agglutination in general can not be accounted for solely by changes in the potential. The results, however, are what might be expected if the salt affected the attractive or "cohesive" force as well as the potential. It was found possible to obtain a comparative measure of the adhesive force by determining the force required to separate two glass discs covered with a film of the bacteria. These experiments confirmed the supposition mentioned above. They showed that high concentrations of salt decreased the attractive force between the organisms. Although the potential required to keep them apart is also decreased, and in very concentrated salt is reduced to zero, the cohesive force is so insignificant that the organisms do not agglutinate, despite the absence of potential.

AGGLUTINATION BY MEANS OF PROTEINS

The agglutinating action of various proteins was found to be the same as that of salts, except that no effect on the cohesive force was noted. The addition of the protein affects the potential and if the potential is decreased below the critical value agglutination occurs. If the potential is *increased* the suspension becomes more stable. This is evidently part of the mechanism of the so-called "protective colloids." The amount of protein required to reduce the potential to the critical value and to cause agglutination varies markedly with the p_H and is much less when the protein and bacteria carry charges of opposite sign. When the bacteria and protein both have the same sign, the charge on the bacteria may be *increased*. This experiment shows conclusively that the union between protein and bacteria is not determined solely by the attraction due to opposite charges since according to this view no combination should occur when the two have the same sign. It appears probable that the union is governed by secondary valence forces and is of the same type as the reactions studied by Langmuir in the formation of surface films. The isoelectric point of organisms coated with a protein is changed to that of the protein.

buffer solutions of high concentration should not be used, but the experiments should be made with dilutions of a suitable acid.

THE MECHANISM OF AGGLUTINATION WITH NORMAL AND IMMUNE SERUM

The effect of normal or immune serum was found to depend on the conditions of the experiment, especially as regards the salt concentration. If dialyzed serum is added to a suspension of organisms in distilled water, the potential of the organisms is gradually decreased but not sufficiently to cause agglutination (in the case of typhoid bacilli), *i. e.*, the potential is still greater than 13 millivolts. If *any* salt solution is now added to this mixture until the potential is decreased to the critical value of 13 millivolts, agglutination occurs. In other words, the immune serum prevents the salt from decreasing the cohesive force between the organisms and the agglutination of organisms treated with immune serum is determined simply by the potential. The agglutination of organisms sensitized with immune serum therefore takes place whenever sufficient electrolyte is added to reduce the potential to the critical value of about 13 millivolts. An excess of trivalent ions or of some divalent ions may cause the potential to change its sign and become greater than the critical value again, in which case the suspension again becomes stable.

If the experiment is performed in the usual way by adding the serum to a suspension of the organisms in salt solution, the mechanism is different. As has already been stated, the addition of ordinary neutral salts such as NaCl in fairly high concentrations to a suspension of bacteria depresses the potential to a very small value but agglutination does not occur owing to the fact that the cohesive force is also decreased, so that although there is no force keeping the organisms apart, neither is there any force to draw them together and no agglutination occurs. If now immune serum is added to this suspension of bacteria in salt solution it is found that very little or no change is caused in the potential but that the cohesive force is increased and agglutination therefore takes place. When the experiment is performed in this way, therefore, the mechanism is just the reverse of the one described above in which the salt was added to the mixture of serum and organisms. When the salt is added to the mixture

of serum and organisms the cohesive force remains constant, but the potential and therefore the repulsion between the organisms is decreased until it is less than the cohesive force and agglutination therefore occurs. When, however, the serum is added to the organisms in the presence of sufficient salt so that the potential is less than 13 millivolts, the cohesive force is increased with increasing concentration of serum until it is greater than the repulsion due to the potential (which is not affected by the addition of the serum) and agglutination again occurs.

INFLUENCE OF THE p_H

It was found that the acidity of the solution markedly affects the amount of either normal or immune serum required to cause agglutination (when the organisms are suspended in low concentrations of salt). The nearer the isoelectric (acid agglutination) point of the organism the less serum is required for agglutination. A determination of the amount of antibody combined with the organism, however, showed no increase in this range. The number of units combined was the same from p_H 3.5 to p_H 8, although the amount of serum required to cause agglutination varied tremendously over this range of p_H . The same amount of antibody when combined with the organisms therefore evidently has a far greater effect in this range of p_H than it has farther from the isoelectric point or else other constituents of the serum have a greater effect near the isoelectric point of the organism. In any case, there seems to be no connection between the sign of charge of the antibody and organism and the combination between the two. This experiment indicates that the serum or protein forms a film on the surface of the organism which then reacts like a particle of the pure protein. A similar mechanism was suggested by Coulter⁴ to account for the effect of serum on the acid-agglutination point of red blood cells.

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⁴ Coulter, C. B.: *J. Gen. Physiol.*, 1921-22, iv, 403.

THE WORK OF U. S. GEOLOGICAL SURVEY

EXPLORATIONS for potash, investigations and explorations of present and possible oil and gas fields, of coal fields, and of ore deposits and mining districts, paleontologic researches, work in chemistry and physics, and investigations of surface and underground water resources are among the year's activities of the United States Geological Survey as mentioned in the annual report of the director, just made public. The number of employees in the Geological Survey holding regular appointments was 877; most of them are field men whose varied work ramifies into every state, in addition to Alaska and other outlying territory of the United States. Besides these a small host of temporary camp field men were employed—rodmen, teamsters, cooks, etc. Some of the most important things that the Geological Survey has to show for its work are geologic surveys which cover a wide scope of practical and scientific activity, embracing 32 states; topographic mapping in 21 states covering 13,544 square miles and including more than 5,000 miles of levels and primary traverse lines; more than 11,000 measurements of stream flow in 31 states and investigations of underground waters in 10 states.

The survey also acted on more than 25,000 cases referred to it in connection with the administration of the public lands; and made a decrease of 3,914,000 acres in outstanding government coal reserves, of 110,000 acres in outstanding petroleum reserves, and of 298,000 acres in phosphate reserves. There was, however, a net increase of 268,000 acres in the area classified as phosphate land. The present acreages classified and reserved by the government as coal, petroleum, oil-shale and phosphate lands are: Coal, 65,000,000; petroleum, 6,500,000; oil-shale, 4,100,000; phosphate, 2,700,000. The survey also made a net increase of 635,000 acres in power-site reserves, designated 382,000 acres in Nevada under the ground-water reclamation act, and made net increases of 5,700 acres in public water reserves, of 3,989,000 acres in enlarged-homestead designations, and of 4,778,000 acres in stock-raising homestead designations. The Washington office distributed 602,000 copies of book publications