THE EFFECT OF IONS UPON AGGLUTINATION.

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1. Introduction.

THE true mechanism of agglutination is still unknown. The first investigation upon it was described in 1899 by Bordet,¹ who showed that for the occurrence of the phenomenon not only the combination of agglutinin and bacteria, but also the presence of sodium chloride, is needed. Bordet found that if the salt was removed from the serum and from the suspension of bacteria by dialysis, and the serum and suspension mixed, agglutination did not occur, but that if a small amount of sodium chloride was added, agglutination promptly took place. According to this view agglutination is a phenomenon of molecular physics—the agglutinin acts on the bacteria or other cells and prepares them for agglutination by altering the relations of molecular attraction between them and the surrounding fluid, whilst the second phase, the loss of motility, clumping, &c., is brought about by the presence of salts as is the sedimentation of clay. Joos² observed that agglutinin was absorbed by bacteria. When the agglutinin-bacteria combination was precipitated by salt, he found that the concentration of salt in the supernatant fluid was decreased, so that he believed that true chemical action took place in agglutination. He distinguished two phases, primary and secondary, of the phenomenon: the absorption of agglutinin by agglutinable substance, and the precipitation of salt with the agglutinin-bacteria combination. Furthermore, he observed that in the secondary phase the precipitating action differs according to the kind of salt, chloride, iodide, bromide, &c., with monovalent cations, and believed that the acid radicals of salts took an important part in the action of precipitation.

Neisser and Friedemann,³ Bechhold⁴ and Friedberger,⁵ investigated the precipitation of inorganic suspensions and of the colloids by several salts which possess negative electric charges, examined the limiting concentration of salt solution in which the action of precipitation took place in 24 hours, and proved distinctly that the really important factor in its action is the cation and not the anion. In addition to the agglutinin and bacteria, therefore, the presence of some cation is necessary for the mechanism of agglutination. In their experiments Joos² and Friedberger⁵ did not, however, use equivalent solutions, while Neisser and Friedemann,³ and Bechhold⁴ used only various light metal salts, and their solutions were equivalent. To fill up these gaps, Porges⁶ made use of the fact that the absorption of agglutinin by bacteria may be produced in the salt-free medium. He added toluol to a suspension of typhoid-agar-culture in distilled water, and to typhoid immunised serum, and dialysed each in running water for three days and in distilled water for two days more. After having ascertained that the water was free from chlorine ions, he mixed each 1 c.cm. of bacteria suspension with 0.5 c.cm. diluted serum and various diluted salt solutions, and observed the limiting concentration of salt in which the agglutination takes place. During his experiments with various dilutions of a normal solution of NaCl, KCl, NH₄Cl, MgCl₂, NaNO₃, KNO₃, NH₄NO₃, Mg(NO₃)₂, Na₂SO₄, K₂SO₄, (NH₄)₂SO₄, MgSO₄, he observed the relation between anions and cations and found that there is only small difference between Cl', NO₃' and SO₄'', but that the effect of Mg'' is 10 times greater than other cations. Buxton and Shaffer,⁷ and Buxton and Teague,⁸ also examined the precipitation of colloids with some simple common salts of mono- to trivalent cations (for example Al'', Fe'', &c.), and proved that there is no difference between the precipitation of agglutinin-bacteria combination and that of inorganic colloids.

The results thus obtained show not only that the agglutination is a physico-chemical phenomenon in which the presence of salts is necessary, but also that these cations play an important part in it.

Such experiments with cations of a valency greater than three had not hitherto been tried, but it was evident that the agglutination is closely related to the valency rule. Accordingly an attempt was made, under the guidance of Prof. K. Miura, to investigate the effect of the valency of salts on the phenomenon of agglutination, and to examine the effect of cations thereon in the following manner.

2. Method of Research.

(A) *Agglutinin-bacteria combination*.—Porges⁶ examined the precipitating action of salts by mixing them with the dialysed serum and bacteria, but as serum is of a complex nature it is not suitable for the investigation of the effect of ions. For my experiments I have prepared the salt-free agglutinin-bacteria in the following manner: Typhoid strains were prepared on 16 to 24 hour slope agar cultures, and the bacilli from 10 cultures suspended in 10 c.cm. of sterilised normal saline solution, care being taken to prevent admixture of agar or condensed water in the agar tube. To this suspension was added 1 c.cm. of typhoid immunised serum with an agglutination titre of 1:10,000. After placing in the thermostat for 30 minutes, 20 c.cm. distilled water were added and the combination centrifuged; the supernatant fluid was removed, a further 20 c.cm. of distilled water added and the mixture again centrifuged. The washing by centrifugation was repeated five times; the mixture was then placed in a dialysis shell, 40 c.cm. of distilled water were added, and the mixture dialysed in running water for five days, and in distilled water for two days, in order to free it completely from chlorine ions. A milky-white and diffusely turbid suspension of agglutinin-bacteria combination was obtained and used in the experiments as follows: Into a series of small test-tubes (previously boiled to free from alkaline matter and electrolytes) was put 1 c.cm. of various salt solutions in different concentration, and to each tube was added one drop of the agglutinin-bacteria suspension (each drop containing about one normal platinum loopful of the bacteria). After well mixing they were kept in the thermostat at 37° C. for two hours and afterwards at the room temperature for 18 hours. Macroscopic observation showed that the agglutinin-bacteria combination was precipitating in the bottom of the tube, while the supernatant fluid was clear; the fluid was more concentrated than the precipitation limit, for if its concentration was lower than the limiting value, it would not have clumped, and the supernatant liquid would have remained turbid. The spontaneous agglutination is to be distinguished easily from the ordinary agglutination phenomenon, for in the case of the former, mere shaking makes the fluid turbid.

(B) *Salts*.—Matsuno⁹ found when investigating the effect of cations upon arsenious sulphide, that simple salts are not appropriate materials for the investigation of the effect of valency on the coagulation of colloidal arsenious sulphide. For example, the concentration of cupric sulphate passes over beyond the limiting value of other bivalent salts as shown on Table I. It dissociates perhaps not only as the formula—

but the hydrolysis would also take place partially in form of—

so that its aqueous solution displays a faint acid reaction. To avoid this inconvenience it was necessary to search for salts with cations of several valencies. The bivalent or trivalent salts are common, but tetravalent salts are difficult to obtain, and pentavalent salts are still unknown. Prof. Shibata of the College of Science, Imperial University, Tokyo, was kind enough to furnish me with cobaltic complexes for this purpose. Though pentavalent salts are unknown

in complex salts of cobalt, yet it was possible to carry out experiments with salts from mono- to hexavalency. The cobaltic complexes were of different chemical composition as shown in Table II.

3. Results of the Experiments.

According to the colloid chemical process, it is evident that a negatively charged colloid begins to show minimum precipitation only after having been neutralised to some degree, and so it is suitable for my purpose to observe the minimum limiting concentration which causes the partial precipitation. Complete precipitation of colloid cannot be said to have made complete neutralisation of ions, for it may be thought that the precipitated substance would absorb more ions than were necessary to the complete precipitation. I have observed in experiments that some common salts—for example, $\text{Cu}(\text{NO}_3)_2$, $\text{Al}_2(\text{SO}_4)_3$, $\text{Al}_2(\text{NO}_3)_6$, &c.—bring about either incomplete agglutination, or no precipitation, in greater concentration than limiting value, but on the contrary complete coagulation takes place at the limiting concentration. This phenomenon is named the “restraint zone” by Neisser and Friedemann³; it is not observed with the solutions of cobaltic complexes, in which, when the concentration is greater than the minimal, complete precipitation takes place, and becomes gradually less complete as the concentration is reduced.

The limiting concentration hereafter mentioned means the lowest concentration at which the least trace of agglutination of the agglutinin-bacteria combination was found.

TABLE I.

The Limiting Concentration for Agglutination by Common Simple Salts.

L.E.C.=Limiting equivalent concentration.

(a) Salts of Monovalent Cations.					
		L.E.C.			L.E.C.
NaCl	..	1/100	NaNO ₃	..	1/100
KCl	..	1/100	Na ₂ SO ₄	..	1/100
KNO ₃	..	1/100			
			Aver.	..	1/100
(b) Salts of Bivalent Cations.					
		L.E.C.			L.E.C.
MgSO ₄	..	1/2000	CuSO ₄	..	1/10000
Ba(NO ₃) ₂	..	1/2000	Cu(NO ₃) ₂	..	1/10000
CoCl ₂	..	1/2000			
			Mean	..	1/2000
(c) Salts of Trivalent Cations.					
		L.E.C.			L.E.C.
Al ₂ (SO ₄) ₃	..	1/20000	Cr ₂ (SO ₄) ₃	..	1/20000
Al ₂ (NO ₃) ₆	..	1/20000			
			Aver.	..	1/20000

TABLE II.

Limiting Concentration for Agglutination by Cobalt Complex Salts.

(a) Salts of Monovalent Cations.			
[Co(NH ₃) ₅ (NO ₂) ₂]Cl	..	..	1/150
[Co(NH ₃) ₅ (CO ₃) ₂]SO ₄	..	..	1/150
		Aver.	1/150
(b) Salts of Bivalent Cations.			
[Co(NH ₃) ₅ Cl]Cl ₂	..	..	1/2000
[Co(NH ₃) ₅ NO ₂]Cl ₂	..	..	1/2000
[Co(NH ₃) ₄ H ₂ O]Cl ₂	..	..	1/1500
		Mean	1/2000
(c) Trivalent Complex Cobaltic Cations.			
[Co(NH ₃) ₅ H ₂ O]Cl ₃	..	..	1/10000
[Co(NH ₃) ₆](NO ₃) ₃	..	..	1/10000
[Co(NH ₃) ₆]Cl ₃	..	..	1/10000
		Aver.	1/10000
(d) Tetravalent Complex Cobaltic Cation.			
[$\text{H}_2\text{O}(\text{NH}_3)_4$ CoNH ₂ Co(NH_3) ₄]Cl ₄	..	..	1/30000
(e) Hexavalent Complex Cobaltic Cations.			
[Co[Co(NH ₃) ₄ (OH)] ₂](SO ₄) ₃	..	..	1/100000
[Co ₄ (OH) ₆ (NH ₃) ₁₂]Cl ₆	..	..	1/100000

In the above experiments it is shown that the limiting concentration of precipitation depends only upon the cations, and this is common for either simple or complex salts. The results obtained by Neisser and Friedemann,³ and Bechhold,⁴ in their experiments with inorganic, and those of Porges,⁶ Buxton and Shaffer,⁷ Buxton and Teague,⁸ with organic colloids, are in complete accord with mine, and, moreover, the important question of the dependence of the agglutination phenomenon upon the cations of the indispensable salts is brought into line. The valency rule established by Freundlich¹⁰ is as follows. Presuming that the neutral salts in their equivalent solution are, despite their valencies, adsorbed in the same manner, the approximate values of concentration of complete precipitation have been obtained by the calculation based on the adsorption curve. His idea can be expressed also as follows: Since the electric charges of mono-, bi-, and tri-valent ions are in the ratio of 1:2:3 the amounts which should be adsorbed causing a complete coagulation should be in the ratio of 3:1.5:1, and if we plot the logarithm of the precipitating concentrations against that of numbers —i.e., 3, 1.5 and 1 for mono-, bi-, and tri-valent ions respectively—it would give a straight line. The above theory has been proved by his experiment with arsenious sulphide colloid and platinum colloid solution.

Before Freundlich, Whetham¹¹ published his research on the effect of valency on the precipitation of hydrosols. He deduced the following equation from a consideration of the probability of collision:

C₁:C₂:C₃=K³:K²:K¹

where C₁, C₂, and C₃ are the molecular concentrations of coagulation of mono-, bi-, and tri-valent ions respectively, K is a constant. If this formula is correct, it must be a straight line when plotted, taking the logarithm of concentration of precipitation and the valency of the ions as its axes of coördinates, but in the present experiment it gave a curve, as shown by Fig. 1, in place of a straight line. The theory of

Freundlich¹⁰ was clearly verified by the investigation of Matsuno⁹; taking the logarithm of the valencies of the cobaltamines as the abscissa and those of the limiting values for precipitation of colloidal arsenic sulphide as ordinate, a straight line (Fig. 2) was obtained, and he was able to deduce the following equation:— $\log S_n + \beta \log n + \alpha = 0$.

From the results of our experiments of agglutination of simple agglutinin-bacteria combination with solution of cobaltic complexes, it is again affirmed that if we plot the logarithm of limiting concentrations of cobaltic complexes as the ordinate and those of the valencies as the abscissa, a straight line is obtained (Fig. 3). Accordingly the equation relating limiting concentration and valencies of cobaltic complexes is

$\log S_n + \beta \log n + \alpha = 0 \dots (1)$,

where S_n is the limiting value of n -valent ion, n the valency and α and β are constants.

When $n=1$, $-\log S_1=a$, the equation (1) will be now,

$$\log S_n + \beta \log n - \log S_1 = 0$$

$$\text{or } \log S = \log S_1 - \beta \log n \dots\dots\dots(2).$$

The value of β in the above equation can be calculated from the equation,

$$\beta = \frac{\log S_1 - \log S_n}{\log n},$$

substituting the experimental data for S_1 and S_n . The results of calculation was as follows:—

For bivalent ions ..	3.733
„ trivalent ions ..	3.620
„ tetravalent ions ..	3.821
„ hexavalent ions ..	3.628
Aver. ..	3.7

This number is applicable only in the case of agglutination of the combination of typhoid bacilli with its agglutinin in the solution of cobaltic complexes.

Putting $\beta=3.7$ in the equation (2), the equation was deduced:— $\log S_n = \log S_1 - 3.7 \log n$. It gave:—

Limiting value equivalent concentrations.

Valency.	Observed.	Calculated.
1 ..	1/150	..
2 ..	1/2000	1/1950
3 ..	1/10000	1/8738
4 ..	1/30000	1/25336
5 ..	—	—
6 ..	1/100000	1.113568

In comparing the calculated limiting values with those obtained by experiment, it is found that close conformity exists.

Since the experiments with common simple salts, for example, CuSO_4 , gave by reason of hydrolysis instead of a straight line a curve, they are not suitable materials for the observation of effect of valencies of salts upon agglutination. The precipitation of agglutinin-bacteria combination is produced through the force of certain amounts of cations and that precipitation follows the valency rule in the same manner as in the case of precipitation of inorganic colloids.

4. Summary.

1. A pure salt free agglutinin-bacteria combination has been prepared by repeated washing of the agglutinin-bacteria mixture with distilled water and subsequent dialysis. As the common simple salts are not suitable, owing to hydrolysis and other causes, for the purpose of testing the effect of ions, the cobaltic complexes of valency from 1 to 6 were used, with which the author has been able to test the effect of positive complex ions.

2. It has been found that the more the valency of the cation (from 1 to 6) increases, the less becomes the limiting concentration with which agglutination takes place; further, it has been found that there exists such close relation between them that when a logarithmic curve is plotted of the limiting values and of the valencies, a straight line is formed. The precipitating action of the agglutinin-bacteria combination is entirely in accordance with the valency rule, and the author has proved that as precipitation occurs through the action of cations, it is a colloid bearing negative electric charges.

The author wishes to express his indebtedness to Prof. K. Miura for guidance and encouragement in this research, to Mr. Y. Shibata, Professor at the College of Science, for much valuable material, and to Dr. T. Itakura for helpful advice.

Addendum.—The above-mentioned findings are not only applicable to the agglutination of the agglutinin-bacteria combination by salts but the same relations seem to appear in the case of hæmagglutination, which is at present in the course of investigation and will be published in a later report.

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SATURNINE ASTHMA:

IS THERE SUCH A MALADY?

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Is there such a malady as saturnine asthma?

History of Case.—Twenty-two years ago I examined for a life insurance company, and passed as a first-class life, a male aged 21. Ten years afterwards he was examined by another medical man for another office, and again was passed as a first-class life. Eight years ago he had pleurisy from which he was said to have made a good recovery. He had good health until 1918 when he began to suffer from shortness of breath on going up an incline. The dyspnoea came on gradually and was believed by the medical men who saw him to be the result of cardiac overstrain. Rest was prescribed, but instead of following out this instruction, patient went on with his business. During 1918 he was attached to the anti-aircraft service, and regularly took his share of night work, attending to office duties during the day. When thus engaged he again began to experience shortness of breath. In January, 1919, he developed bronchitis, in the course of which asthma supervened. Six months afterwards, as tubercle bacilli were found in the sputum, patient was sent to a sanatorium in Scotland, where he was informed that his malady was not bronchitis and asthma, but that the so-called asthma was due to some toxic cause, the nature of which was not ascertained.

Prior to this the patient had been losing weight, but during the three months he was in the sanatorium in 1919 he gained 22 lb. in weight and came home. In September, 1920, he returned to the sanatorium. It was now noticed that his finger-nails were black. Vomiting at this stage supervened, and after a residence of three months in the sanatorium tachycardia developed. At the time no explanation was forthcoming as to the blackening of the finger-nails, the vomiting, and tachycardia. The asthmatic seizures kept recurring and were severe. After a rest in bed for several weeks the cardiac condition considerably improved. To iodide of potassium, given to relieve the asthma, patient exhibited marked intolerance; instead of giving relief it brought on respiratory spasm attended by an acute rash on the skin. At this stage he consulted a London physician.

Last February he was sent to me by Dr. John Macfadyen of Newcastle-on-Tyne. On examining the patient wheezing râles could be heard pretty well all over the chest attended by prolonged expiration; over the right apex posteriorly dry friction could be heard, with here and there small moist râles. There was

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