

morphological changes with a thin lamella of coacervate lying directly against the central vacuole. The rest of the coacervate, however, must then vacuolize i.e. the change in the colloid proportion takes place here under the formation in the parietal coacervate of new vacuoles, containing equilibrium liquid.

When, therefore, we observe the colloid proportion in the coacervate it becomes clear that the inflow effect consists in vacuolization, in spite of the fact that the coacervate becomes richer in water at the same time.

We wonder what the effect will be on outflow. On return to the original pH the proportion of the two colloids will return to the original one. Here too we must consider whether the colloid displacement between coacervate and equilibrium liquid may be effected sufficiently rapidly by diffusion only.

As for this, however, we are in a more favourable position than with inflow, because now the parietal coacervate layer is full of vacuoles (containing equilibrium liquid). When for a moment we leave out of consideration the fact that on return to the original pH the coacervate becomes poorer in water, so when we exclusively observe the change of the colloid proportions, two possibilities are seen to exist:

a. the colloid displacement by diffusion is sufficiently rapid, in which case there will be no new vacuolization and so the picture caused on inflow will be retained,

b. the colloid displacement by diffusion is not rapid enough, in which case a new generation of vacuoles must be formed in addition to those formed on inflow.

A summation of the three effects discussed I, II and III may lead to the expectation that the total effect on inflow will be vacuolization in the parietal coacervate and on outflow that the coacervate remains vacuolized (new vacuoles may even form), new coacervate drops forming in the central vacuole.

These expectations are in accordance with the in- and outflow effects observed.

SUMMARY.

1. We studied the morphological changes in consequence of pH variations of an uncharged complex coacervate enclosed in the cells of a celloidin membrane.
2. Vacuolization of the parietal coacervate takes place both on sufficient pH increase and decrease.
3. On return to the original pH the coacervate at first remains vacuolized, while new coacervate drops are formed in the central vacuole.
4. Considering that the uncharged complex coacervate becomes richer in water on increase as well as on decrease of the pH, 2. is unexpected.
5. On pH change the colloid proportion in the coacervate is also modified, as is seen from new data concerning the effect of the pH on the composition of a coacervate and equilibrium liquid at constant proportion of the colloids in the total system.
6. The morphological changes mentioned in 2 and 3 may be understood from the summation of three effects resulting from pH decrease or increase:
 - a. the increase of the water percentage of the coacervate,
 - b. the increase of the colloid percentage in the equilibrium liquid,
 - c. the modification of the colloid proportion in the coacervate.

Biochemistry. — *Effect of neutral salts on the composition of complex coacervate (gelatine + gum arabic) and equilibrium liquid at constant pH and constant mixing proportion of the two colloids in the total system.* By H. G. BUNGENBERG DE JONG and E. G. HOSKAM. (Communicated by Prof. H. R. KRUYT.)

(Communicated at the meeting of November 29, 1941.)

1. First method of investigation.

Although the question put in the title can only be answered directly by analysis, the experiments rescribed in the following pages enable us to answer the question indirectly. We followed two methods, which led to the same conclusion.

The first method is the simplest experimentally, as we avoid the preparation of isohydric gelatine and gum arabic sols, necessary in the second method.

It makes use of the fact that when in a number of mixing proportions of gelatine and gum arabic which are constant within each series, the coacervate volume is determined as a function of the added quantity of HCl, the coacervate volume curve is generally asymmetrical, becoming symmetrical with a certain mixing proportion.

Starting from purified gelatine¹⁾ and gum arabic²⁾ we made 2½% (air dry) mixed stock solutions, such that the proportion of gelatine and gum arabic changes mutually. This mixing proportion we express in what follows in %A (= gum arabic) of the colloid mixture.

In sedimentation tubes provided at their lower ends with narrower cylindrical tubes divided into 0.1 cc, is pipetted 10 cc stock solution and then a cc HCl 0.1 N + (2.5 — a) cc H₂O. After mixing the tubes are placed in the thermostat at 40° and after one night the coacervate volume is noted.

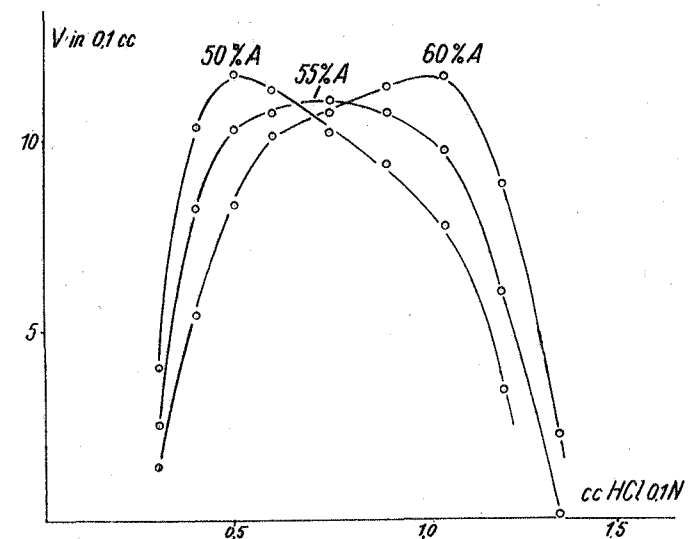


Fig. 1. Shape of the coacervate volume curves in some mixing proportions of the two colloids in the total system.

¹⁾ F00 extra of the "Lijm- en Gelatinefabriek 'Delft'" at Delft, purified by a method described previously (Kolloid Beihefte 43, 256, 1936), a modification of LOEB's method.

²⁾ Gomme Sénégal petite boule blanche I, of Allan et Robert, Paris.

In Fig. 1 are shown the coacervate volume curves for 50, 55 and 60% A. We see that the ones for 50% A and 60% A are very asymmetrical, but that for 55% A has its maximum (0.74 cc HCl 0.1 N) practically in the centre of the HCl concentration section, in which coacervation takes place.

These maxima (determinable by the construction of bisecting lines, see Fig. 2) have shifted considerably to the left in the case of 45 and 50% A (0.46, resp. 0.53 cc HCl 0.1 N), and to the right in the case of 60 and 65% A (0.88 resp. 1.0 cc HCl 0.1 N).

For the investigation of the effect of neutral salts we select a colloid mixture (55% A) whose curve is practically symmetrical with HCl and now we examine whether in the presence of salt the coacervate volume curve becomes asymmetrical.

We shall not here discuss all the experimental material, restricting ourselves to a single instance, see Fig. 2, namely a comparison of a blank series with series in which

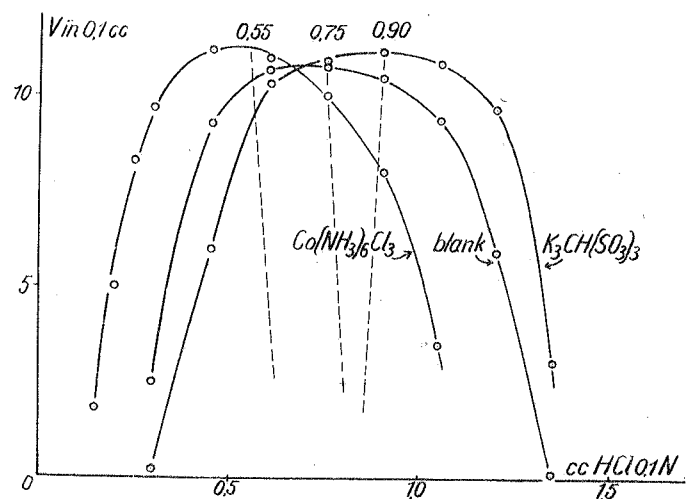


Fig. 2. Effect of some salts on the shape of the coacervate volume curve with constant mixing proportion of the two colloids in the total system.

6 m aeq. p. L. $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ resp. $\text{K}_3\text{CH}(\text{SO}_3)_3$ are present, but in which otherwise the final concentration of the colloids in the mixtures are practically the same as in Fig. 1 (we always use 5 cc 55% A containing stock sol with a total colloid concentration of 5%, to 12.5 cc final volume, so that there is 7.5 cc left for HCl and salt solution).

With the aid of bisecting lines in Fig. 2 we find that the maximum is shifted by $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ to smaller added HCl quantities (0.55 cc), by $\text{K}_3\text{CH}(\text{SO}_3)_3$ to greater ones (0.90 cc) than is necessary in the blank series (0.75 cc).

In this way we measured for 7 salts, each time at 3 concentrations, coacervate volumes as function of the added quantities of HCl, and the position of the maximum was determined graphically. Figure 3 shows the results obtained, from which we see that as regards the shifting of the maximum the salts arrange themselves in the series:

(Increasing pH) $3-1 \dots 2-1 \dots 1-1 \dots 1-2 \dots 1-3$ (decreasing pH).

KCl has here no influence (type $1-1$) and $2-1$ and $3-1$ shift the pH of optimal coacervation to higher, $1-2$ and $1-3$ to lower values.

We would also point out that the order in which the salts are arranged here is the same as occurs in the so-called "continuous valence rule":

(relative positivation) $3-1 \dots 2-1 \dots 1-1 \dots 1-2 \dots 1-3$ (relative negatization)

This rule applies to the effects of salts on the electrophoretic velocity of complex

coacervate drops (at constant pH and mixing proportions of the colloids in the total system)¹⁾.

This rule should not be confused with the so-called "double valence rule":

$$3-1 > 2-1 > 1-1 \\ 1-3 > 1-2 > 1-1$$

which applies to the neutralizing effect of neutral salts on complex coacervation¹⁾. The

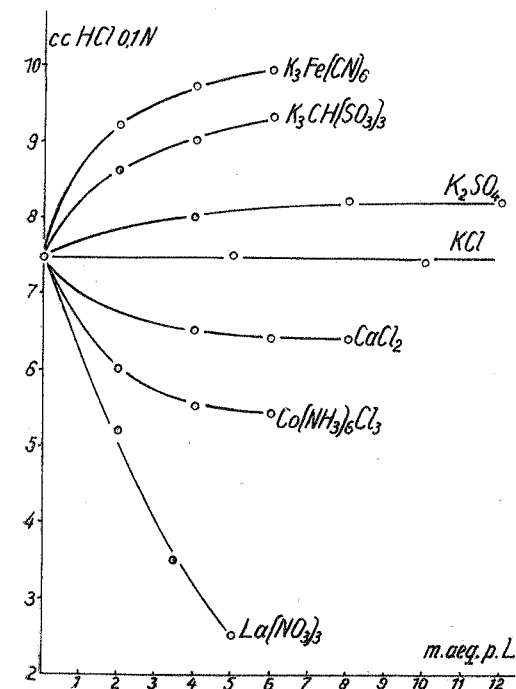


Fig. 3. Shifting of the maximum of the coacervate volume curve (in cc 0.1 N HCl) owing to salts.

experimental material necessary for the above experiments shows for a constant mixing proportion (55% A) and a constant added quantity of HCl (0.75 cc 0.1 N) the following neutralization concentrations (coacervation volume = 0):

	$\text{K}_3\text{Fe}(\text{CN})_6$	$\text{K}_3\text{CH}(\text{SO}_3)_3$	K_2SO_4	KCl	CaCl_2	$\text{CO}(\text{NH}_3)_6\text{Cl}_3$	$\text{La}(\text{NO}_3)_3$
Neutralization with m. aeq. p. L.	9	19	28	33	18	9	6

Also in this neutralization the two salts of type $3-1$ and the two salts of $1-3$ do not act with equal strength, so that here besides the valence of the ions other factors should be considered²⁾.

2. Second method of investigation.

In the second method of investigation the experiments are arranged in such a way

¹⁾ H. G. BUNGENBERG DE JONG and W. A. L. DEKKER, *Kolloid. Beihefte* **43**, 143 (1935).

²⁾ Probably they may be ascribed to coefficients: thus with $\text{K}_3\text{Fe}(\text{CN})_6$ the coacervate layers and equilibrium liquids become rapidly bluish-green and $\text{La}(\text{NO}_3)_3$ causes a decrease of pH (at 5 m. aeq. p. L. the pH of our systems decreases from ca. 3.7 to 3.4).

that we modify the mixing proportion at constant pH, this enabling us to determine the shifting of the mixing proportion of optimal coacervation.

We then made separate stock solutions of the two colloids (5 g air dry + 100 cc dist. water) and determined first for them pH titration curves at 40°, preparing mixtures of the following composition: 20 cc stock solution + a cc 0.1 N HCl + (30 - a) cc H₂O. From these curves we could see how much HCl was to be added in order to prepare isohydric gelatine ($a=3.8$) and gum arabic ($a=2.1$) sols of pH 3.70. We made two such sols, only taking 10 cc H₂O less, the final volume not being 50 cc but 40 cc.

In sedimentation tubes we placed 5 cc water resp. 30 m aeq. salt solution, then b cc gum arabic solution and (20 - b) cc gelatine sol.

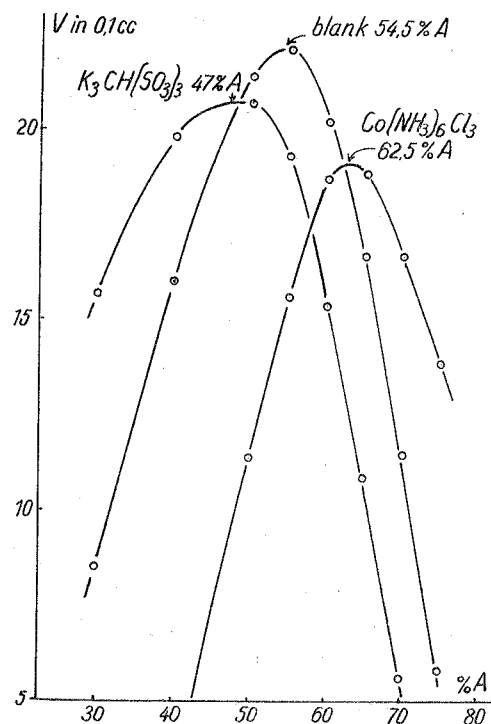


Fig. 4. Shifting of the optimal mixing proportion of the isohydric sols owing to 6 m. aeq. p. L. $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ resp. $\text{K}_3\text{CH}(\text{SO}_3)_3$.

The final concentration of the colloids is now the same as in the determination of the pH titration curves, so that in this way we realize isohydric mixing series. On three successive days we compared in this way blank II - $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ - $\text{K}_3\text{CH}(\text{SO}_3)_3$; blank I - KCl - K_2SO_4 and CaCl_2 - $\text{La}(\text{NO}_3)_3$ - $\text{K}_3\text{Fe}(\text{CN})_6$. The two blank series were slightly different in the absolute values of the coacervate volume, but (construction of a bisecting line) they give practically the same values for the mixing proportion of optimal coacervation (54 resp. 54.5 % A).

Table I contains the results of these experimental series, Fig. 4 showing the curves of a blank series and of the series with 6 m. aeq. $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ and $\text{K}_3\text{CH}(\text{SO}_3)_3$.

Leaving again out of consideration the mutual difference between the two salts of type 3-1 and those between 1-3, we find for the intensity and direction of the shifting of the optimal mixing proportion the series:

(% A increases) 3-1...2-1...1-1...1-2...1-3 (% A decreases)

i.e. the same order at which we had arrived by the first method of investigation.

Again KCl (1-1) has no influence here and the shifting owing to 3-1 and 2-1 is the opposite of that owing to 1-2 and 1-3

3. Agreement of the results obtained by the two methods of investigation.

By the first method of investigation in 1. we found that at constant mixing proportion (being that of optimal coacervation without salt) neutral salts shift the pH according to the *continuous valence rule*, polyvalent cations causing shifting to higher and polyvalent anions to a lower pH:

(increasing pH) 3-1...2-1...1-1...1-2...1-3 (decreasing pH).

From this it may be deduced directly how with constant pH salts affect the mixing

TABLE I.

Coacervate volumes (in 0.1 cc) in the absence and in the presence of 6 m. aeq. p. L. salt as function of the mixing proportion of the two isohydric sols (pH 3.70).

Mix. prop. isohydr. sols in % A	$\text{K}_3\text{Fe}(\text{CN})_6$	$\text{K}_3\text{CH}(\text{SO}_3)_3$	K_2SO_4	KCl	Blank I	Blank II	CaCl_2	$\text{Co}(\text{NH}_3)_6\text{Cl}_3$	$\text{La}(\text{NO}_3)_3$
10	5.3								
20	11.6								
30	16.5	15.7	12.0	8.5	7.7	8.5	3.6	0	
35	18.2								
40	18.6	19.8	18.4	16.7	15.9	16.0	12.8	2.2	
45	17.7								
50	16.4	20.7	21.5	21.1	20.9	21.4	19.6	11.4	
55	13.5	19.3	21.4	21.8	21.4	22.1	20.5	15.6	0.7
60		15.4	18.8	20.5	20.8	20.2	20.6	18.7	4.1
65		10.9	15.2	17.6	16.8	16.7	18.9	18.8	7.6
70		5.6	9.7	12.2	12.0	11.5	14.4	16.7	9.5
75		1.6	4.3	6.5	6.0	5.8	9.7	13.9	9.5
80									7.0
85									4.4
90									1.4
Mix. prop. optimal coacerv.	39.5 % A	47 % A	51.50 % A	54 % A	54 % A	54.5 % A	56.50 % A	62.5 % A	72 % A

Graphically, as discussed above, we determined the position of the maxima, given in the lowest horizontal row of the table.

proportion of optimal coacervation. With this in mind we will again consider Fig. 2: With the mixing proportion selected of 55 % A without salt the maximum of the coacervate volume curve is found to be at 0.75 cc HCl 0.1 N. Now the coacervates at pH values higher, resp. lower than those of the maximum are positively, resp. negatively charged, while the uncharged coacervate is found at or very near the pH of the maximum (corresponding to 0.75 cc HCl 0.1 N).

With the latter pH we are, however, when $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ is present, already on the right descending branch of the curve, i.e. there where the coacervate charge is positive. In order to bring the coacervate at the point of reversal of charge at this pH, we should have to add isohydrically a certain quantity of A (negative gum arabic solution) to the 55 % colloid mixture. From this it follows that in the presence of $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ and at constant pH the mixing proportion of optimal coacervation has shifted to colloid mixtures richer in A.

Analogously we deduce that in the presence of $\text{K}_3\text{CH}(\text{SO}_3)_3$ and with constant pH the mixing proportion of optimal coacervation has shifted to colloid mixtures of lower A percentage.

From this it follows in conformity with the result in 2., that at constant pH salts must affect the mixing proportion of optimal coacervation as follows:

(optimal mix. prop. shifts to systems of higher A percentage) in which 1-1 (KCl) does not affect the mixing proportion of optimal coacervation applying to the blank series.

(optimal mix. prop. shifts to systems of lower A percentage)

4. *Conclusions for the change in composition of a complex coacervate owing to salts at constant pH and constant mixing proportion of the two colloids in the total system.*

From previous investigations¹⁾ we know that — at least when no salt is added — the proportion of gelatine and gum arabic in the complex coacervate varies (with constant pH) with the mixing proportion of the two colloids in the total system. With the mixing proportion of optimal coacervation, the A/G proportion in the coacervate is equal to that of the equilibrium liquid and therefore to that in the total system.

With mixing proportions richer in A than those of optimal coacervation the coacervate also takes up A , but in such a way that the shifting of A/G in the coacervate is less than in the total system. Likewise the coacervate can still take up G from mixing proportions richer in G than those of the optimal mixing proportion, but again the shifting of A/G in the coacervate is less than in the total system.

So there is a certain tendency in the complex coacervate to maintain with constant pH the composition belonging to the mixing proportion of optimal coacervation.

When we remember these properties of the complex coacervates and presume that they persist in the presence of salts, we can deduce how the salts will change the A/G proportion in the coacervate at constant pH and constant mixing proportion of the two colloids in the total system.

Let us suppose that with constant mixing proportion in the total system we select that one which without salt is the optimal mixing proportion. In the presence of $K_3CH(SO_3)_3$ however, the mixing proportion given is no longer the optimal one, but it contains too much A (for the optimal mixing proportion is poorer in A than it was without salt). With the mixing proportion given the coacervate will still take up A , but a relatively limited quantity on account of the persistency discussed above. The consequence will be that in the presence of $K_3CH(SO_3)_3$ the A/G proportion in the coacervate will be smaller than that of the coacervate formed in the absence of salts. In the same way the conclusion is reasoned out that with the same pH and mixing proportion of the sols the A/G proportion in the coacervate formed in the presence of $Co(NH_3)_6Cl_3$ will be greater than in the coacervate formed in the absence of salt. Thus we arrive at the conclusion that the *continuous valence rule* must also be applicable to the modification of the colloid proportion in the coacervate when salts are added at constant pH and mixing proportion of the two colloids, namely:

(A/G increase in the coacervate) $3-1 \dots 2-1 \dots 1-1 \dots 1-2 \dots 1-3$ (A/G decrease in the coacervate)

As we are concerned with constant mixing proportion of A and G in the total system, A/G in the equilibrium liquid will necessarily shift in a reversed sense.

5. *Verification of the conclusion for the case of $CaCl_2$.*

During a previous investigation¹⁾ of complex coacervation we also investigated the effect of $CaCl_2$ on the composition of coacervate and equilibrium liquid (the complex coacervation of gelatine and arabic acid). The investigation really aimed at verifying the increase of the water percentage of a complex coacervate owing to neutral salts, which assumption had been based on different grounds. While the investigation confirmed

¹⁾ H. G. BUNGENBERG DE JONG and W. A. L. DEKKER, loc. cit.

this supposition an additional effect of $CaCl_2$ was noted, namely the shifting of optimal mixing proportion to mixtures of a higher A percentage (so in accordance with 2).

From these analysis results we can also see if — in accordance with what has been discussed in 4. — at constant pH and mixing proportion in the total system, the complex coacervate gains relatively in A by the addition of $CaCl_2$, so whether the equilibrium liquid necessarily becomes poorer in A . The following Table (II) shows the results for the mixing proportion of 45% A .

TABLE II.

CaCl ₂ m. aeq. p. L.	Coacervate			Equilibrium liquid			A/G	
	% A + G	% G	% A	% A + G	% G	% A	coacervate	equilibrium liquid
0	15.90	8.96	6.94	0.39	0.23	0.16	0.77	0.70
5	12.81	7.06	5.72	0.84	0.51	0.30	0.81	0.59
7.5	11.94	6.40	5.50	1.04	0.63	0.37	0.86	0.59 ¹⁾

The table first shows the general effect of a neutral salt: the water percentage of the coacervate increases (% $A + G$ decreases) and the equilibrium liquid becomes richer in colloids (% $A + G$ increases). Moreover it is seen from the last two columns that A/G does indeed increase in the coacervate and that A/G in the equilibrium liquid decreases. The change of $A + G$ as well as of A/G are also clearly visible in Fig. 5, in which the

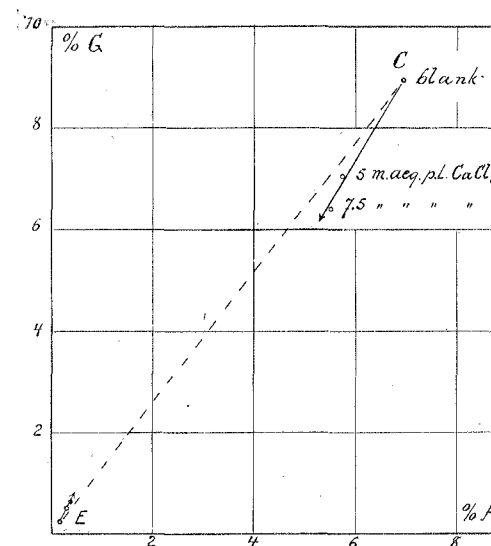


Fig. 5. Effect of $CaCl_2$ on the composition of coacervate (C), resp. equilibrium liquid (E).

¹⁾ The material gathered in the table was taken from three separate experimental series. Owing to the fact that in each of these series the isohydric gelatine and arabic acid sols were of a slightly different concentration, irregularities such as the equality of A/G in the equilibrium liquid for 5 and 7.5 m. aeq. $CaCl_2$ should not be considered real, although these figures are not equal in the coacervate. As a matter of fact the analysis figures of the equilibrium liquid are always much less accurate than those of the coacervate. In all the other mixing proportions (31.6; 35; 40; 50; 51.6%) we shall always see that the $CaCl_2$ increases the proportion of A/G in the coacervate, but from the analysis figures of the equilibrium liquid we see deviations in some mixing proportions from the expected decrease of A/G , which we cannot, however, consider real.

analysis figures (%A and %G) of Table II are set out. In this connection we should notice the courses of the points representing the composition of coacervate (C) and equilibrium liquid (E).

If these points should move towards each other only along the dotted connecting line, this would only prove that the water percentage of the coacervate increases ($A + G$ of the coacervate decreases), likewise that the colloid percentage of the equilibrium liquid increases, without any change being brought about in the proportion of A/G in the coacervate.

The figure shows that the course of the coacervate point deviates downwards from the connecting line, that the course of the point of the equilibrium liquid deviates upwards from the connecting line. This means that A/G increases in the coacervate but that it decreases in the equilibrium liquid.

The case discussed of CaCl_2 is the only one in which analysis of the A and G percentages were made, so that we do not dispose of further material to verify the conclusions drawn in the previous section. On the ground of those conclusions we may expect that for salts 1-1 the coacervate and the equilibrium liquid points will move towards each other, approximately along the connecting line, that for a salt 3-1 the deviations will be in the same direction as for CaCl_2 (2-1) but greater, that for a salt 1-2 and even more for a salt 1-3 the deviation from the connecting line will be the reverse of those for 2-1. Compare scheme Fig. 8.

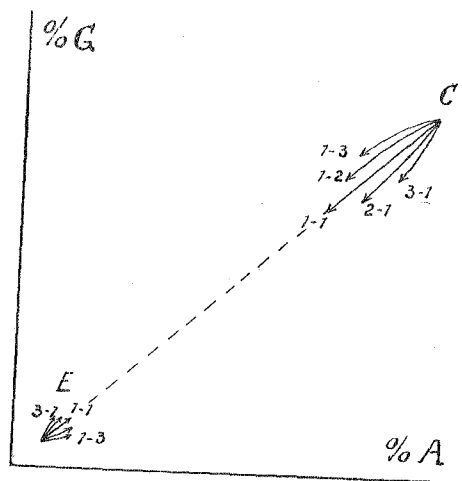


Fig. 6. Scheme of the effect of neutral salts on the composition of coacervate and equilibrium liquid.

Summary.

1. At constant pH and constant mixing proportion of the colloids (gelatine, gum arabic) in the total system the addition of salts causes a change, not only of the water percentage in a complex coacervate, but also in the colloid proportion in the coacervate.
2. The continuous valence rule is applicable to the change of the colloid proportion, namely: 3-1...2-1...1-1...1-2...1-3, in which 1-1 does not modify the proportion, 2-1 and even more 3-1 increase the gum arabic percentage of the coacervate, whereas 1-2 and even more 1-3 increase the gelatine percentage of the coacervate.
3. The proportion of the two colloids in the equilibrium liquid is modified in a reversed sense from that in the coacervate.

Biochemistry. — *Tissues of prismatic cells containing Biocolloids. V. Morphological changes of the complex coacervate gelatine-gum arabic owing to the addition of salts resp. non-electrolytes to the liquid flowing past the membrane.* By H. G. BUNGENBERG DE JONG and B. KOK. (Communicated by Prof. J. VAN DER HOEVE.)

(Communicated at the meeting of November 29, 1941.)

Introduction and methods.

In the previous communication we discussed the effect of a pH change¹⁾. In this communication follow our results as regards the effect of neutral salts and of some non-electrolytes. For colloid preparations employed and apparatus we refer to that communication.

As regards the method, we first brought about coacervation in a newly prepared membrane by conducting past it 0.01 N acetic acid, then we changed to a solution of a salt, resp. non-electrolyte in 0.01 N acetic acid, observing the *inflow effects*. Finally in order to study the *outflow effects* 0.01 N acetic acid is again conducted past the membrane, after which the membrane is removed and replaced by a freshly prepared one.

I. Inflow and outflow effects with neutral salts.

a. Effect of neutral salts on the complex coacervate.

We know that salts have a neutralizing effect on complex coacervation and the more strongly (i.e. with smaller concentrations) in proportion as with unchanging valence (e.g. monovalent) of the cation the valence of the anion increases, likewise when with unchanging valence of the anion the valence of the cation increases. We again checked this rule in our colloid preparations measuring the volume of the coacervate, which demixes on otherwise equal conditions on variation of the salt concentration.

In sedimentation tubes whose cylindrical lower ends were narrowed and divided into 0.1 c.c., we placed 1 cc HCl 0.1 N + a cc salt solution + (6.5 + a) dist. water.

After placing in the thermostat at 40° we added to each tube 5 cc sol mixture (6 g. gum arabic + 5 g. gelatine + 190 cc H₂O), the contents of the tube were mixed and then left in the thermostat. The next morning the coacervate volume was read (estimated in 0.01 cc). The results are given in the following table:

TABLE I. Coacervate volume in 0.1 cc in the presents of salts Blank = 13.1.

Concentr. m. aeq. p. L.	K ₃ FeCy ₆	K ₂ SO ₄	KCl	CaCl ₂	La (NO ₃) ₃
2	12.0	—	—	—	12.5
4	10.5	13.0	13.1	12.8	9.5
6	7.7	—	—	—	2.7
8	5.3	12.2	12.9	10.4	0
10	3.3	—	—	—	—
12	0	10.8	11.9	4.5	—
16	—	8.1	10.8	0	—
20	—	3.9	7.6	—	—
24	—	0	1.8	—	—
28	—	—	0	—	—
Neutralization conc. in m. aeq. p. L.	ca 12	ca 22	ca 25	ca 14	ca 7

¹⁾ H. G. BUNGENBERG DE JONG and B. KOK, Proc. Ned. Akad. v. Wetensch., Amsterdam, 45, 51 (1942).