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.

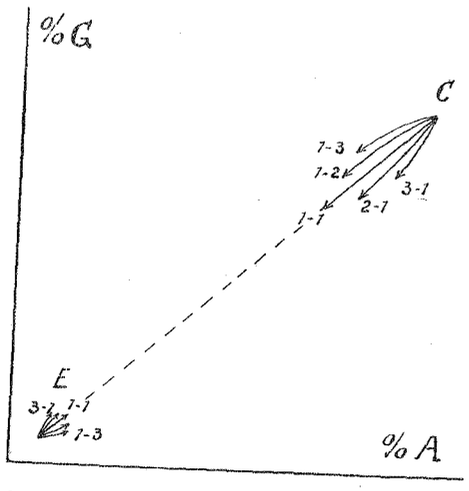


Fig. 6. Scheme of the effect of neutral salts on the composition of coacervate and equilibrium liquid.

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 change [1]). 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 H_2O), 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_3FeCy_6	K_2SO_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).

Graphically the neutralization concentrations mentioned in the lowest horizontal row of the table are found, which proves the validity of the valence rule mentioned:

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

"Double valence rule"

This rule may be foreseen from the screening effect of the cation on the arabinate-colloid anion and of the anion on the gelatine-colloid cation.

As the screening effect increases with the valence of the ions, and the mutual electric attraction of the two colloidions possesses the character of a product, it follows that for neutralization of the complex coacervation the double valence rule must apply. It is further to be expected that *the water percentage of the coacervate must increase in salts concentrations preceding the neutralization.*

This conclusion had formerly been confirmed by analyses in the case of the effect of $CaCl_2$ on complex coacervates of gelatine and arabic acid sols, the results of which we give here (left) for an arbitrary mixing proportion (50 %). From an investigation made lately as to the effect of the temperature we can also take material in confirmation of this conclusion for KCl. There are given the results for an arbitrarily selected temperature (40°) and constant mixing proportion and pH (right).

TABLE II.

Effect of $CaCl_2$			Effect of KCl		
Salt conc. in m. aeq. p L.	% A + G coacervate	% A + G equil. liquid	Salt conc. in m. aeq. p. L.	% A + G coacervate	% A + G equil. liquid
0	15.24	0.35	0	13.20	0.50
5	13.05	0.65	5	12.02	0.69
7.5	12.00	0.85	10	10.90	0.96
			20	8.84	1.63

In the two tables we see that the dryweight of the coacervate decreases (water percentage increases) and the dryweight of the equilibrium liquid increases on increase of the salt concentration. The mutual mixability of the two liquids (coacervate and equilibrium liquid) increases therefore as we approach the neutralization concentration.

b. Expectations as to the nature of inflow and outflow effects based on a).

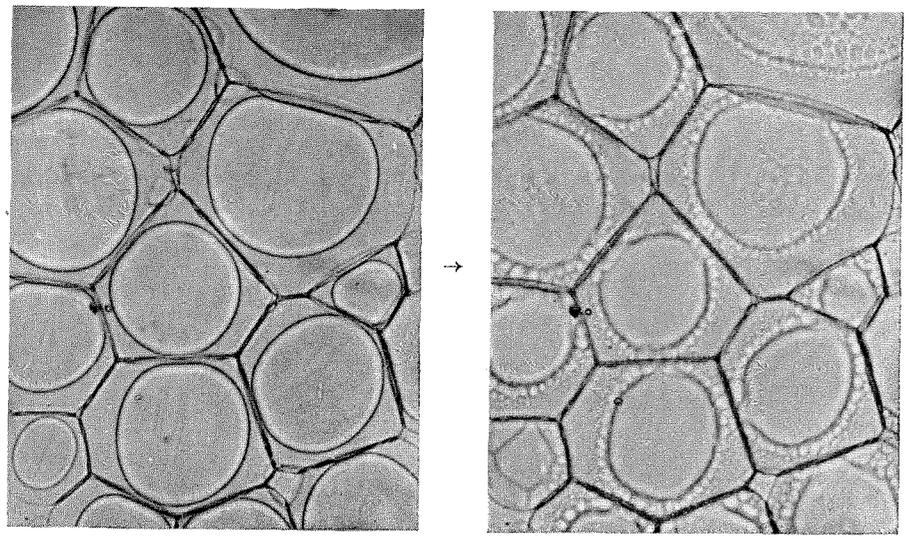
As neutral salts increase the water percentage of the coacervate, while the colloid percentage of the equilibrium liquid also increases, we cannot expect any morphological changes on inflow. On outflow of the salt the waterpercentage of the coacervate decreases again, like the colloid percentage of the equilibrium liquid. Here we can indeed expect morphological changes, namely vacuolization of the parietal coacervate and formation of new coacervate drops in the large central vacuole (which contains the equilibrium liquid).

c. Inflow effects. Distinction of four concentration sections.

It is found experimentally that for each salt four concentration sections may be distinguished, depending on the nature of the morphological processes on inflow. Based as these sections are on the appreciation of microscopic pictures, their limits cannot be indicated. Of course, they pass into each other without any clear demarcation. We distinguish sections:

α. in which no morphological effects occur;
β. in which equally divided vacuolization of the coacervate takes place;
γ. in which the vacuolization is clearly localized or at least begins to appear in certain places of the coacervate;
δ. in which the coacervate entirely dissolves. The following survey gives the salt

PLATE 1.



A *B*

D *C*

In- and outflow cycle with $K_3Fe(CN)_6$.
A: Initial state.
B and *C*: Inflow effect.
D: Outflow effect.

concentrations investigated in milli-aequivalent pL arranged in four columns, corresponding to the concentration sections mentioned.

TABLE III.

Salt	α No vacuolization	β Homogeneous vacuolization	γ Localised vacuolization	δ Neutralization	Neutralization concentration cp. a
$K_3Fe(CN)_6$	0.3	0.75—1.5—3	5—10	15	12
K_2SO_4	1.5	3—5—10—20	(25)	25	22
KCl	5	10—15	20	25—30—40	25
$CaCl_2$	1—2	5	10	15	14
$Co(NH_3)_6Cl_3$	1	3	5	10	—
$La(NO_3)_3$	1	2.5—5	—	—	7

From the survey it is clear that the neutralization concentrations have the same order of magnitude for the complex coacervate enclosed in the celloidin cells as found in *a*.

It is further noticeable that the boundaries between sections α/β resp. β/γ lie at higher concentrations as the salt has greater difficulty in neutralizing the coacervate. These boundaries rise in the order $K_3Fe(CN)_6$—K_2SO_4—KCl, similarly in the order $La(NO_3)_3$ or $Co(NH_3)_6Cl_3$—$CaCl_2$—KCl.

This suggests a connection between the morphological changes on inflow and the neutralizing effect of neutral salts (Double valence rule). But this leads to a contradiction, as neutral salts at concentrations preceding neutralization increase the water percentage of the coacervate, so that no vacuolization is to be expected.

Concentration sections α and δ are not the most interesting to us. With the relatively small salt concentrations in α the attendant internal changes in the composition of the coacervate can apparently be sufficiently brought about by diffusion so that vacuolization is not enforced.

In section δ, morphological processes belonging in γ precede the neutralization in the cases in which in γ the vacuolization processes are very intense and rapid (e.g. $K_3Fe(CN)_6$). When the changes in γ are slower and less intense they are absent in δ. This is the case with KCl at 30 and 40 m. aeq. p. L. with $CaCl_2$ at 15 m. aeq. and with $Co(NH_3)_6Cl_3$ at 10 m. aeq. p. L. So in these cases there is no vacuolization in the parietal coacervate. The central vacuole is generally seen to become rapidly smaller and the bounding face: central vacuole coacervate, which at first was clearly visible is soon obscured. All this is accounted for by the now reversed proportion of the tempo and the intensity of the neutralization and vacuolization processes.

d. Effect of the nature of the salt on the character of the in- and outflow effects.

An instance of marked deviation from the expectations mentioned in *b.* is furnished by the inflow and outflow effects with $K_3Fe(CN)_6$, (salt type 1—3), which will be discussed in connection with four microphotographs of Plate I.

A. Shows a part of the celloidin membrane after the complex coacervate formed with 0.01 N acetic acid has become entirely parietal and free from vacuoles.

B. Shows that after a short period of inflow with 10 m.aeq. $K_3Fe(CN)_6$ containing 0.01 N acetic acid, there is *vacuolization of the parietal coacervate* and that this process sets in along the bounding face coacervate/central vacuole. This vacuolization process now becomes very intensive, extending over the entire parietal coacervate. The vacuoles become larger, flatten each other so that a foam structure is formed. Many foam lamellae burst so that a relatively small number of large foam vacuoles are left.

C. Shows such a foam structure after some time of inflow, after the foam-forming processes have practically ceased.

D. Shows the condition after a short period of outflow with 0.01 N acetic acid. The foam lamellae burst and *no vacuoles are formed in the parietal coacervate*[1]), while a great number of new coacervate drops are formed in the central vacuole. These gradually coalesce with each other and with the parietal coacervate, so that if one waits long enough one sees again the picture of microphotograph *A*.

The picture given in *D* is not different in any other way from the stage also found in the original coacervation with acetic acid 0.01 N and which gradually led to the condition in *A*. So in cycle *A—B—C—D—A* the absence of coacervation in the central vacuole during inflow and the process of coacervation in the central vacuole on outflow are in conformity with the expectation of *b*. The vacuolization of the parietal coacervate on inflow and practically its absence on outflow, however, are not in accordance with what was expected.

A picture entirely differing in many details is given by the in- and outflow cycles with $La(NO_3)_3$ or with $Co(NH_3)_6Cl$ (Salt type 3—1) while the other salts form a gradual transition between these two extremes (1—3 and 3—1) arranging themselves in the series:

$$K_3Fe(CN)_6 - K_2SO_4 - KCl - CaCl_2 - Co(NH_3)_6Cl_3 \text{ resp. } La(NO)_3$$
$$(1—3) \ldots (1—2) \ldots (1—1) \ldots (2—1) \ldots (3—1)$$

Compare fig. 1, which gives a scheme of the actual points of difference between $K_3Fe(CN)_6$, KCl and $Co(NH_3)_6Cl_3$.

The following changes occur from left to right in this salt series:

1. The velocity and the intensity of the vacuolization on *inflow* decrease considerably.

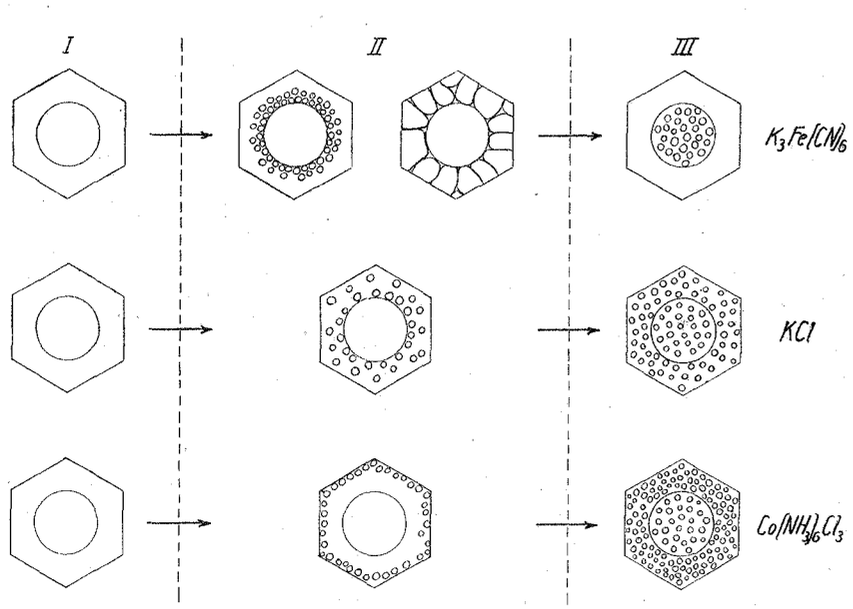


Fig. 1. In- and outflow effects with $K_3Fe(CN)_6$, KCl and $Co(NH_3)_6Cl_3$.
I. initial state.
II. inflow effects.
III. outflow effects.

[1]) On outflow there are some indications of very weak vacuolization. A great number of very small points is formed (probably vacuoles) which, however, soon disappear.

With 2—1 and especially with 3—1 for instance, vacuolization is very slight and slow.

2. The localization of the vacuoles first appearing on inflow in concentration section γ changes gradually. With $K_3Fe(CN)_6$ and K_2SO_4 they occur round the central vacuole, with $CaCl_2$ and $Co(NH_3)_6Cl_3$ on the other hand, round the walls of the cell. In KCl, where this localization process is rather vague, it is perhaps like $K_3Fe(CN)_6$ and K_2SO_4.

3. Secondary changes of the vacuoles formed on inflow soon recede to the background from left to right. Foam formation is very marked with $K_3Fe(CN)_6$, with K_2SO_4 it is possible at most to speak of a passing tendency to foam formation, any other indication with the other salts being absent.

4. The velocity and especially the intensity of vacuolization on *outflow* increase considerably from left to right in the series. With $K_3Fe(CN)_6$ vacuolization is practically absent. With K_2SO_4 it is already fairly noticeable, increasing considerably in the order of KCl—$CaCl_2$—$Co(NH_3)_6Cl_3$. On outflow vacuolization we have not been able to state with any certainty any details concerning localization resp. secondary changes of the vacuoles (analogous to points 2 and 3 above).

e. Details concerning the formation and disappearance of vacuoles.

When the vacuolization is rapid, there are generally at first a great many small vacuoles, whose number usually decreases fairly rapidly owing to mutual coalescence or to coalescence with the central vacuole (the latter process is much retarted with foam formation).

When vacuoles have formed on inflow, and one does not wait till they have all disappeared, these which remain often undergo changes of diminishing volume and (or) number on outflow. This is most evident in small vacuoles.

When inflow is begun with a parietal coacervate which has not yet become entirely free from vacuoles, it is seen that with certain salts (KCl, $CaCl_2$, $Co(NH_3)_6Cl_3$) these vacuoles become smaller or disappear in concentration section α.

All this might be taken as an argument in favour of the "neutralizing effect" of neutral salts, viz. an increase of the water percentage of the coacervate. But such an accelerated disappearance of vacuoles is also seen on outflow after inflow with certain other salts ($K_3Fe(CN)_6$, K_2SO_4, possibly also KCl). In accordance with the expectations in *b*. we may expect vacuolization on outflow, because the coacervate becomes poorer in water. There is no reason therefore for the disappearance of vacuoles which have formed on inflow.

It is even the rule, that when either on inflow, or on outflow there is vacuolization, vacuoles which had formed owing the previous process, nevertheless disappear, while simultaneously or shortly afterwards new vacuolization occurs. We will moreover mention the fact that a new generation of vacuoles arises ever deeper in the membrane, i.e. on the side of the coacervate which is turned towards the medium liquid flowing past the membrane.

Finally we mention that vacuolization processes, especially when they are not intense, are much more noticeable in the larger cells of the celloidin membrane than in small ones. In large cells the rate of the exchange of matter between coacervate and central vacuoles is retarded by the greater thickness of the parietal coacervate layer. A slight change in composition which can be brought about in the small cells with sufficient rapidity by diffusion while the coacervate remains homogeneous, will be too slow in larger cells, so that this change is now brought about under the formation of vacuoles.

f. Discussion.

While describing the in- and outflow effects we have repeatedly pointed out, that the expectations expressed in *b*. based on the effect of salts on the water percentage of a complex coacervate, are not at all in accordance with the effects found experimentally.

There we are rather in the same position as we were in the previous communication

in connection with the effect of the pH. There too we found vacuolizations on inflow, although the water percentage of the coacervate also increases. A solution of the difficulty was given by further observation of the simultaneous change of the colloid proportion in the coacervate.

Analogously the question may here be asked if the fact that on inflow with neutral salts vacuolization practically always occurs, is not to be ascribed to a coeffect of the salt, causing a change in the colloid proportion in the coacervate.

Such a coeffect of $CaCl_2$ on complex coacervates we had observed in a previous investigation of the complex coacervation of gelatine (positive) + arabic acid (negative) [1].

We did not know, however, if this coeffect is also active in the case of gelatine + gum arabic, and if it is also brought about by other neutral salts. As we must know this, however, in order to arrive at an interpretation of the in- and outflow effects described, we lately made some investigations, the result of which was that generally neutral salts cause a change in the colloid proportion of the coacervate with constant pH and constant mixing proportion of the colloids in the total system (these are the conditions under which the in- and outflow effects were studied) [2].

As regards intensity and direction this change depends on the nature of the salts, which arrange themselves in the order:

A/G in the coacervate increases	3—1 ... 2—1 ... 1—1 ... 1—2 ... 1—3	A/G in the coacervate decreases

in which 1—1 has practically no effect on *A*/*G* (= proportion of gum arabic and gelatine in the coacervate) [3].

This is exactly the same order of the salts which we found above in the description of the in- and outflow effects (see *d*).

This takes away in principle the apparent inconsistency that vacuolizations occur on inflow in spite of the fact that the coacervate can only become richer in water in consequence of added salts. For owing to the change of the colloid proportion a certain quantity of *A*. resp. *G*. must moreover leave the coacervate and as the diffusion velocity of the colloids is only slight, this occasions expulsion in the form of vacuoles (in which there separates the new equilibrium liquid belonging to the new colloid composition). Some details in *e*. can now also be accounted for, e.g. the disappearance on outflow of some vacuoles belonging to the inflow generation and the simultaneous or subsequent formation of a new vacuolization. The small vacuoles of the inflow generation embedded in the coacervate disappear then on outflow, because their location is very favourable for reversibility (the colloid containing equilibrium liquid originally expelled is taken up again in the coacervate, the latter using it to recover its original colloid proportion).

The new generation of vacuoles which form on outflow is then to be ascribed to the diminishing of the waterpercentage of the coacervate.

Although, therefore we understand in principle the formation of vacuoles on inflow with salts, there is not yet full accordance. It is true that in the in- and outflow effects we also find the series:

3—1 ... 2—1 ... 1—1 ... 1—2 ... 1—3,

but we did not find that the inflow vacuolization in this series decreases from 3—1 to 2—1, that it is absent or very weak with 1—1 to increase via 1—2 and 1—3.

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[1]) H. G. BUNGENBERG DE JONG and W. A. L. DEKKER, Kolloid Beihefte **43**, 213 (1936).

[2]) H. G. BUNGENBERG DE JONG and E. G. HOSKAM, Proc. Ned. Akad. v. Wetensch., Amsterdam, **45**, 59 (1942).

[3]) Compare in communication IV of this series the very slight effect of KCl on the pH with reversal of charge of the complex coacervate.

This was to be expected from the result of the investigation cited, in which we saw that *A*/*G* in the coacervate increases with 3—1 and 2—1, is constant with 1—1 and decreases with 1—2 and 1—3.

We rather get the impression that for the coacervate in the celloidin membrane it is not salt 1—1 which causes the least change in the *A*/*G* proportion in the coacervate, but that it is salts 2—1 and 3—1 which have that effect.

For the in- and outflow effects caused by these salts come nearest the expectations given in *b* (where we did not take the *A*/*G* change into account); the inflow vacuolization is very weak here and on outflow there is strong vacuolization of the parietal coacervate and formation of new coacervate drops in the central vacuole.

It is not impossible that for the coacervate in the celloidin membrane the shifting of the point of neutralization in the salt series has been moved from 1—1 to 2—1 or to a place between 2—1 and 3—1. For this coacervate is not quite comparable with the one we examined in sedimentation tubes ("vitro"). In the latter we always retain in the total system the Ca salt which is formed from the counterions of the two colloids (Ca ions of gum arabic and Cl, resp. acetate ions of gelatine). But in the membrane this is removed by the medium liquid which flows continually past it. Since, as regards the main problem: the formation of vacuoles on inflow with salts we have yet found a satisfactory solution, there is no point in trying to find an explanation of the many other details observed (e.g. the location of the vacuoles).

We only note that the evident foam formation with $K_3Fe(CN)_6$ is reminiscent of analogous foam formations previously studied, for which we found that they depend on negativation [1]). In this connection we would point out that relative positivation and relative negativation is also brought about with salts and that to this the long-known, so called "continuous valence rule" is applicable, in which the order of the salts is the same as in the series discussed:

relative positivation	3—1 ... 2—1 ... 1—1 ... 1—2 ... 1—3	relative negativation

With the complex coacervate gelatine-gum arabic we found in communication IV (see Table on p. 68) of this series that ("in vitro") KCl is again very near the neutralization point in this series. $K_3Fe(CN)_6$ (1—3) in this series of salts is therefore the strongest negativing salt and probably connected with this in the fact that here the vacuoles formed on inflow undergo the secondary changes mentioned (foam formation).

II. In- and outflow effects with some non-electrolytes.

a. Effects of glucose on the complex coacervate and expectations based on it for the nature of in- and outflow effects.

With the aid of the coacervate volume method we made ourselves acquainted with the effect of glucose (see above Ia). But here we did not work with constant, but with varying pH. Three experimental series were set in, in which we started from a 55 %. A containing sol mixture as employed in *b*. The composition of the mixture in these 3 series was:

A. 5 cc 55 % stocksol + 5 cc dist. H_2O + *a* cc HCl 0.1 N + (2.5—*a*) cc dist. H_2O.
B. 5 cc 55 % stocksol + 5 cc 25 % glucose + *a* cc HCl 0.1 N + (2.5—*a*) cc dist. H_2O.
C. 5 cc 55 % stocksol + 5 cc 50 % glucose + *a* cc HCl 0.1 N + (2.5—*a*) cc dist. H_2O.

When the results are set out graphically (Table IV) it is seen that 10 % resp. 20 % glucose:

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[1]) H. G. BUNGENBERG DE JONG and O. BANK, Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **42**, 274 (1939).

H. G. BUNGENBERG DE JONG, O. BANK und E. G. HOSKAM, Protoplasma **34**, 30 (1940).

a. slightly increases the maximum of the coacervate volume curve;

b. clearly, but not greatly diminishes the section of the added quantities of HCl (so the pH section) in which coacervation takes place.

Increase of the maximum of the coacervate volume curve and change of the coacervation section are phenomena known form a previous investigation to be characteristic of the neutralizing effect of an agent [1]).

From these preliminary experiments we conclude that *10 % and 20 % glucose increase the waterpercentage of the coacervate.*

So the expectations for the nature of the in- and outflow effects wtih glucose are the same in principle as we expressed for salts in I *b*: No morphological effects on inflow and if the increase of the waterpercentage in consequence of the glucose has been sufficient, vacuolization of the parietal coacervate and formation of coacervate drops in the great central vacuole on outflow.

TABLE IV.

Effect of glucose on the coacervate volume (in 0.1 cc).

cc HCl 0.1 N	Blank	10 % glucose	20 % glucose
0.3	0.7	0	0
0.4	7.3	7.7	6.3
0.5	11.6	11.9	11.9
0.6	13.0	13.4	13.6
0.9	13.2	13.6	13.7
1.0	12.8	12.9	12.8
1.2	9.5	7.8	6.1
1.4	0	0	0

b. In- and outflow effects with glucose.

Glucose is practically inactive with concentrations which in the case of electrolytes cause considerable effects (10 milli mol). We must here choose the concentrations much higher to observe notable effects e.g. 10 % glucose (= 5/9 molary): so there is vacuolization of the parietal coacervate in which the vacuoles are preferably formed near the bounding face coacervate/central vacuole. Outflow causes vacuolization which is more intensive and in which the many vacuoles formed rapidly coalesce to a smaller number of larger vacuoles. On outflow there are no changes in the central vacuole.

The microphotographs (see plate II) show a similar cycle:

A. original coacervate formed with 0.01 N acetic acid;

B. inflow effect with 10 % glucose containing 0.01 N acetic acid;

C and *D.* successive stages on inflow with 0.01 N acetic acid. In these microphotographs there is one cell (right upper corner, with circular vacuole), which clearly demonstrates that on inflow the diameter of the central vacuole becomes a little smaller and that on outflow it first becomes smaller again (*C*) and then larger (*D*).

Certain matters of detail, observed with salts also occur with glucose e.g. that on outflow vacuolization small vacuoles belonging to the inflow generation first or simultaneously become smaller or disappear. These effects are clear, however, when the glucose concentration is taken somewhat smaller (5 or 2½ %).

c. In- and outflow effects with other non-electrolytes.

We note here that the same in- and outflow effects occur with aequimolecular solutions (5/9 mol p.L.) of saccharose, glycerine and ethylalcohol. The intensity of the effects greatly decreases in the order glucose — glycerine — alcohol.

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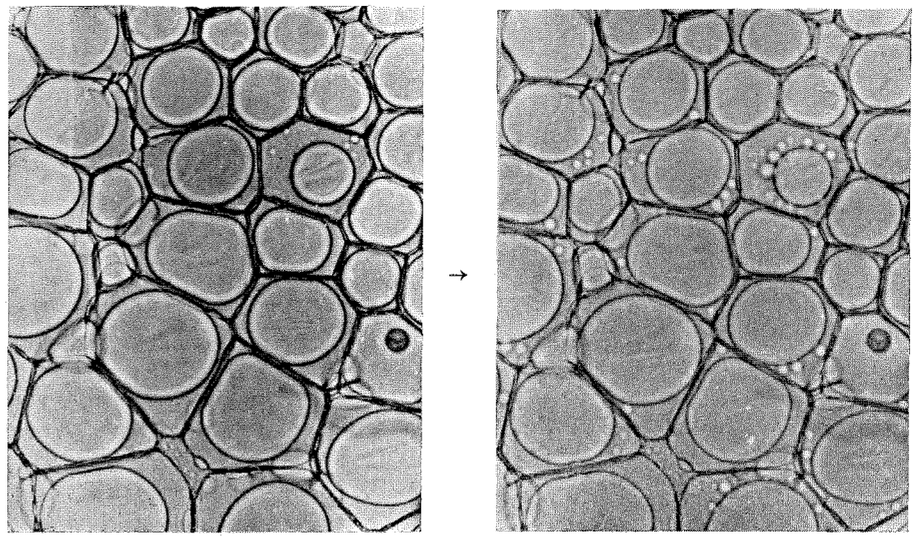
[1]) H. G. BUNGENBERG DE JONG and W. A. L. DEKKER, loc. cit. compare p. 249, Table XI.

PLATE 2



A *B* *D* *C*

In- and outflow cycle with glucose.

A: Initial state.
B: Inflow effect.
C and *D*: Outflow effect.