Biochemistry. — *Tissues of prismatic celloidin cells containing biocolloids.* **III.** *a. Behaviour of an embedded complex-coacervate in the electric field. b. Polarisation phenomena.* By H. G. BUNGENBERG DE JONG and D. R. KREGER. (Communicated by Prof. J. VAN DER HOEVE).

(Communicated at the meeting of April 27, 1940.)

1. *Introduction and Methods.*

The method described in a previous communication [1]) for embedding biocolloids in celloidin membranes enables us not only to investigate the coacervation phenomena dependent upon an altered milieu, but also to study the influence of the electric field upon the resulting coacervates.

For this purpose the membranes, spread on a water-surface and fixed on a cuvette, as has been fully described in the earlier communication above-mentioned, were exposed to the influences to be studied and examined under the microscope.

This cuvette has been arranged so that the object-glass can be laid on it with the membrane downwards, and any selected fluid allowed to flow over it and, at the same time, an electric current may be applied. The flushing fluid flows off along the electrodes, so that the electrolysis products are carried away. Fluid and cuvette are so heated that the temperature of the membrane is never lower than 30°.

As objects of study the complex coacervates of gelatine and gum arabic were selected, for reason that this combination, which has been studied exhaustively before [2]), rapidly forms parietal coacervates in the celloidin cells. The stock solution embedded in the tissue was always the same, viz. 6 gr. gum arabic + 5 gr. gelatine + 200 cc distilled water.

In this the coacervation was brought about at different pH, with or without 10 m. aeq. KCl. The pH variation was obtained by allowing acetic acid of different concentrations to flow through the cuvette. Reversal of charge of the complex coacervate at the selected proportion of gelatine and gum arabic is near pH 3.35 (0.01 N acetic acid). The coacervation can be attained at all pH values between pH 4 (0.0005 N acetic acid) and PH 2.65 (0.2 N acetic acid) [3]).

---

[1]) See Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, XLIII, 512 (1940).

[2]) H. G. BUNGENBERG DE JONG and W. A. L. DEKKER, Kolloid Beihefte **43**, 143, 213 (1936).

[3]) Cathaphoretic determination at 36° in a microscopical cuvette yielded pH 3.32 as reversal of charge of the free coacervate drops, and pH 3.28 with 10 m. aeq. KCl.

The influence of the electric field was examined on negative, uncharged as well as on positive coacervates.

For the strength of the direct current field 30 V/cm was taken, as a rule, if there was no KCl in the acetic acid solution used to flush the membrane, and 10 V/cM when 10 m. aeq. KCl was present. The salt accélerates the phenomena very materially, so that, with 10 V/cM and KCl, the process is still appreciably more rapid than with 30 V/cM without the addition of salt.

The phenomena in the electric field were studied on ± 100 membranes. As a rule a new membrane was prepared for each experiment.

The description which here follows of these phenomena has been made as concise as possible, and will be published in more detail at another place (in "Protoplasm").

2. *Phenomena in the electric field.*

These are:

1. If we look through the microscope immediately after the object-glass has been laid on the cuvette, it will be possible, as was earlier described in detail, to see the coacervation taking place.

A current of short duration will show that the droplets and vacuoles in process of formation behave in accordance with the sign of charge of the coacervate, as this is determined by the pH of the flushing acetic acid, i.e. in the case of negative coacervates they migrate to the anode and in the case of positive coacervates to the cathode.

2. When the coacervate has completely formed and has taken up its topographical ultimate position (Fig. 1a), the current being applied, vacuolisation takes place in the parietal coacervate both right and left of the vacuole (Fig. 1 *b*). (See microphoto A.)

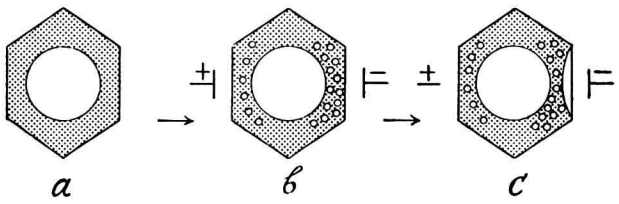


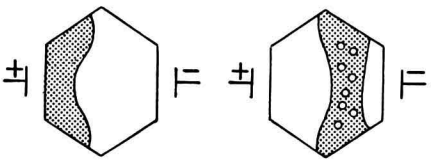
Fig. 1.

The vacuolisation in the coacervate right of the vacuole ("right" is here always the cathode side) is invariably much more marked than that to the left of the vacuole, while, moreover, at the right wall of the cell a secondary vacuole is formed, which expounds to the left (Fig. 1c). This always takes place at the cathode side, irrespective of the sign of charge of the coacervate. See microphoto B.

3. After a short time (± one minute, dependent on the voltage and

presence of KCl), we see in most of the cells containing the positive coacervate, the vacuole on the right and the coacervate on the left (Fig. 2a).

In the case of the negative coacervate, then in most cells the coacervate will be on the right and the vacuole on the left. In this "right" coacervate the vacuolisation described above and the formation of the secondary vacuole continues to take place (Fig. 2b).



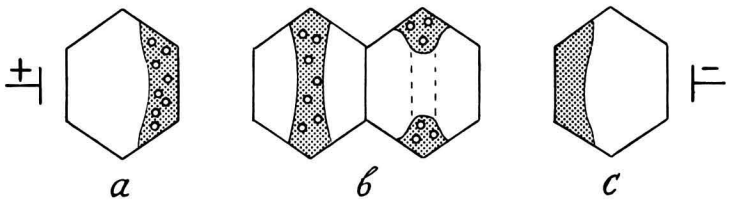
Coac. positive Coac. negative

a Fig. 2. *b*

4. Again, after a short time (± 3 minutes according to the voltage, etc.) a difference will be noticed between the right and left halves of the cell-groups.

Then, in the positive as well as in the negative coacervate, we see in the left half of the cell-groups the coacervate at the right in the cell (Fig. 3a), whereas in the right half of the cell-groups, it will generally be to the left in the cell (Fig. 3c). See microphoto C. The coacervate standing at the right side (cathode) in the cells (Fig. 3a) continues vacuolising, especially if KCl is present; the coacervate on the left side (Fig. 3c) does not do so and is frequently almost vacuole-free.

In the larger cells in the middle of the cell-groups a condition now arises, whereby the coacervate divides the cell, like a partition, into two parts, or stands like two heaps opposite each other. These heaps are often connected by thin coacervate strips along the upper and lower wall of the cell. In Figure 3b these conditions are seen.

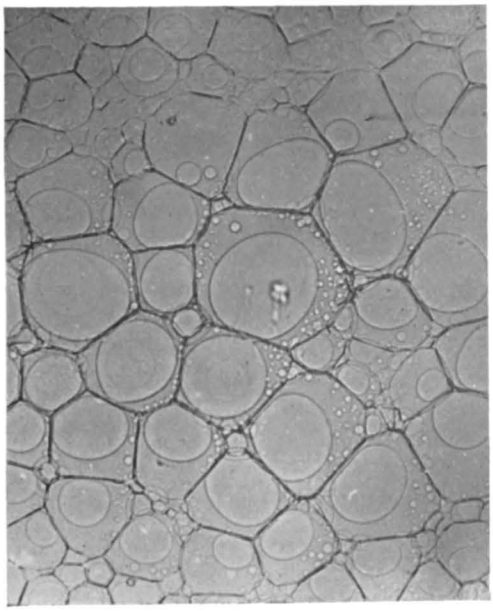


a *b* *c*

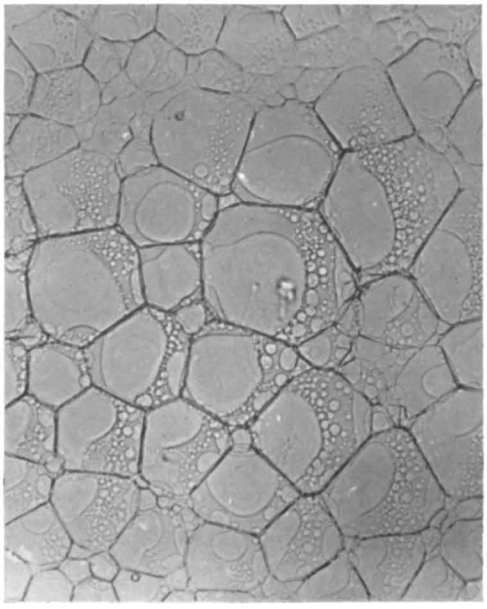
Fig. 3.

It may be mentioned as a peculiarity that the coacervate partitions are a conspicuous feature of nearly the entire cell-group when the polarity is reversed during the process.

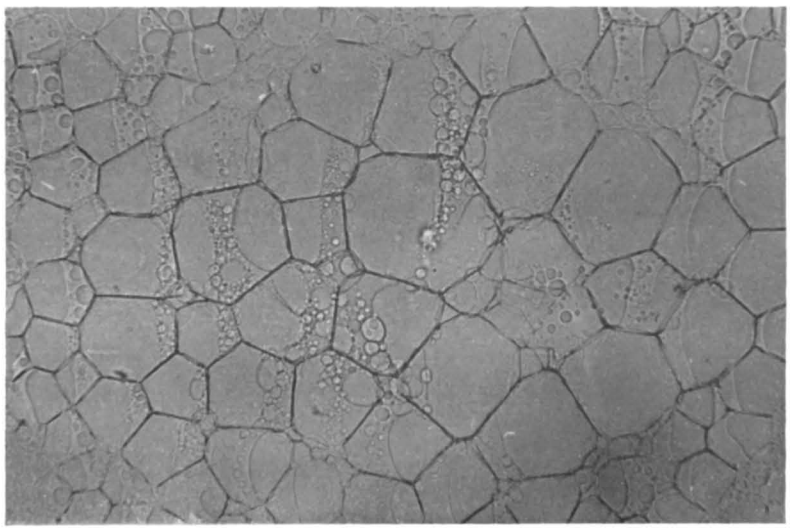
After this, if the current be continued, no fundamental changes take place, except that the coacervate in the cells becomes quantitavely less and less.



A



B



C

3. *Differentiation of the phenomena in the electric field into two groups.*

We have seen in § 2 that the action of the electric field leads to an ultimate condition in which, practically speaking, no further changes occur and in which only the slightest difference, or none at all, between positive and negative coacervate is to be observed. In the course of the process, however, there do appear phenomena which vary with the sign of charge of the coacervate (e.g. the movement of the coacervate droplets and vacuoles during the coacervation process and the position of the coacervate and vacuole with respect to each other in Fig. 2). The phenomena which, in the case of free complex coacervate droplets, occur in the electric field were dealt with fully on a former occasion [1]). It then appeared that in the positive coacervate these are the reverse of those in the negative. Some of the phenomena seen in § 2 may be attributed to this (e.g. the shifting of the vacuoles).

The other phenomena in § 2, and which are the most noticeable ones, are never observed in the case of free coacervate droplets. In this respect it is typical that they are independent of the sign of charge of the coacervate, so that they must be ascribed to the presence of the surrounding celloidin walls.

4. *Working hypothesis concerning the part played by the celloidin walls.*

If we assume that in the electric field concentration differences of the ions, in particular of the H ions, arise between either side of the celloidin walls of the cell-compartiments [2]), the principal phenomena (i.e. those whereby the sign of charge of the coacervate has no part) can be explained.

It is obvious that we may assume that in consequence of the said polarisation, the pH will rise on the anode side of the celloidin membrane but fall on the cathode side (confirmation of this hypothesis will be found in the following paragraphs).

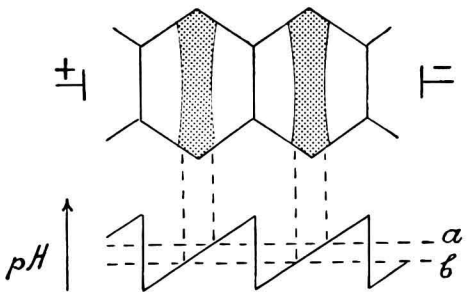


Fig. 4.

[1]) H. G. BUNGENBERG DE JONG and W. A. L. DEKKER, loc. cit. see p. 201—203. See also H. G. BUNGENBERG DE JONG and W. A. L. DEKKER, Biochem. Z. **221**, 403 (1930).

[2]) BETHE and TOROPOFF, Z. physikal. Chem. **39**, 613 (1902).

Considering some contiguous cells, we might expect in every cell-compartment the pH, consequently, to rise from left to right (see Fig. 4).

In connection with the fact that, with a given ratio of gum arabic and gelatine, the complex coacervation is only possible in a certain pH-range (in Fig. 4 between the lines a and b), the typical position of the coacervate in the middle cells of a group during the ultimate stationary condition (Fig. 3b) is comprehensible.

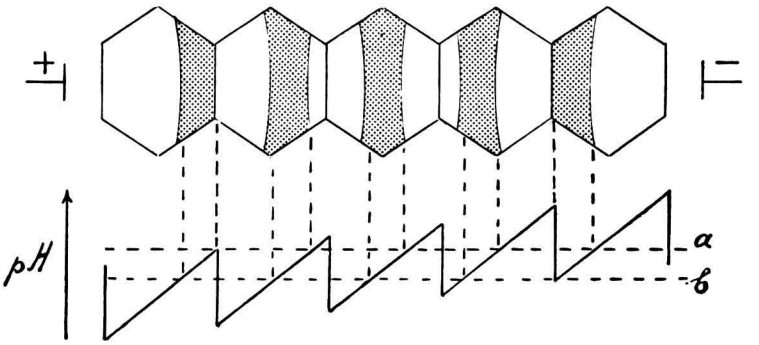


Fig. 5.

In these cells the pH is then too low in the left half, and too high in the right half, and the coacervate can, therefore, exist only in an intermediate zone perpendicular to the field.

If, in the final stationary stadium, we regard, however, not merely the middle cells of a cell-group, but also the cells on either side, we shall come to the conclusion that the average pH in the cells rises from left to right in one cellgroup (left anode, right cathode).

For we notice that in the left half of a cell-group the coacervate is no longer situated in the middle, but at the right side-wall, and similarly that in the right half of a cell-group the coacervate is just at the left side. See fig. 3*a*, 3*c* and microphoto C.

The pH range in which the coacervate can exist is, thus, in the left cells quite at the right side of the cell, i.e. the average pH is lower here than in the middle cells. Inversely, in the right cells the pH range in which there can be a coacervate, is just quite at the left in the cells, i.e. the average pH is higher here than in the middle cells (see Fig. 5).

We cannot discuss here further the other details of § 2, but will merely state that their explanation may advantageously be based upon the two hypotheses discussed above, viz. in consequence of the polarisation of the celloidin walls there develops a) a pH gradient inside each cell, and b) a gradient of the average pH over the whole cell-group.

In the following paragraphs an account will be given of experiments which confirm both these hypotheses.

5. *Macromodel for the origin of pH differences on both sides of a membrane after applying an electric field over the membrane.*

For this a U-shaped tube was used, one arm of which is divided by two perforated plate-glass slides lying one upon the other (see Fig. 6). The upper one is cemented to the upper half of the tube, the lower to the lower half. When a cellophane membrane has been inserted between the slides, the upper part of the tube can be laid loosely upon the lower half.

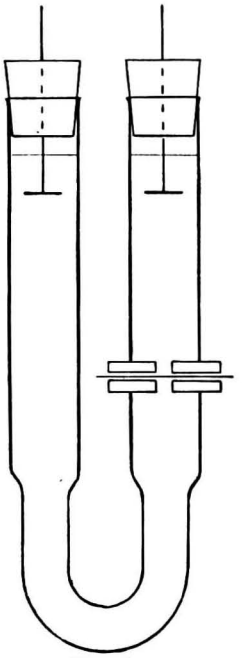


Fig. 6.

The cuvette is filled with a solution of bromthymol-blue in distilled water [1]) up to the upper edge of the slide, the piece of cellophane is laid upon this slide, the upper part then laid down upon it, and the two arms of the tube filled up equally, whereupon 2 electrodes are put into each. To get the potential gradient over the membrane as large as possible, the arms of the cuvette are taken very wide, ± 3.5 cM, and the bore in the slides not more than ± 7 mm in diameter. On the vertical outside edge of both plate-glasses, a spot was made thoroughly transparent, so that any staining in the columns of liquid on either side of the membrane could be observed by looking through the plate-glasses lengthways.

When the current was applied (± 60 V), blue staining appeared in the brownish-yellow fluid on the anode side of the membrane. The same result was obtained with 10 m. aeq. KCl.

This experiment shows that the shifting of the pH in the electric field, as was assumed above in § 4, (the pH rises on the anode side of the membrane) does actually take place, that is to say, with a cellophane membrane. In the following paragraphs we shall see that the same is the case with the membranes of the microscopic cells in the celloidin tissue.

6. *Demonstration of the pH gradient inside the cells and over the entire cell-group by means of bromphenol-blue.*

If the pH differences occurring left and right in the cell, as well as in the cell-group are indeed the cause of the principal phenomena described in § 2, they must be so great that they can be shown with indicators. The difficulty here is to get an indicator with a suitable range in such a high concentration in the cell that this very thin layer of fluid, no thicker than the height of one cell, will allow an observable colour change.

---

[1]) The stock solution: 0.2 gr. bromthymol-blue + 3.2 cc NaOH o.1 N dissolved in 500 cc. distilled water, was diluted five times with distilled water.

No results are to be expected unless an indicator is used which, in one of its forms, is accumulated (adsorbed) in the complex-coacervate gum-arabic-gelatine.

For our purpose bromphenol-blue (pH 3.0 yellow, pH 4.6 blue) proved suitable. From a bromphenol-blue solution, even if pH is such as to have an intermediate shade, or a more pronounced blue colour, the yellow form is nevertheless always adsorbed by the complex-coacervate. The yellow form is the non-dissociated form, and the binding must be looked for here in the general affinity of phenol-groups with proteins. This last results in dehydration, and if we follow the behaviour of the complex-coacervate in the cell-membrane in the electric field, after the yellow staining with bromphenol-blue, we shall also see distinct phenomena which indicate a less aqueous, and thereby a more viscous, state of the coacervate. For this experiment the celloidin membrane was flushed with a mixture of 80 vol. % 0.01 N acetic acid + 20 vol. % bromphenol-blue stock solution [1]). When, after 5 minutes a plainly observable staining is seen (light yellowish-green if looked at microscopically in bluelight (clear blue sky) the experiment proper can be proceeded with, either at once, or after a short rinsing of the cuvette with 80 vol. % 0.01N acetic acid + 20 vol. % distilled water.

When the current was applied there was, according to the rule, a strong vacuolisation on the cathode side. The expounding of the secondary vacuoles to the left was very slow and the coacervate made the impression of being very viscous. After some time partitions of green coacervate appeared in nearly all the cells of a shape which differed in some respects from the coacervate partitions, as these have been described above.

About 5 minutes after the current was applied the vacuole fluid in the right half (cathode side) of the cell began to stain blue in some places, especially in the right cell-groups.

In a short time most of the cells in the right cell-group-halves on the cathode side were coloured distinctly blue. This was the case hardly anywhere in the left cell-group halves. The coacervate generally in the middle of the cells remained green, which, especially at the left side of the partitions and in the cells at the left (anode side) of a cell-group became more a yellowish-green.

Herewith the pH gradient, a) inside the cell and, b) over the whole cell-group, has been demonstrated.

7. *Demonstration of the pH gradient inside the cells and over the entire cell-group by means of resorcin.*

As we stated above, phenols exercise in general a dehydrating effect upon proteins. If the charge of the protein is small, phenols cause floccu-

---

[1]) 0.2 gr. bromidephenol-blue + 2 cc NaOH 0.1 N dissolved in 500 cc distilled water.

lation or coacervation. Also with gelatinsol we find that resorcin brings about coacervation in a fairly limited pH range round about the I.E.P.

We may make use of this to inquire, in another way than with the aid of indicators, whether the assumed pH shifts actually occur in the electric field.

If gelatine only be enbedded in the membrane and it be flushed with a resorcin solution with a pH which lies outside the pH range in which the coacervation takes place, it should be possible that, in applying the field, the pH on *one* side of the cells is raised or lowered to such a degree that it comes within the coacervation area and we shall see the coacervation take place here.

In consequence of the change of the average cell pH from left to right in a cell-group, this phenomenon will be more distinct again in the one half of the group of cells than in the other.

Such a condition can indeed be realised. For this a 5 % gelatine solution in distilled water is embedded in the membrane.

This was then flushed with a 0.01 N acetic acid solution (pH 3.35), and this solution was followed, without any break, by an acetic acid solution of the same concentration in which, however, 4 % resorcin had been dissolved. This solution was passed through the cuvette for 10 minutes, after which there was not yet any coacervation to be seen in the cells. When the current was applied the coacervation appeared in most of the cells in numberless tiny droplets against the wall on the right side (see Fig. 7 A). In the right half of the cell-group the coacervation extended farther towards the middle of the cells than on the left side, whereas in many of the cells quite to the left in the cell-group there was no coacervation.The pH of the wash was lower than the coacervation range. As has been exposed above, the pH will become higher on the anode side of the membranes, on the cathode side lower, when the current is applied. The appearance of the coacervate in the cells on the right side is thus in accordance with this.

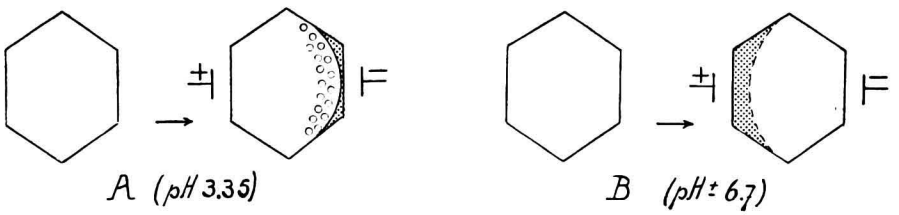


Fig. 7.

Now, taking a medium which has a pH lying above the coacervation area, we should, in accordance with the same reasoning as above, be able, when the current is applied, to bring about coacervation in the left cell-half, since pH is here lowered.

A pH of ± 7 proved to be the best suited for this, since a buffer solution had already been tried in the preceding experiment to attain a certain pH and had yielded no coacervation, possible owing to the eliminating of the pH differences by the buffer; this pH was obtained by neutralising the acid reaction of the 4 % resorcin solution (per 100 cc 10 cc NaOH 0.1 N was added). With this solution bromthymol-blue was greenish-blue.

This solution was washed for 10 minutes over the membrane with embedded gelatine, after which still no coacervation occurred. When the field was applied, coacervation very quickly occurred (± 20 sec.) as was to be expected, at the left of the cells (Fig. 7 B). Moreover, the difference between anode and cathode sides of a cell-group was again observed, namely on the anode (left) side the phenomenon was stronger than on the right side, so that a change of the average pH for the whole cell-group was evinced here in accordance with the hypothesis in § 4.

When the current was cut off the coacervate disappeared within a few seconds.

March 1940. *Laboratorium for Medical Chemistry at Leiden.*