

Biochemistry. — *Complex systems of biocolloids. II. Specific factors of importance to the intensity of the complex relations, their significance in particular with regard to the formation of the tricomplex systems.* By H. G. BUNGENBERG DE JONG. (Communicated by Prof. J. VAN DER HOEVE).

(Communicated at the meeting of June 25, 1938.)

1. Introduction.

After discussing in the preceding communication the general properties of the complex biocolloid systems and their classification from the most general point of view possible, we shall consider in this communication the question which are the factors determining the very striking differences between the biocolloids mutually as well as between the crystalloid ions mutually with regard to the tendency to form complex colloid systems. Originally this formed an object for experimental research.

A more or less satisfactory survey of these factors may at this moment already be given for the dicomplex systems¹⁾, thanks to more elaborate comparative researches concerning complex and auto-complex coacervation and flocculation respectively.

Originally the colloid-chemical point of view²⁾ was taken as basis for interpretation of the experimental data. When it became apparent that for the understanding of the complex colloid systems electrochemical points of view may just as well and in some cases even better serve as basis for interpretation, the projected theories on a colloid-chemical foundation were not all at once useless. They appeared to contain so many essential elements, which might as well be considered as typically electrochemical, that their renewed interpretation from the new point of view did not offer special difficulties³⁾.

In the following we shall discuss in the first two paragraphs specific factors determining the intensity of the complex relations in dicomplex systems. After that it will be easy to indicate in broad outlines the conditions promoting the formation of unicomplex systems.

We shall, however, particularly discuss here the conditions favourable to the formation of tricomplex systems.

¹⁾ H. G. BUNGENBERG DE JONG and collaborators, *Rec. Trav. Chim.* **53**, 163, 171, 607, 622, 737, 747 (1934); **54**, 1, 17 (1935).

²⁾ H. G. BUNGENBERG DE JONG, *Kolloid Z.*, **79**, 223, 334 (1937), cf. Chapters IV—VII.

³⁾ H. G. BUNGENBERG DE JONG, *Kolloid Z.*, **80**, 221, 350 (1937), cf. Chapters III—V.

They can, namely, be foreseen from those which apply to the uni- and dicomplex systems and it will be seen that the expectations pronounced on this ground are indeed corroborated by the experiments.

2. Factors determining the intensity of the complex relations between colloid cations and colloid anions.

In the course of the researches on complex coacervation and complex flocculation respectively specific differences were observed between the biocolloids mutually¹⁾: positive gelatin forms complex coacervation with negative nucleinate and with negative gum arabic, with negative agar only a slight opalescence occurs, the complex coacervation or flocculation failing entirely with negative soluble amyllum and negative glycogen.

When it is examined in the above-mentioned cases how strong the NaCl concentration must be in order to neutralize exactly the coacervation at the mixing-ratio which each time is most favourable to the optimal coacervation, it appears that at one and the same P_H this is strongest for the combination gelatin + nucleinate, lower with gelatin gum + arabic, while the slight turbidity in the combination gelatin + agar is neutralized already by a very low NaCl concentration. In the combinations of gelatin with soluble amyllum or glycogen, yielding only clear mixtures, the required NaCl concentration consequently is zero.

The negative complex components may consequently be placed in a series, the intensity of the complex relations decreasing from left to right with regard to the same positive complex component and each time at the most favourable mixing-ratios, which becomes manifest by a diminishing tendency to form complex coacervation and a reduced resistance to added neutral salt:

nucleinate > arabinat > agar > soluble amyllum > glycogen.

Already before, in analogous researches on the complex coacervation and flocculation of positive gelatin with a number of negative sols of phosphatides of different origin, analogous differences between the latter were observed and at the same time it was stated that in the combinations with positive clupeine for the phosphatide preparations the same sequence occurs with regard to the neutralization by neutral salt, but that the neutral-salt concentrations required for the neutralization are now considerably higher than in the combinations with positive gelatin²⁾.

The same is found by comparison of the combinations of the above-mentioned series of negative complex components (nucleinate, arabinat, etc.) with clupeine on the one hand and with positive gelatin on the other hand.

From this it appears that also for the positive complex components of

¹⁾ H. G. BUNGENBERG DE JONG and A. DE HAAN, *Bioch. Z.*, **263**, 33 (1933).

²⁾ H. G. BUNGENBERG DE JONG and R. F. WESTERKAMP, *Biochem. Z.*, **234**, 367 (1931).

complex coacervation or complex flocculation a series may be formed, in which combined with one and the same negative complex component they bring about differences in the intensity of the complex relations, viz. this decreases from left to right in the order

clupeine > gelatin.

Consequently the intensity of the complex relations each time at the most favourable mixing-ratio is dependent on the negative as well as the positive complex component.

If now we turn to the mixing-ratios of optimal coacervation, varying for each combination, analogous regularities are found. However, now we have not to consider a property of the formed complex coacervate, viz. the extent of its neutral-salt resistance, but the mixing-ratios of the components, varying for each combination, in order to obtain the optimal coacervation at one and the same P_H . If gelatin is taken as positive complex component, at P_H 3.5 less nucleinate than gum arabic is required to obtain the optimal coacervation with a given amount of gelatin:

nucleinate < arabinat.

The same difference between nucleinate and arabinat also occurs in order to obtain the optimal coacervation, if clupeine is taken as positive complex component, but at the same time it becomes apparent that these amounts of both negative biocolloids are now larger than in the corresponding combinations with gelatin at the same P_H .

Consequently to the quantities of clupeine and gelatin respectively, required at the same P_H to obtain optimal coacervation with each time the same quantity of a special negative biocolloid, applies:

clupeine < gelatin.

Now it is of importance that exactly at or at any rate close to the mixing-ratio of optimal coacervation the complex coacervate is electrophoretically uncharged, while with excess of the negative complex component the coacervate is negatively charged, with excess of the positive complex component positively. The mixing-ratio of the optimal coacervation thus has the character of a ratio of masses of the two oppositely charged biocolloids, while they compensate each other electrically.

The above-mentioned series of sequences of the biocolloids for the tendency to form complex coacervation or neutral-salt resistance and for the varying amounts required for mutual electrical compensation, may be explained if we assume that the quantity of charge per gram biocolloid decreases from left to right in the following order:

nucleinate > arabinat > agar > amyllum
and clupeine > gelatin

These series, considered from a colloid-chemical point of view, may then be regarded as series in which from left to right the *density of charge*

on the surface of the particles decreases (by some very simplified suppositions, e.g. that the volume of the particles in the different biocolloids does not vary greatly, etc.). However, this may be expressed as well *electrochemically* by saying that in these series the *equivalent weight* increases from left to right.

From the study of auto-complex coacervation a method developed enabling us to measure these magnitudes quantitatively.

For negative biocolloids Hexol nitrate is used, which yields the so-called reciprocal Hexol numbers. However, we may also determine the equivalent weights from chemical analyses and find then that the reciprocal Hexol number and equivalent weight down to a systematic difference of about 10—15 % always yield the same number, in spite of the fact that the order of magnitude of these numbers may highly vary¹⁾.

These researches indeed confirm that the order drawn up of the negative biocolloids is also that of their reciprocal Hexol numbers and equivalent weights respectively.

Reciprocal Hexol numbers:

Na-nucleinate	<	Na-arbinat	<	Na-agar	<	Amyllum
294		1068		2264		26000

Not yet published researches concerning the two mentioned proteins likewise show that at the same P_H of 3.5 clupeine has a far greater density of charge than gelatin, i.e. a much smaller equivalent weight.

Recapitulating we find that *for the intensity of the complex relations between colloid cation and colloid anion the equivalent weight is the most important factor*. Considering that the molecular weight of the biocolloids generally is very high, we usually have to deal with highly polyvalent colloid ions and evidently it is the degree of polyvalence which is so important to complex coacervation. According as the equivalent weights are smaller, i.e. the colloid ions are more polyvalent, their mutual complex relations are stronger and a higher concentration of KCl is required for the neutralization of the coacervation.

The question whether yet other factors in addition to the equivalent weight are of importance to the strength of the complex relations can as yet not be answered. It is not unlikely that not only the number of charges per gram colloid are of importance but also the nature of the ionized groups (e.g. phosphate-, carboxyl-, sulphate groups). This factor, which by the side of the equivalent weight probably is only of secondary importance to the intensity of the complex relations between colloid cations and colloid anions, comes more to the foreground in auto-complex coacervation (where one of the voluminous colloid ions is replaced by a much smaller crystalloid ion), to which we shall revert below.

¹⁾ H. G. BUNGENBERG DE JONG and P. H. TEUNISSEN, *Kolloid Beih.*, 47, 254 (1938).

3. *Factors determining the intensity of the complex relation between colloid anions and crystalloid cations.*

a. *Significance of the density of charge of biocolloids.*

Analogous conditions to those found in the preceding paragraph with regard to the significance of the density of charge apply also to the intensity of the complex relations between colloid anions and crystalloid cations. The tendency to form auto-complex coacervation or flocculation with a given crystalloid cation increases according as the negative biocolloid has a greater density of charge, i.e. has a smaller equivalent weight. At the same time the neutral-salt resistance also increases in this direction. On the other hand, generally speaking, the tendency of a given negative biocolloid to form auto-complex coacervation or flocculation with various cations likewise increases with the valency of the cation.

However, this rule is not always applicable, e.g. not to the behaviour of sulphate colloids towards monatomic cations, which we shall discuss in c).

b. *Measurements of the relative affinities of a number of cations to a given negative biocolloid.*

It appeared from electrophoretic measurements that almost every negative biocolloid can obtain reversal of charge by means of numerous salts. Comparison of the concentrations at the reversal of charge of a number of chlorides or nitrates shows that this is higher according as the tendency to form auto-complex flocculation or coacervation with these salts is smaller. In cases where the sol remains stable, i.e. the neutral-salt resistance is zero, the concentrations at the reversal of charge may yet be determined by electrophoretic measurements on SiO_2 particles suspended in the sol, which are covered by a colloid film.

Such measurements¹⁾ give a survey of the relative affinity of a number of cations to one given biocolloid, while consequently we may also include in this research cations whose maximal intensity of the complex relations with the colloid anion is yet too small for coacervation or flocculation.

As far as it is necessary in order to understand the conditions for the formation of the tricomplex systems, some of the most important results may be briefly described below.

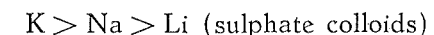
c. *Significance of the polarizability of the ionized groups of the colloid anion with regard to the intensity of the complex relations with small anorganic cations.*

In the case of complex relations between small cations (consisting of one atom) and colloid anions it appeared that here the polarizability of the ionized groups of the colloid anion is of great importance, viz. whether this is greater or smaller than that of the water molecule. To biocolloids with

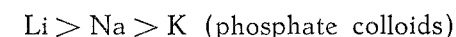
¹⁾ P. H. TEUNISSEN and H. G. BUNGENBERG DE JONG, *Kolloid Beihefte* 48, 33 (1938).

phosphate- or carboxyl groups which are more polarizable than water applies that the tendency to enter into complex relations with cations consisting of one atom strongly increases with the valency of the cation, on the other hand with biocolloids with sulphate groups (these are less polarizable than water) this pronounced influence of the valency is absent or even the reverse is found.

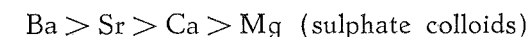
These and other details concerning the specific differences of the cations, which are the result of differences in volume and polarizing capacity, with regard to the tendency to enter into complex relations with biocolloid anions, become manifest in the sequences of the concentrations at the reversal of charge and have been published elsewhere¹⁾. Since it is important in the discussions further on (§ 5 ff.) of the tricomplex systems, it may be mentioned here that for the sulphate colloids the affinity of the three smallest alkali cations increases with growing volume:



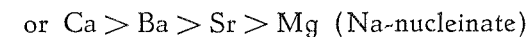
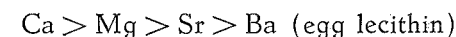
whereas for phosphate colloids it diminishes with increasing volume of these cations:



With the alkaline earth cations we find analogous conditions:

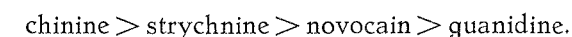


although with the phosphate colloids not a complete reversal of this series occurs but so-called transitional series, e.g.:



As was to be expected, the mentioned difference in polarizability of the ionogenic groups plays no part in the intensity of the complex relations between large complex or organic cations and colloid anions. Owing to their size, they have no polarizing effect.

It was found²⁾ that the complex relations between colloid anions and organic cations with phosphate- and carboxyl- as well as sulphate colloids decrease from left to right, always in the same order:



Further examination even showed that the three largest alkali cations already behave as organic cations: with the phosphate-, carboxyl- as well as the sulphate colloids they take the same order $\text{Cs} > \text{Rb} > \text{K}$. Their volumes, consequently, are already so large that practically they have no longer a polarizing effect and thus with egg lecithin a transitional series is formed:



¹⁾ P. H. TEUNISSEN and H. G. BUNGENBERG DE JONG, *Kolloid Beihefte* 48, 33 (1938).

²⁾ H. G. BUNGENBERG DE JONG and J. G. WAKKIE, *Biochem. Z.* 297, 70 and 221 (1938).

Concerning the connection of structure and differences in affinity of organic cations towards colloid anions in due time systematic researches will be published in *Kolloid Beihefte*, as well as analogous researches on the sequences of affinity of anorganic and organic anions towards positively charged biocolloids.

4. *Specific properties of biocolloids and crystalloid ions promoting the formation of uni- and dicomplex colloid systems.*

In the two preceding paragraphs we discussed already which factors increase the intensity of the complex relations in the case of interaction between colloid ions mutually and colloid ions and crystalloid ions mutually. They are at the same time the factors which promote the formation of dicomplex colloid systems.

As regards the analogous question in the case of the unicomplex systems it may be expected that here also the density of charge, e.g. of the protein-multipolar ions at the I.E.P. will be of the greatest importance. Here we meet with the experimental difficulty that we can easily determine the maximal positive or negative density of charge on either side of the I.E.P. but have great trouble with that at the I.E.P. itself, where positive and negative charges compensate each other outwards. In analogy with our experiences with the dicomplex systems it may consequently be expected that, if an at random distribution of both charges is assumed, the density of charge at the I.E.P. in isolabile proteins (globulins) will be greater than in isostable proteins (albumins, gelatin). With the former the neutral-salt resistance of the unicomplex colloid system is larger than zero, with the latter it is zero.

5. *General remarks concerning the complex relations in the tricomplex systems.*

A detailed description of the factors favouring the formation of tricomplex systems may be preceded by a general discussion. In the first place it has to be taken into account that the ions occurring in the diagram reproduced in the 1st communication may be joined into the two diagrams for uni- and dicomplex systems. We may compare fig. 1, leaving undecided whether the complex relations a of the unicomplex system and similarly

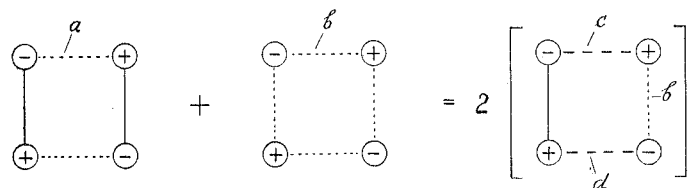


Fig. 1.

b of the dicomplex system are sufficiently intensive to make these systems indeed possible.

In the tricomplex system by the side of b two other complex relations

occur, c and d , and consequently it is obvious that the answer to the question whether in a given case a tricomplex system will be formed will depend on the intensity of the complex relations in the tricomplex system with regard to those in the unicomplex and dicomplex systems together.

If $2c + 2d + 2b > 2a + 4b$ or $c + d > a + b$, the tricomplex system might be formed at the cost of the uni- and dicomplex systems.

Here we should take into account that the diagrams have exclusively formal significance and reproduce the complex relations only between 4 charges, whereas in reality these patterns have to be continued 3 dimensionally.

In any case it is evident that the realization of tricomplex systems will be promoted when the complex relations c and d are more intensive than a and b . This has been expressed in the diagrams by representing a and b by dots, c and d by dashes.

6. *Significance of the density of charge of the participating biocolloids with regard to the tendency to form tricomplex flocculation. Phosphatides as tricomplex components.*

The condition formulated in the preceding paragraph is not the only one, since $c + d + b$ itself also must exceed a certain minimum value in order that a tricomplex system may be formed. The significance of the density of charge discussed in §§ 2, 3 and 4 applies here as well: When the condition $c + d > a + b$ has been satisfied, the tendency to form tricomplex flocculation or coacervation will increase with rising density of charge of the participating biocolloids.

Here we may briefly refer to a seeming incongruity: Tricomplex flocculation was first observed with phosphatide sols and it is more pronounced in egg lecithin than in the phosphatide of soya beans. These phosphatides behave as strong complex components in tricomplex systems, which points to a great density of charge of these phosphatides. These two phosphatides, however, are only weak complex components with regard to the formation of dicomplex colloid systems (e.g. complex coacervation with positive gelatin), which is in perfect agreement with their relatively small density of charge found experimentally. The reciprocal Hexol numbers amount to about 20.000 (egg lecithin) and 4000 respectively (soya bean phosphatide, soluble in alcohol).

This incongruity, however, is only seeming and is due to the complicated conditions existing here, to which we shall refer further on in § 10. It may suffice here to point out that the density of charge, which is of importance to the zwitter ion or multipolar ion for the tendency to form tricomplex systems, is not accessible to direct experimental determination (§ 4). That this is indeed great for phosphatides is apparent from the relatively small molecular weight of about 800. On the other hand, the density of charge which is of importance to the formation of dicomplex systems and may be determined by the method of the reciprocal Hexol numbers is that of

the small surplus of negative charges over the positive charges and in the case of the phosphatide sols it is produced by admixtures (phosphatidic acid, etc.). From this it follows that the phosphatides will tend more to tricomplex formation and less to dicomplex formation according as they are purer, i.e. freer from these negative admixtures.

7. *Realization of tricomplex flocculations of the type I.E. protein or phosphatide + colloid anion + crystalloid cation with small crystalloid cations (e.g. Ca, Li).*

The tricomplex systems can be considered important in biology only when they appear to be realizable with participation of physiologically occurring biocolloids and crystalloid ions and besides can exist at P_H 6—8.

We shall, therefore, first restrict ourselves to the combination:

I.E. protein + colloid anion + Ca ion (cf. fig. 2) and on the basis of the condition $c + d > a + b$ discussed in § 5 find out how the protein and

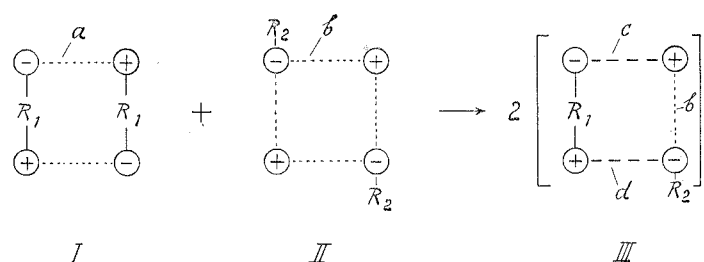


Fig. 2.

colloid anion have to be chosen in order that only the tricomplex system (fig. 2 III) and not the uni- and dicomplex systems (fig. 2 I and II resp.) may be formed. Consequently we shall take an isostable protein (a small), while the choice of the colloid anion has to be such that b is small and besides c as well as d are large.

If we want to produce tricomplex flocculation with Ca, i.e. with a strongly polarizing (for small) cation, it will be in all respects advisable to choose a colloid anion which is much less polarizable than the negative ionized group of the protein. Since the polarizability of the negative groups of the biocolloids decreases in the order:

phosphate group > carboxyl group > sulphate group¹⁾

it will be best to choose a sulphate colloid as colloid anion. Thus it is attained that $c > b$ and besides that the absolute value of b will be small, which reduces the possibility of formation of the dicomplex system: Ca + colloid anion.

However, the complex relation d does not obtain a small intensity by the choice of a sulphate colloid as colloid anion, since the positive ionized group of the protein multipolar ion may be compared to a large organic cation and polarization phenomena do not occur here (§ 3, c).

¹⁾ P. H. TEUNISSEN and H. G. BUNGENBERG DE JONG, loc. cit.

Consequently, in order to make d large, it will be desirable to take a colloid anion with great density of charge.

Indeed the tricomplex flocculation now succeeds already with cations of a low valency (e.g. Ca, Li), if in accordance with the above we take as protein an isostable protein (gelatin, egg albumin) and as essentially negative biocolloid a sulphate colloid with great density of charge (carrageen).

These combinations, as well as the analogous ones with egg lecithin, satisfy completely what has been stated above: In mixtures of I.E. gelatin with sodium carrageen, likewise of I.E. gelatin with $CaCl_2$, or sodium carrageen with $CaCl_2$ no flocculation is formed, but it does take place in mixtures in which gelatin, carrageen and Ca are simultaneously present.

Moreover, it is interesting from a biological point of view that, although the I.E.P. of gelatin is 4.7, tricomplex flocculation still takes place strongly at P_H 6—8 and does not fail until the P_H values amount to about 10 and more.

More elaborate researches on the mentioned combinations and analogous ones, examined in collaboration with C. H. RERING, will in due time be published elsewhere, while some more of the obtained results in connection with this theory will be briefly discussed in the following.

8. *Significance of the nature of the ionogenic group of the colloid anion with regard to the tendency to form tricomplex flocculation.*

Comparative experiments on the intensity of the tricomplex flocculation in the mixtures lecithin + carrageen + Ca and lecithin + pectate + Ca showed that this is smaller in the latter case and still smaller or practically absent in the case lecithin + nucleinate + Ca.

It is true, carrageen, pectate and nucleinate have not exactly the same density of charge, but at any rate in the same order of magnitude and the observed differences in intensity of the tricomplex flocculation can therefore hardly be due to these differences in density of charge.

However, an explanation is found in the different polarizability of the ionogenic groups. This decreases in the following series from left to right:

phosphate group > carboxyl group > water > sulphate group¹⁾.

Owing to the slighter polarizability of the carboxyl group with regard to the phosphate group, in the combination lecithin + pectate + Ca formation of tricomplex systems is still possible but, since Ca + pectate itself produces a dicomplex system, this formation becomes manifest by an increased flocculation in the presence of lecithin. In the combination: lecithin + nucleinate + Ca the factors are not at all favourable to the formation of tricomplex systems. Here also Ca with nucleinate forms a

¹⁾ P. H. TEUNISSEN and H. G. BUNGENBERG DE JONG, loc. cit.

dicomplex system but the nucleinate and lecithin now have the same ionogenic group (phosphate group).

In the presence of lecithin, therefore, the Ca turbidity of the nucleinate is not or hardly increased.

9. *Specific differences of the cations with regard to the tendency to form tricomplex flocculation.*

It is to be expected that, on comparison of small anorganic cations of equal valency, the tricomplex flocculation with egg lecithin + carrageen or gelatin + carrageen will be stronger according as the cation has a greater capacity to produce polarization. Consequently, if we compare the 3 smallest alkali cations, we may expect that the tricomplex flocculation will decrease in the order: $\text{Li} > \text{Na} > \text{K}$, which is indeed the case. In both cases the Li ion is highly active, the Na ion only feebly, while the K ion does not possess the capacity to form tricomplex flocculation.

This enables us to neutralize a tricomplex flocculation formed with Li or Na resp. by means of KCl, so a marked "antagonism" sets in between two monovalent cations. This exceptional position of the K ion is perhaps biologically important.

Of the alkaline earth cations in both cases the Ca ion always has the strongest capacity to form tricomplex systems, the order for lecithin being: $\text{Ca} > \text{Mg} > \text{Sr} > \text{Ba}$ and in the case of gelatin: $\text{Ca} > \text{Ba} > \text{Sr} > \text{Mg}$.

These sequences may be foreseen from the quantitative measurements concerning the concentrations at the reversal of charge of negative lecithin and negative gelatin resp. on the one hand and carrageen on the other, which we will discuss in a following publication.

10. *Tricomplex systems in which by the side of the complex relations still other binding forces exist between the complex components.*

The study of the properties of phosphatide sols revealed that the negative charge which they usually exhibit is due to admixtures, for which may be taken fatty acids and phosphatidic acids, which owing to VAN DER WAALS forces are so intensively bound to the phosphatide zwitter ions that they are only very imperfectly removed by repeated precipitations with organic solvents (e.g. ether, acetone).

With the soya bean phosphatide, insoluble in alcohol, we found the ratio $\text{P} : \text{N} = 2 : 1$ and besides a reciprocal Hexol number of about 800¹⁾. In all probability we have to deal here with a mixture of phosphatide + phosphatidic acid in the proportion 1 : 1.

The perfectly clear sols of this phosphatide preparation coacervate with the alkaline earth metals, but not with NaCl or KCl. Very likely the coacervation with Ca, etc. is a tricomplex coacervation. From what has been said above (§ 8) about the absence of tricomplex flocculation in the combination egg lecithin + nucleinate + CaCl_2 , we should not expect

1) H. G. BUNGENBERG DE JONG and P. H. TEUNISSEN, loc. cit., p. 290.

tricomplex flocculation here either, where the zwitter ion and the phosphatidic-acid anion both have phosphate groups. Where this is found, the cause will be the fact that the constellation, which for the rest is unfavourable from the standpoint of the complex relations, is overcompensated by the VAN DER WAALS forces, which contribute their share to the mutual binding of the complex components.

From this example we see that, in cases where symplex relations occur as well, tricomplex systems may be formed, which from the standpoint of the complex relations only would not be present. This consideration also seems to be of importance in biology.

Summary.

1. A survey is given of the specific factors which are of importance to the intensity of the complex relations between colloid ions mutually and between colloid ions and crystalloid ions, while their significance is discussed with regard to the formation of uni-, di- and tricomplex colloid systems.

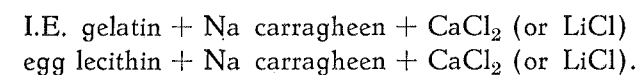
2. The intensity of the complex relations is always promoted by a low equivalent weight (great density of charge) of the biocolloids having part in the complex systems.

3. By means of measurements of the concentrations at the reversal of charge it is possible to obtain data concerning the sequences in which crystalloid cations may be placed with respect to increasing affinity to the colloid anion.

4. In the case of small anorganic cations specific differences in the sequences mentioned in 3. occur between phosphate-, carboxyl- and sulphate colloids, which are based on differences in polarizability of the negatively charged ionized groups of these biocolloids.

5. From theoretical considerations we may expect that tricomplex flocculation, in which a small anorganic cation takes part by the side of a negative biocolloid, will be promoted if we take as negative biocolloid a sulphate colloid of great density of charge.

6. The expectation pronounced in 5. is confirmed, since tricomplex flocculation is found in the combinations:



7. The sequences, to be expected from theoretical points of view, of the alkaline earth cations and alkali cations as regards the intensity of the tricomplex flocculations mentioned in 6. are corroborated by the experiments.

8. Tricomplex systems, which from the standpoint of the complex relations may be called unfavourable, may yet be formed, if symplex relations are simultaneously present to a sufficient extent.

May 1938.

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