

Another object was that visually indications may also be obtained as to the increase or decrease caused by variables of the partial mixability of the two coexisting coacervates. Increase (decrease) of it will become manifest as decrease (increase) of the contrast in intensity of the green colours of the two coacervates.

*Summary.*

1. An auxiliary apparatus for the study of morphological changes of coacervate drops is described.
2. We determined the pH section in which coexisting coacervates occur with colloid proportion gelatine : gum arabic : nucleinate = 3 : 1 : 1.
3. The composite drops of simple construction formed under favourable conditions, consisting of the coacervates mentioned in 2. were further studied with respect to dyes, salts, foreign particles and cooling.
4. Dyes effect the coacervate of high nuclein percentage far more than the coacervate of high arabinat percentage. Toluidin blue causes metachromasy.
5. KCl, K<sub>2</sub>SO<sub>4</sub> and K<sub>3</sub>CH(SO<sub>3</sub>)<sub>3</sub> neutralize the coacervate shell of high arabinat percentage on increasing by smaller concentrations, on the other hand CaCl<sub>2</sub> and with smaller concentrations Co(NH<sub>3</sub>)<sub>6</sub>Cl<sub>3</sub> cause strong vacuolization of the enclosed coacervate drop of high nucleinate percentage, until finally it becomes a hollow sphere with a fairly thick wall.
6. Foreign particles are taken up by the composite coacervate drops and localized on the separation plane of the two coacervates.
7. On slow cooling in their own medium vacuoles are formed in the coacervate of high arabinat percentage; they form a wreath round the coacervate of high nucleinate percentage.
8. The behaviour of gelatinized objects obtained by pouring the coacervate into cold water with respect to salts and stains is discussed in detail.

*Leiden, Laboratory for Medical Chemistry.*

**Biochemistry.** — *Specific influence of cations on the water percentage of phosphatide coacervates.* By H. G. BUNGENBERG DE JONG and G. G. P. SAUBERT. (Communicated by Prof. H. R. KRUYT.)

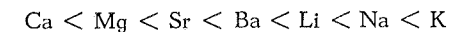
(Communicated at the meeting of February 28, 1942.)

1. *Introduction.*

The phosphatide trade preparations are to be considered as mixtures of phosphatides, phosphatidic acids and impurities (e.g. fats, oils etc.).

Each of these three classes plays its part in the colloid-chemical behaviour of these preparations. The phosphatidic acids are strongly bound to the phosphatides by LONDEN-V. D. WAALS and electrostatic forces and therefore their separation is not brought about by solvents (e.g. solution in aether and precipitation with acetone). Fats etc. are bound by LONDEN-V. D. WAALS forces to a less extent so that solvents can bring about more or less complete separation from the phosphatide-phosphatidic acid mixture. This process, however, causes a great change in the colloid chemical behaviour. Whereas sols of the original preparation flocculate (resp. coacervate) with salts (e.g. CaCl<sub>2</sub>, NaCl, etc.), this does not happen to the sols of the preparation purified with aether-acetone. When for the sol preparation fats, fatty acids etc. are added, these sols recover this property: they are "sensitized". The impurities present in the preparation such as fats etc., therefore play the part of *sensitizers*. The part of the phosphatidic acids is an entirely different one. Whereas the perfectly pure phosphatide (e.g. Egglecithine) has in i.e.p. which lies close to the neutral point, the i.e.p. shifts to considerably lower pH values by a slight percentage of phosphatidic acid. On this account phosphatidic acids give a pronounced "acidoid" character to the phosphatide preparation. It is owing to their presence that especially the cation of the salt is all important for the behaviour of phosphatide preparations with respect to salts.

In sensitized sols (e.g. of unpurified preparations, resp. of purified preparations to which a known sensitizer has been added) the effect of the cations may be studied in connection with the flocculation resp. coacervation phenomena. For each salt there is flocculation resp. coacervation in a certain section of concentrations. It was seen that variations are evident among the cations, in which not only the valency of the cation becomes manifest, but in which there also occur marked specific variations between cations of the same valency. To most of the phosphatides examined the following series applies, in which the concentration of optimal flocculation resp. coacervation increases from left to right:



The same series occurs when electrophoretically (with quartz particles suspended in them) the concentration is determined with which reversal of charge takes place (from negative to positive) of sols of purified phosphatide preparations<sup>1)</sup>. So this concentration is low for Ca and increases in the series mentioned from left to right.

This cation series often occurs in physiological experiments (e.g. concerning the effect of salts on permeability), so that the presumption seems warranted that systems of a phosphatide + phosphatidic acid character take part in the protoplasmic membrane. But there are also indications that cholesterol (sterines) has a densifying effect on the plasmic membrane. It has appeared that cholesterol acts as a strong sensitizer on purified phosphatide preparation, so that the supposition is warranted that the same three classes of substances: phosphatide + phosphatidic acid + sensitizer are intricate parts of the plasmic membrane as are found in the usual phosphatide preparations of

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

trade. Elsewhere — on the ground of experiments — the theory of flocculation with salts of these sensibilized sols has been worked out (autocomplex flocculation, resp. auto-complex coacervation) <sup>1)</sup>, although at present we prefer a slightly different formulation of the systems formed (tricomplex systems). From this theory it may be foreseen that under comparable circumstances (compared each time at the optimal salt concentration, i.e. at the first approximation of the electrophoretic point of reversal of charge) the water percentage of these systems must also increase from left to right in the order of the cation series mentioned. Further, that at a constant and too large CaCl<sub>2</sub> concentration the water percentage must increase on the addition of NaCl.

These two points, which play a fundamental part in the theory of the protoplasmic membrane as autocomplex (tricomplex) phosphatide system, will be further investigated in the following pages.

## 2. Methods.

One of us has worked out a method of preparing phosphatide sols, which with salts produce sufficiently liquid coacervates at room temperature. On account of this they are suitable for comparing the mutual effect of salts with the aid of the determination of the coacervate volume. This method is actually a partial desensibilization of the original phosphatide preparation: 20 g "planticin alcohol solvable 90—95%" of RIEDEL-DE HAËN is shaken with 200 cc 96% alcohol at room temperature, when ca. 85% being dissolved. This solution is poured into an Erlenmeyer of 200 cc which is placed in a thermosflask filled with 1 l water of 5°. After six hours a certain fraction has separated and deposited against the walls and on the bottom. This fraction is sensibilized to a greater extent than the phosphatide remaining in solution. The remaining clear solution is poured out in a thin jet into 800 cc dist. water under constant mixing and the sol obtained is liberated from alcohol by dialysts during 3 to 4 days (Sterndialysator; dialysis at 6°). The concentration of sols obtained in this way is of the order of 1%. It is now seen that a certain subsequent heating treatment is necessary for the sol to produce sufficiently liquid coacervates at room temperature (e.g. heating for ½ hour to 90°, resp. 24 hours to 40°).

The importance of coacervability at room temperature is in the possibility rapidly to execute more extensive series of experiments. For then it is possible after coacervation to centrifuge the sedimentation tubes. This can namely only be done when the centrifuging is done at the same temperature as the coacervation, as the temperature has a very great influence on the water percentage of the phosphatide coacervate and hence on the coacervate volume.

In a series of flasks we made mixtures of the composition: a cc salt solution + (20—a) cc dist. water, adding 5 cc phosphatide sol to each mixture. After shaking each time 5 cc from each mixture is pipetted into two sedimentation tubes. The tubes are placed into the hollows of a wooden block. Four of these blocks, each with 6 sedimentation tubes are then placed in the holders of a large "Ecco" centrifuge, so that 24 tubes can be centrifuged simultaneously (20 min. at 2000 rotations per minute <sup>2)</sup>).

### Results.

#### A. Reversal of charge concentration of sol IV A.

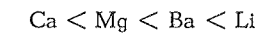
Of mixtures of the composition indicated in 2. electrophoretic measurements were made with some salts. The results are given in the following table:

<sup>1)</sup> H. G. BUNGENBERG DE JONG und R. F. WESTERKAMP, Bioch. Z. 248, 131, 309, 335 (1932).

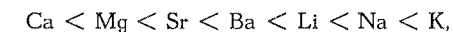
<sup>2)</sup> For further description see G. G. P. SAUBERT, The influence of alcohols on the protoplasmic membrane and colloid models. Recueil des Travaux Botaniques Néerlandais XXXIV, 710 (1937) compare p. 733—755.

Log. C salt C in aeq. p.l.	Electrophoretic velocity			In arbitrarily selected units.
	Ca Cl <sub>2</sub>	Mg Cl <sub>2</sub>	Ba Cl <sub>2</sub>	Li Cl
0.60 — 2	— 63	— 113		
0.78 — 2	— 16	— 42	— 108	
0.90 — 2	+ 18			
0.00 — 1	+ 46		— 50	
0.08 — 1		+ 42		
0.20 — 1		+ 51	— 3	
0.30 — 1			+ 11	
0.60 — 1				— 135
0.78 — 1				— 61
0.00				+ 7
0.18				+ 46
Reversal of charge at log C =	0.83 — 2	0.93 — 2	0.02 — 1	0.97 — 1

So we see that the reversal of charge concentration from left to right increases in the order:



So these determinations were only made for the sake of control, that the order



which we have repeatedly determined for this type of phosphatides (in sensibilized as well as in desensibilized preparations) is not changed by the new method of sol preparation (partial desensibilization).

#### B. Comparison of the coacervate volumes with each other after coacervation of sol IV A with CaCl<sub>2</sub> MgCl<sub>2</sub> SrCl<sub>2</sub> BaCl<sub>2</sub>.

In the following table we give the results (each figure being the average of 2 duplicate determinations, differing no more than 0.2) of coacervate volume measurements with CaCl<sub>2</sub>, MgCl<sub>2</sub>, SrCl<sub>2</sub> and BaCl<sub>2</sub>, in sections of the salt concentration round about the points of reversal of charge.

Coacervate volumes (in 0.01 cc)

Conc. m. aeq. p.l.	CaCl <sub>2</sub>	Mg Cl <sub>2</sub>	Sr Cl <sub>2</sub>	Ba Cl <sub>2</sub>
20	6.0	7.8	—	—
40	5.7	7.2	10.6	11.5
70	5.6	7.0	—	—
80	—	—	9.2	9.5
120	—	—	9.2	9.4
150	5.9	6.8	—	—
160	—	—	9.3	9.7
200	5.8	7.0	—	10.0
300	5.8	7.1	—	—
400	—	—	—	11.4

Theoretically it is to be expected that at or near the point of reversal of charge the water percentage of the coacervate is minimal; further, that at these minima the water percentage will increase from left to right in the order Ca < Mg < Sr < Ba.

As on the coacervation of phosphatide sols these variations of the water percentage are at once reflected in the changes of the coacervate volume:

- A. the coacervate volume curves must be curves with a minimum,
- B. these minima are expected near the reversal of charge concentrations.

This character of the curves is especially evident in  $\text{BaCl}_2$  and  $\text{SrCl}_2$ , but the minimum is much less pronounced in Mg and Ca and the curve branches which rise more suddenly are here outside the section of salt concentrations examined. It is further to be expected that the coacervate volume will increase from left to right in the order:

$$\text{Ca} < \text{Mg} < \text{Sr} < \text{Ba},$$

which was indeed found experimentally (see also Fig. 1).

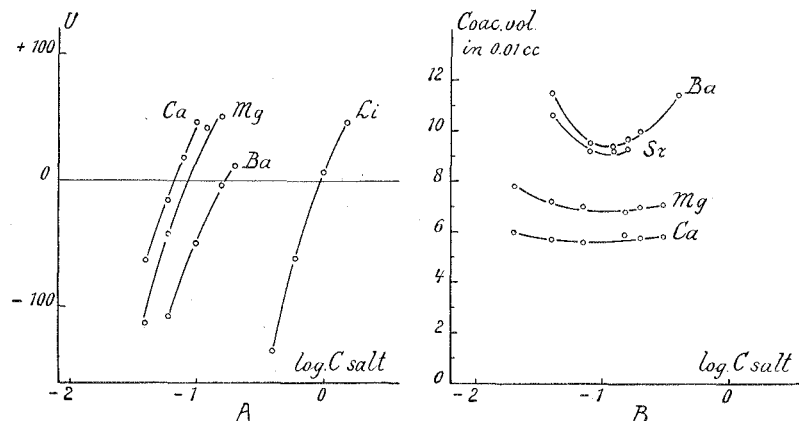


Fig 1.

### C. Comparison of Ca, Mg, Sr, Ba, Li and Na with another sol.

Analogous experiments were made with another sol, only with this difference that in order to obtain larger coacervate volumes, not 5 cc but 10 cc sol was present in the final volume of 25 cc. The results are given in the following table: (See p. 405).

Here we see that although in this series we used twice as much sol, the coacervate volumes are less than twice as large. Compare for instance in the previous table the values for  $\text{CaCl}_2$  (there averagely 5.8 here 7.0 instead of 11.6). This indicates that the sol used here is sensibilized to a greater extent than the previous one. In agreement with this is the fact that the minimal character of the curves is even less marked here. As is seen from the table the only indication of this is the decrease of the coacervate volume with increasing NaCl concentrations.

Unfortunately our technique did not allow of the investigation of higher NaCl concentrations, as with 1040 m.aeq. and higher the specific gravity of the coacervates was less than that of the NaCl-solution, so that they came up on top, instead of being deposited as a layer in the calibrated narrow tube at the lower end of the sedimentation tubes. Hence the expected increase of the coacervate volume with higher NaCl-concentration could not be measured.

As for all the other salts the coacervate volume depends comparatively little on the salt concentration we have taken the average of the coacervate volumes, in order to compare the specific effect of the cations with each other (compare the lowest horizontal line of the table).

So here we do indeed see the expected order of the coacervate volumes:

$$\text{Ca} < \text{Mg} < \text{Sr} < \text{Ba} < \text{Li} < \text{Na},$$

Coacervate volumes in 0.01 cc.

Conc. m. aeq. p.l.	Ca	Mg	Sr	Ba	Li	Na
20	7.4	—	—	—	—	—
40	—	8.0	8.6	8.8	—	—
50	7.0	—	—	—	—	—
70	—	7.8	—	—	—	—
80	7.0	—	8.6	8.6	—	—
100	6.9	7.9	—	—	—	—
120	—	—	8.5	—	—	—
140	—	—	—	8.9	—	—
150	6.5 <sup>1)</sup>	7.9	—	—	—	—
160	6.9	—	8.7	—	—	—
200	7.1	8.0	—	9.0	—	—
240	—	—	8.9	—	—	—
300	7.0	7.9	—	—	—	—
320	—	—	8.8	—	—	—
400	—	—	—	9.4	10.0	—
480	—	—	—	—	—	13.3
600	—	—	—	—	9.7	—
640	—	—	—	—	—	12.9
800	—	—	—	—	9.9	12.7
1000	—	—	—	—	10.0	—
1040	—	—	—	—	—	— <sup>2)</sup>
1200	—	—	—	—	10.0	— <sup>2)</sup>
	7.04	7.92	8.68	8.94	9.92	12.97

indicating that the water percentage increases in this series, which is also the one of increasing reversal of charge concentrations (decreasing affinity of the cation for the phosphatide system).

With the glass electrode we measured the pH of a number of coacervated mixtures, which gave the following results:

$\text{CaCl}_2$	20 m.aeq. = 3.37	200 m.aeq. = 3.37	
$\text{MgCl}_2$	40 " = 3.37	150 " = 3.34	300 m.aeq. = 3.35
$\text{SrCl}_2$	40 " = 3.47	160 " = 3.45	320 " = 3.41
$\text{BaCl}_2$	40 " = 3.44	400 " = 3.37	
$\text{LiCl}_2$	400 " = 3.38	1200 " = 3.34	
NaCl	480 " = 3.35	1440 " = 3.32	

Although there are slight variations in pH it does not by any means follow that the salts themselves have a systematic effect on the pH of the coacervated systems. In this case therefore, the specific cation effect cannot be attributed to the consequences of primarily occasioned pH changes.

### D. Antagonism $\text{CaCl}_2$ —NaCl.

In some series we measured the effect of increasing NaCl-concentrations with constant  $\text{CaCl}_2$ -concentration. The following is the result of the series with 20 m.aeq.  $\text{CaCl}_2$ .

<sup>1)</sup> This value, which is probably incorrect, has been left out of consideration in calculating the average.

<sup>2)</sup> The specific gravity of the coacervates is less than that of the NaCl solution.

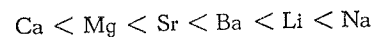
Effect of NaCl on the coacervate volume with 20 m. aeq. CaCl<sub>2</sub>

Conc. NaCl in m. aeq p.l.	Coacervate volume in 0.01 cc.
—	3.35
20	3.3
50	3.6
120	4.0
400	4.6
560	4.4

We see here that as may be expected from the theory of the autocomplex systems, NaCl causes an increase of the water percentage (here = coacervate volume). But this influence will be the less evident, as the CaCl<sub>2</sub> concentration which is kept constant is chosen higher. With 80 resp. 160 m.aeq. CaCl<sub>2</sub> this can no longer be seen as a pronounced increase of the coacervate volume.

## SUMMARY.

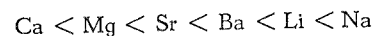
1. We measured the coacervate volumes of phosphatide sols coacervated with salts (chlorides), the order of increasing volume was found to be:



2. This order is the one of increasing reversal of charge concentration.

3. The theory of autocomplex coacervation foresees that in the order of increasing reversal of charge concentrations the water percentage of the coacervate will increase with optimal coacervation.

With phosphatide coacervates the coacervate volume is a measure for the water percentage of the coacervate and as moreover, the reversal of charge concentrations increase in the order:



the results of 1 may be fully expected.

4. With not too great CaCl<sub>2</sub> concentrations the coacervate volume increases with increasing NaCl concentration. This effect (increase of the water percentage) is also to be foreseen from the theory of autocomplex coacervation.

5. The significance of the foregoing for the problem of the nature of the protoplasmic membrane was touched upon.

Leiden, Laboratory for Medical Chemistry.

**Anatomy.** — *Biologic-anatomical Investigations on the Bipedal Gait and Upright Posture in Mammals, with Special Reference to a Little Goat, born without Forelegs.* II. By E. J. SLIJPER (Utrecht). (From the Institute of Veterinary Anatomy of the State University, Utrecht, Holland; Director Prof. Dr. G. KREDIET.)

(Communicated at the meeting of March 28, 1942.)

5. **Length of the ilium, m. gluteus medius.** HOWELL (25) and ELFTMAN (14) tried to demonstrate, that in bipedal Rodents and Marsupials the ilium was proportionally shorter than in their quadrupedal relatives. WATERMAN (64) on the contrary believes, that in upright going Primates the ilium is longer than in quadrupedal monkeys. These authors, however, used either the length of the whole ilium, or the length of the iliac blade as a fixed dimension to compare with the postsacral part of the ilium. For this postsacral part is the only part of the ilium, which is directly connected with the transmission of the body-weight to the supporting leg. My own researches surely showed that only the body-length may be used as a standard dimension, with which the dimensions of the pelvis may be compared.

The data given in table 3 show, that in all bipedal and upright going mammals, with the exception of man, the ilium has been lengthened. In most mammals this lengthening exclusively has been brought about by a lengthening of the presacral part of the ilium (the iliac blade). Only in hanging-climbing mammals the postsacral part too is a little elongated. It is further shown, that the length of the postsacral part of the ilium only to a very small extent depends on statical or mechanical forces. The length of this part is chiefly connected with the demands of space in the pelvis. Together with the length of the sacrum, the width of the lumbo-sacral and the width of the ilio-lumbar angle, the length of the postsacral part of the ilium determines the position of the pelvic inlet. The longer the sacrum and the narrower the ilio-lumbar angle are, the longer the postsacral part of the ilium must be, in order to bring the pelvic inlet in a plane that lies caudal to the last sacral vertebra (see for example *Capra hircus* L. and the Primates).

As we have seen above, in bipedal mammals the ilium has been elongated by an increase in length of its presacral part. This is easy to understand, because the length of the ilium determines the length of the fibres of the m. gluteus medius. In consequence it determines the width of the angle that the upright or semi-upright body can make with the horizontal plane. Hence in the series of climbing, bipedal jumping and hanging-climbing mammals, the length of the ilium and in consequence the length of the gluteal fibres increase gradually. But in man, whose body is perfectly upright and kept in balance on the lower extremities, the ilium is comparatively short and the m. gluteus medius shows a comparatively weak development. The broadening of the ala ilii is connected with the broadening of the whole body in anthropoids and man [SLIJPER (61)].

In the bipedal goat, which could not very easily attain an upright posture since it had no tail acting as a counterweight to the body, one might have expected, that the ilium would have been very long. Table 1, however, shows that this bone is nearly as long as in the control-animal. This may easily be understood since in the goat — as in most Ungulates — the length of the fibres of the m. gluteus medius only to a certain extent depends on the length of the ilium. In the greater part of the Ungulates the muscle originates not only from the ala ilii but also, by the so-called gluteal tongue, from the superficial aponeurosis of the m. longissimus dorsi in the lumbar region cranial to the iliac crest (fig. 4). This gluteal tongue is absent in *Proboscidea* [CUVIER (11), MIALL and GREENWOOD (40), EALES (13)], *Rhinocerotidae* [HAUGHTON (22)], *Camelidae* (own observations) and *Dicotyles tajacu* (L.) [CUVIER (11)]. The tongue is com-