

Biochemistry. — *Some instances of tricomplex flocculation. II. The flocculation of egglecithin and carraghene sols with crystalloid cations.* By H. G. BUNGENBERG DE JONG and C. H. RERING. (Communicated by Prof. H. R. KRUYT).

(Communicated at the meeting of May 30, 1942.)

1. *Preparation of the lecithin and carraghene sols.*

For the preparation of the lecithine sols we started from lecithinum ex ovo puris. of "Kahlbaum". 6 gr. of this preparation was dissolved in a tap funnel in 60 cc alcohol + 60 cc aceton and then precipitated with 60 cc dist. water. After whirling the liquid the double refracting precipitation is collected in the froth on top and the main mass of the remaining liquid can be removed by opening the tap. The precipitate is then washed in the funnel with a mixture of 30 cc alcohol + 30 cc aceton + 30 cc H₂O; after removing the washing liquid the precipitate is dissolved in 10 cc aether, the lecithin is then precipitated with 30 cc aceton, after which the superfluous liquid is removed. The latter process with aether and aceton was then repeated twice. The precipitate is then dissolved in 40 cc aethyl alcohol and with the aid of the apparatus described previously¹⁾ and while the solution is being vigorously stirred it is squirted in a thin jet at 70° c into 160 cc dist. water. Then follows dialysis in a dialysator of the type described in the 1st Communication of this series, during 4 hours against 4 l dist. water, and after the water has been replaced by fresh, again during 16 hours. During the dialysis the sol volume increases (e.g. with sol 1 from 200 cc to 252 cc). The sols thus obtained are more opalescent than the sols from soybean phosphatide, however, in a layer of 10 cm they are still quite transparent. The concentration of these sols varied a little, but was about 0.5 %. The carraghene sols of ca. 0.1 % were prepared as described in the first Communication of this series.

2. *Specific effect of the cations in tricomplex flocculation: Egglecithin + carraghene + crystalloid cation.*

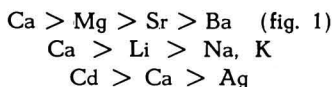
The lecithin sol, when mixed with a solution of CdCl₂, AgNO₃, MgCl₂, CaCl₂, SrCl₂, BaCl₂, LiCl, NaCl, KCl resp. does not flocculate. The carraghene sol behaves in the same way. Neither is there flocculation when the phosphatide sol is mixed with the carraghene sol.

It appears that of the salts examined Ag, Cd, Mg, Ca, Sr, Ba, Ag and Li cause flocculation of the solmixtures but that they remain perfectly clear with Na and K.

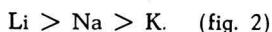
In these combinations we again start from the clear mixture of 2 vol. egglecithin sol + 1 vol carraghene sol. The composition of the mixtures, the turbidity of which was measured after 30 min. was:

a cc salt solution + (23—a) cc dist.water + 2 cc sol mixture, the salt itself, value a and the concentration of the salt solution added being varied.

In a number of different series (partly with different lecithin sol individuals) we found:



In order to determine the sequence of ions Na and K which do not flocculate in a watery medium, we used a weak alcohol containing medium, as in Comm. I (25 vol % alcohol and 2 cc sol mixture in 25 cc final concentration). Thus we found the order:



¹⁾ BUNGENBERG DE JONG and WESTERKAMP, Biochem. Z. **234**, 347 (1931).

So here there is weak tricomplex flocculation also with KCl, in contradistinction to the behaviour of the soybean phosphatide, where it did not occur.

Owing to the greater variation of the concentration of the individual lecithin sols and the greater variability of these sols in course of time, it was not possible to get the same

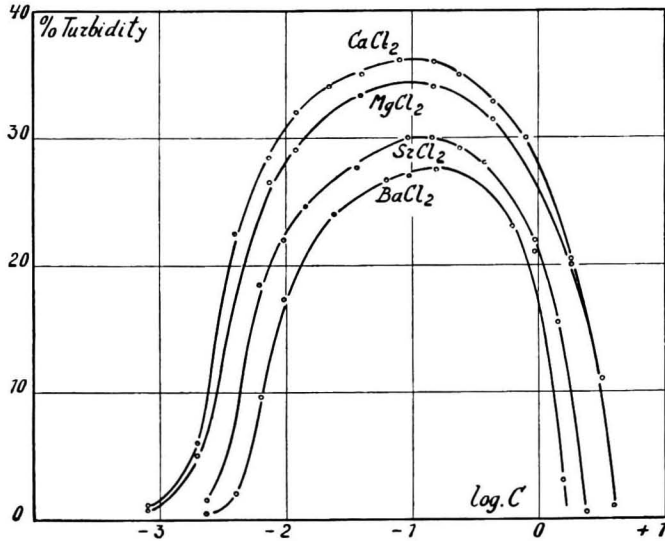
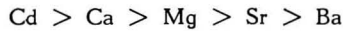


Fig. 1.

certainty as in Comm. I for the soybean phosphatide, of the sequence of all the cations mentioned, from a comparison of the maximal turbidity, but we have certainty as to the sequence of the bivalent cations with



and of the monovalent cations with



The sequences are the same as found in Comm. I and are to be expected here on the

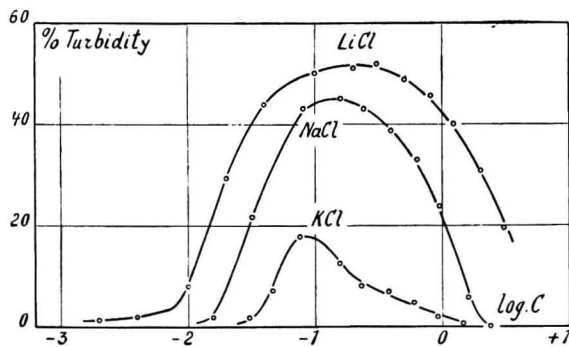
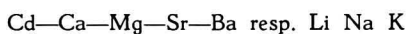


Fig. 2.

following grounds: in Comm. I we saw that the tendency to tricomplex flocculation is theoretically dependent on the value of $\log \frac{C_1}{C_2}$ in which C_1 is the reversal of charge concentration of the caraghene sol and C_2 the reversal of charge concentration of the phosphatide sol.

As the spreading of values C_1 is comparatively small for the various cations, whereas the spreading of values C_2 is very considerable, the sequence of the values $\log \frac{C_1}{C_2}$ for the various cations is practically determined by the order of the values of C_2 . This is apparent when in Table 1 of Comm. I columns 3 and 4 are compared. The sequence of the cations for which $\log \frac{C_1}{C_2}$ increases is there actually the same as in which $\log C_2$ decreases.

As according to previous measurements¹⁾ the reversal of charge concentrations of egglecithin sols also increase from left to right in the following series:



it may be expected that in these orders the degree of tricomplex flocculation must decrease from left to right.

3. *Is the anion of the salt used for the tricomplex flocculation indifferent?*

According to the theory we have advanced that the flocculations occurring here are tricomplex, three charged components:

colloid amphoteric + colloid anion + crystalloid cation are real and hence it would be immaterial if we cause flocculation with CaCl_2 , $\text{Ca}(\text{NO}_3)_2$, CaBr_2 etc.

We may add the following details: the salt curves are in general maximum curves and the indifference mentioned is to be expected most for the left ascending branch of the curves. For the right descending branch (with higher concentration) the generally neutralizing effect of the salts is ever more apparent which is characteristic of salts for complex systems (so here too for the salt which in smaller concentration caused the tricomplex flocculation). As with the neutralizing effect both ions of a salt are active, it is possible that in the right hand curve branches there is a spreading dependent on the anion chosen. When we choose a comparatively weak tricomplex causing cation (e.g. Li), there is the possibility that the anion spreading mentioned last will also manifest itself in the left ascending branch.

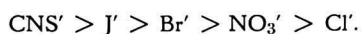
But it may be expected that the latter spreading will be much slighter or entirely absent when we choose a strong tricomplex causing cation (e. g. Ca).

So we chose for the experiment a simultaneous comparison of salts CaCl_2 , CaBr_2 , CaJ_2 , $\text{Ca}(\text{NO}_3)_2$ and $\text{Ca}(\text{CNS})_2$, dividing the programme over two working days. On the first day a comparison of the salts was made of the left ascending curve branch (concentration section from 0.7—100 m.aeq.p.l.), on the second day we made a comparison of the right descending branch (concentration section from 100—3500 m.aeq.pl.).

In conformity with the expectation mentioned above we found of the left ascending branch that the individual salt curves come together within the experimental error: $\text{CNS} = \text{J} = \text{Br} = \text{NO}_3 = \text{Cl}$.

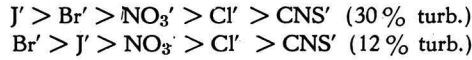
Of the right descending branch specific differences did indeed occur between the anions, the character of which at first sight seems complicated (see Fig. 3 in which the right descending branches are pictured, connected with it on the left — dotted — the ascending branch, alike for all the salts, the separate measuring points being omitted).

As we shall see in the following section, on neutralization with salts of type 1—1 of tricomplex flocculation produced with a constant quantity of Ca or Li the following sequence occurs:



¹⁾ H. G. BUNGENBERG DE JONG and P. H. TEUNISSEN, *Kolloid Beihefte* 48, 33 (1938). Actually the tricomplex flocculation with all the sols mentioned should be measured simultaneously with one lecithin sol individual, in order to arrive at the composition of an uninterrupted series, which is not feasible.

This order is actually also to be expected for the descending branches of Fig. 3. But with a turbidity of ca. 30%, 12% resp. the order is here:



that is, the CNS in the series has shifted to the extreme right, and with higher salt

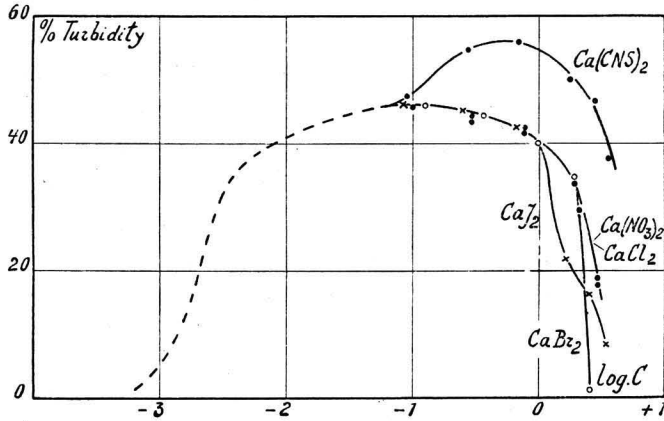


Fig. 3.

concentrations the J ion has also this tendency to shift to the right in the anion series.

Such abnormal behaviour of the CNS and the J ion is to be expected, if the effect of the ions is not exclusively a neutralizing one, but if they also have a tendency to cause with the phosphatide and the Ca ion a tricomplex flocculation of type:

colloid amphoteric + crystalloid anion + crystalloid cation.

This tendency must be very pronounced in the CNS' ion, and in a lesser degree it must also exist in the J' ion.

This tendency does indeed exist, as is seen from the occurrence of flocculation of the egg lecithin sol itself, so in the absence of carraghene, with $Ca(CNS)_2$ and CaJ_2 compare Fig. 4, which refers to mixtures of this composition:

a cc salt solution + (23-a) cc H_2O + 2 cc (2 vol lecithin sol + 1 vol dist. H_2O).

This, therefore, accounts for the anion spreading of Fig. 3.

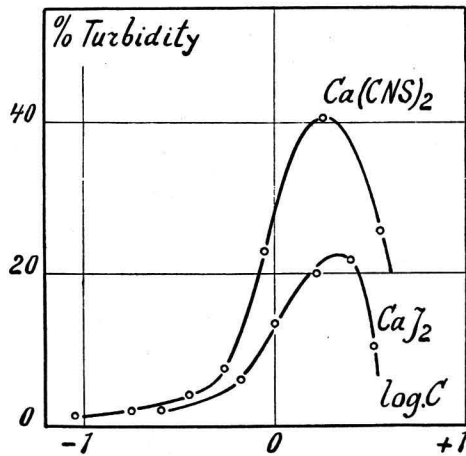


Fig. 4.

4. *Specific cation and anion sequences on the neutralization of Ca, Li resp. tricomplex flocculation.*

In what follows we shall restrict ourselves to the neutralizing effects of salts of type 1—1 near the maximal Ca resp. Li tricomplex flocculation¹⁾. We always added 2 cc sol mixture (2 vol phosphatide sol + 1 vol carraghene sol) to 25 cc final volume.

We began with the experiments announced in Comm. I concerning the tricomplex flocculation with *soybean phosphatide*.

Fig. 5a refers to the neutralization of the CaCl_2 flocculation by LiCl, NaCl and KCl (1 cc CaCl_2 1 N + 2 cc sol mixture 2 : 1 + a cc salt solution + (22—a) cc dist. water);

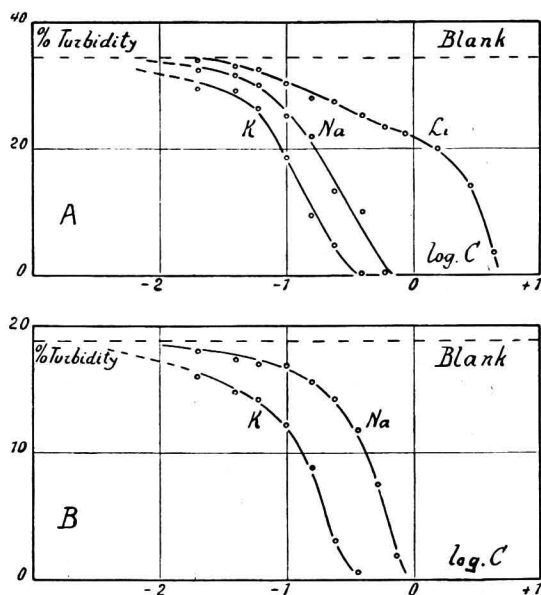
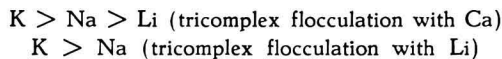


Fig. 5.

similarly Fig. 5b refers to the neutralization of the LiCl flocculation through NaCl and KCl (5 cc LiCl 5 n + 2 cc sol mixture 2 : 1 + a cc salt solution + (18—a) cc dist. water). In these figures the turbidity of the mixtures in which there is only 1 cc CaCl_2 1 N resp. 5 cc LiCl 5 N is indicated by the dotted horizontal line. We see that the cations arrange themselves in the following series, the neutralizing effect decreasing from left to right:



In the same way as indicated above for the neutralization of the tricomplex flocculation with CaCl_2 by KCl, NaCl and LiCl we examined in a second series the neutralization by KCl, KBr, KJ, KNO_3 and KCNS and found that $\text{CNS} > \text{J} > \text{NO}_3, \text{Br}, \text{Cl}$.

So there is no evident difference between the three anions named last, while the neutralizing effect of J is a little stronger and that of CNS markedly stronger than the effect of J.

¹⁾ Properly speaking it was to be expected that on neutralization of the tricomplex flocculation the valence rule of the anions would also occur. Experiments concerning the neutralization of the CaCl_2 tricomplex flocculation with KCl, K_2SO_4 , $\text{K}_3\text{Fe}(\text{CN})_6$, $\text{K}_4\text{Fe}(\text{CN})_6$ showed complications which are possibly owing to the tendency present in some of these anions (e.g. $\text{Fe}(\text{CN})_6'''$) of forming with phosphatide + Ca tricomplex systems of type colloid amphoteric + crystalloid anion + crystalloid cation.

When we proceed to the neutralizing effect of the salts of type 1—1 on the complex flocculation with *egglecthin*, we find there the same cation sequences, while the spreading of the anions is more favourable here, so that the order of all five anions mentioned above now becomes known.

The procedure is entirely the same as described above, except the quantity of LiCl added (2 cc 5 N) to reach the maximal tricomplex flocculation. For the cations we find:

$K > Na > Li$ (tricomplex flocculation with Ca) see Fig. 6a

$K > Na$ (tricomplex flocculation with Li) see Fig. 6b

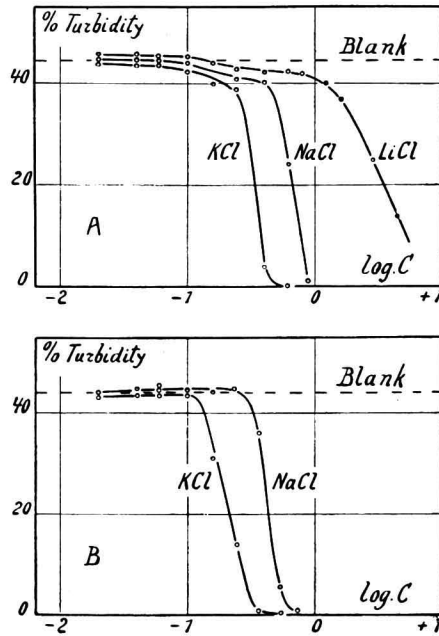


Fig. 6.

for the anions we find:

$CNS > J > Br > NO_3 > Cl$ (tricomplex flocculation with Ca) see Fig. 7a

$CNS > J > Br > NO_3 > Cl$ (tricomplex flocculation with Li) see Fig. 7b.

Previous investigations by BANK and HOSKAM¹⁾ now enable us to draw conclusions from these cation and anion sequences, concerning the way in which the added salt neutralizes the flocculation.

These investigators found namely that on the neutralization of complex flocculation the specific ion sequences occur which are also found on reversal of charge of each of the colloids participating in the complex.

On complexes to which, for instance, positive gelatine takes part they find on the neutralization:



that is to say the same order in which the reversal of charge concentration increases (i.e. the affinity of the anion for the colloid cation decreases)²⁾. The cation sequence on the neutralization of the complex gelatine (positive) + arabinat (negative) resp. + carraghene (negative) is:



¹⁾ O. BANK and E. G. HOSKAM, *Protoplasma*, **34**, 188 (1940).

²⁾ L. TEUNISSEN-VAN ZIJP. Diss. Leiden 1939.

that is to say the order in which from left to right with the carraghene as well as with the arabinatate the reversal of charge takes place with greater difficulty (the affinity

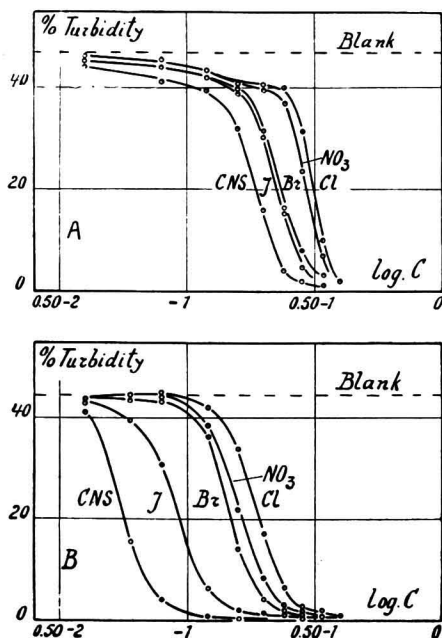


Fig. 7.

decreases). But on neutralization of the complex gelatine (positive) + nucleinate (negative) resp. phosphatide (negative) the sequence is

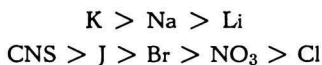


that is the order in which from left to right the reversal of charge of the two phosphate colloids takes place with greater difficulty (in other words the affinity of the cations to the phosphate group of the colloid decreases).

Returning to our problem we have however to consider that with the tricomplex systems there are three "salt-bonds". In the case of phosphatide + carraghene + Ca they are:

- a. Phosphate group of the phosphatide amphotoion..... Ca.
- b. Quaternary ammonium group of the amphotoion..... carraghene.
- c. Carraghene..... Ca.

By screening the addition of salts will cause each of these bonds to become weaker, so that the ultimate neutralization is the result of the three separate actions. But it is likely that the two bonds a and c (crystalloid ion-colloid) are much weaker and therefore more easily neutralized than bond b (colloid-colloid). If this is so the weakening of bond b must dominate the neutralization of the tricomplex flocculation through neutral salts, that is the specific sequences found:



must also fit in with the carraghene and the quaternary ammonium group of the phosphatide amphotoion, in connection with the investigation by BANK and HOSKAM cited.

As a matter of fact the sequence in which the reversal of charge concentrations for carraghene increase is K—Na—Li, while it is highly probable that the order of decreasing affinity of the positive group of the phosphatide is for anions: CNS > J > Br >

$\text{NO}_3 > \text{Cl}$. For it is to be expected that this is the general affinity order for organic bases. Hence it is found with the positive gelatine.

As a matter of fact there are sufficient instances known in organic chemistry in which the iodides and especially the rhodanides of bases are the most insoluble.

Summary.

1. The problems investigated in the first Communication were extended to the combination egglecithin + carraghene + crystalloid cation, practically identical results being obtained.

2. When tricomplex flocculation is brought about with a neutral salt the accompanying anion does not play a part.

3. On the neutralization of the tricomplex flocculation (with Ca, Li resp.) by neutral salts there are specific differences of the cations and anions:



which indicates that the neutralization is determined by the degree in which the bond between carraghene and the positive group of the phosphatide amphotoion is weakened.

4. Egglecithin sol is flocculated by $\text{Ca}(\text{CNS})_2$ and CaJ_2 . This flocculation is of the tricomplex type: amphotoion + crystalloid anion + crystalloid cation.

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