Biochemistry. — *Tissues of prismatic cells containing Biocolloids.* VI. *Location of coëxisting coacervates and equilibrium liquid in the cells. Morphological model of the plant cell.* By H. G. BUNGENBERG DE JONG. (Communicated by Prof. J. VAN DER HOEVE.)

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

1. *Introduction.*

After enclosing a mixture of gelatine and gum arabic sols in the celloidin membrane, coacervation occurs in the cells of the celloidin membrane, when an acid medium liquid is conducted past it. In the final condition we find that the complex coacervate has become parietal and that the equilibrium liquid has collected in one large "vacuole" surrounded by the coacervate [1]). This relative location is reminiscent of the analogous location of cell wall-cytoplasm-central vacuole in the mature plant cell. In the previous communications we have nearly exclusively occupied ourselves with the properties of this object of study (behaviour in the electric field, effect of a variation in the composition of the medium flowing past).

For biologists however, a somewhat more complicated object of study, consisting of two coëxisting coacervates and equilibrium liquid enclosed in the cells of the celloidin membrane might be even more interesting. Previous investigations [2]) have shown that from a suitable selected mixture of gelatine + gum arabic + Na-yeast nucleinate coëxisting coacervates are formed after acidification to a certain pH. These coacervates are distinct from each other because the one contains mostly nucleic acid besides gelatine (and a little gum arabic) the other containing mainly gum arabic besides gelatine (and a little nucleic acid). It appears that after the coacervation the drops of the two coacervates do not float in the equilibrium liquid loose from each other, but that those of the complex coacervate of high nucleinate percentage are taken up in those of the coacervate of high arabinate percentage. This location is analogous to that of nucleus, cytoplasm and surrounding medium in monocellular animal objects.

We first asked ourselves the question what will be the position with regard to each other of the two coacervates and the equilibrium liquid when we occasion complex coacervation of the mixture of the three sols (gelatine, gum arabic, nucleinate) in the cells of the celloidin membrane. Next analogously as in the complex coacervate gelatine + gum arabic we should study the properties of this object. In what follows we mainly communicate our experiences concerning the first question and further we shall discuss why for the time being it is not possible to carry out a deeper systematic investigation.

2. *Difficulties in connection with the enclosure of yeast nucleinate and some other colloids in the celloidin membrane.*

Whereas the method followed thus far for enclosing colloids in the cells of a celloidin membrane yields good results with gum arabic, with gelatine and with a mixture of the two, this is not the case with Na-yeast nucleinate, K-chondroitine sulphate and Clupeine. It is true that it is possible to enclose these colloids, but the celloidin membrane which

1) H. G. BUNGENBERG DE JONG and B. KOK, Proc. Ned. Akad. v. Wetensch., Amsterdam, **45**, 67 (1942). Compare Plate I A.

2) H. G. BUNGENBERG DE JONG und A. DE HAAN, Biochem. Ztschr. **263**, 33 (1933).

encloses the cells on all sides is more or les permeable to them, so that they diffuse to the medium liquid flowing past the membrane with the result that the cells are soon free from colloids.

As is to be expected these colloids also enter the cells when they are dissolved in the flowing medium liquid.

This is evident from the coacervation phenomena in the cells when only gelatine has been enclosed in the membrane and e.g. 0.01 N acetic acid containing very little nucleinate (e.g. 0.1—0.01 %) is conducted past the membrane. When after that only 0.01 N acetic acid is conducted past the membrane the coacervation gradually disappears. From these experiments it appears that the permeability of the celloidin membrane is not due to the presence of Na nucleinate during the formation of the membranes.

Nevertheless the nucleinate has a certain effect on the appearance of the membranes; the cells are not so large and visible defects are more numerous. Disturbances of this nature may cause the disintegration of the colloidin membrane when they become great. We know form experience that they may also proceed form fats and fatlike substances. The appearance of the membranes in which nucleinate has been enclosed improves indeed, when before enclosing it, the Na nucleinate solution is shaken in a separating funnel with CCl_4 which removes possible traces of fat. This preliminary process does not remove the principle difficulty; the permeability of the celloidin membrane to nucleinate.

We have in vain tried to remove the difficulty by the addition of various organic substances to the emulsification liquid (aether + amylalcohol + celloidin). Owing to this failure it is not as yet possible systematically to investigate nucleinate containing colloid mixtures whose mixing proportion is known, in the cells of the celloidin membrane. It is possible to obtain coacervation phenomena with them, but the enclosed system eventually loses nucleinate, more or less rapidly, so that the mixing proportion is continually changing in the direction of systems of lower nucleinate percentage.

3. *Complex coacervation of a mixture of gelatine, gum arabic and yeast nucleinate sols in the cells of the celloidin membrane.*

For the enclosing we used a mixed sol consisting of 3 g gelatine [1]), 1 g gum arabic [2]), 1 g Na-yeast nucleinate [3]), 250 cc dist. water.

This stock solution was first shaken with CCl_4. For enclosing we used a 2½ % solution of celloidin in amylalcohol + aether (1 : 1). For the further technique of the preparation of membranes [4]) and method of investigation we refer to previous communications [5]).

The best results were obtained by at a comparatively low temperature (the water leaving the cuvette is ca. 30°, so 2° to 3° higher in the cuvette) conducting past the membrane a buffer consisting of 10 m. aeq. p. L. Na acetate + 100 m. mol. p. L. acetic acid. Complex coacervation then occurs, of which a number of morphological pictures is given in fig. 1.

In fig. 1A the vacuolized complex coacervate of high nucleinate percentage forms a cohering mass hung in strings from the coacervate of high arabinate percentage (the vacualization is not indicated in the figure). This condition is seen in the early stages of complex coacervation when the celloidin membrane has been prepared from an emulsion of the colloid stock solution in the ether-amyl alcoholid celloidin solution, which has been left at room temperature for some considerable time. The emulsified drops of the

1) Gelatine for bacteriological purposes of the "Lijm- en gelatinefabriek Delft" at Delft.

2) Gomme senegal, petite boule blanche I" of the firm of Allan et Robert, Paris.

3) Na-nukleinat aus Hefe, of Schering-Kahlbaum, Berlin.

4) H. G. BUNGENBERG DE JONG, B. KOK and D. R. KREGER, Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **43**, 512 (1940).

5) H. G. BUNGENBERG DE JONG and B. KOK, loc. cit.

colloid stock solution have then apparently passed into a condition of gelation. Such pictures in which a central mass is connected with the wall by strings has been previously described for the complex coacervate gelatine + gum arabic and there the conditions for the formation were identical. The only difference is that here the central mass consists

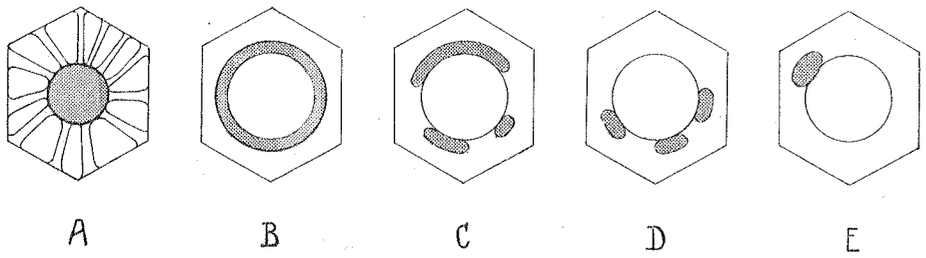

Fig. 1.

of one large cohering mass of the coacervation of high nucleinate percentage or of a greater number of smaller masses packed closely together, which becomes apparent on staining with methylgreen [1]).

The formation of strings is the outward sign that the coacervate is not, or rather, not yet in a very liquid condition, but in a plastic intermediate stage between liquidity and solid gelation.

Therefore the picture does not remain like this with the temperature employed; at least the coacervate of high arabinate percentage becomes very liquid, whereas the coacervate of high nuclein percentage becomes liquid, but remains highly viscous. In course of time the other pictures of fig. 1 may arise, which otherwise will also arise when the emulsion used in casting the membranes has been left at room temperature during a shorter period of time.

After a confusion of forming coacervate drops those of the complex coacervate of high arabinate percentage soon coalsce to one coacervate which in course of time becomes free from the vacuoles first enclosed. For the location of the coacervate of high nucleinate percentage (grey in the figure) we then find the pictures of fig. 1 B—E, that is to say it is always located against the central vacuole. From the discussion in 5 c it may be concluded that the condition of fig. 1 B, in which the central vacuole seems quite surrounded by a layer of the coacervate of high nucleinate percentage is only a picture which may occur incidentally when the preparation is sharply focussed at a certain depth.

4. *Changes in course of time.*

The pictures of fig. 1 B—E are changed gradually in course of time, because nucleic acid is continually removed from the cell. As coëxisting coacervates in the gelatine — gum arabic — nucleinate system at a given pH are only possible in a certain section of mixing proportions of the three colloids, the relative mixability of the coëxisting coacervates increases on continued withdrawal of one of the three colloids (here nucleinate) and when too much nucleinate has been drained off coëxisting coacervates are no longer possible. Before this occurs the coacervate of high nucleinate percentage becomes richer in water,

[1]) In a medium of only diluted acetic acid methylgreen strongly stains the coacervate of high nuclein percentage, whereas the coëxisting coacervate of high arabinate percentage is weakly or not at all stained. When to the buffer used here we add a little methylgreen the coacervate of high nuclein percentage is very weakly stained, the coacervate of high arabinate percentage is not stained. The great decrease of the intensity of the staining is due to the presence of a salt (Na acetate) in the medium liquid.
Compare communication II.

which may find expression in the microscopic picture as a (not very striking) increase of volume of the coacervate of high nucleinate percentage. Soon however the decrease of volume of this coacervate preponderates in consequence of the increased mutual solubility of the two coacervates.

So when the medium liquid continues to be conducted past the membrane we see that gradually the volume of the coacervate of high nucleinate percentage decreases and finally disappears altogether.

When the coacervate of high nucleinate percentage is at first divided into several cohering masses (fig. 1 C and D) these do not disappear simultaneously, but the mass which was originally largest continues longest, so that the condition of fig. 1 E, — in which there is one coacervate drop of high nucleinate percentage on the boundary of the coacervate of high arabinate percentage and the large central vacuole — is always passed.

5. *Discussion.*

a. What will be the final condition when the nucleinate cannot be removed?

In 4. we have seen that pictures as in fig. 1 E are always passed on dissolution of the complex coacervate of high nucleinate percentage which is divided into several distinct masses.

The condition pictured in fig. 1 E however acquires a more fundamental significance in view of the following considerations. When we suppose that it is experimentally possible to make the celloidin membrane sufficiently impermeable, so that no nucleinate is lost, it is to be expected that after sufficient duration a condition will yet arise as in fig. 1 E. The coacervate of high nucleinate percentage divided into several cohering masses in fig. 1 C and D possesses greater boundary face energy than if it had coalesced to one cohering mass.

With the comparatively low temperature (ca. 33°) at which we have worked the coacervations are however so viscous, that convection currents in the cells become extremely slight so that the separate masses have no opportunity of coming into contact and coalescing.

b. Morphological model of the plant cell.

The morphological picture of fig. 1 E, which as we have seen in *a* would be the final condition if the celloidin membrane was not permeable to nucleinate is much like that of a mature plant cell. There too a cohering body rich in colloids — the nucleus — is embedded in a liquid rich in colloids — the cytoplasm — which encloses another liquid poor in colloids — the central vacuole.

c. The location of the coacervate of high nucleinate percentage against the central vacuole.

As regards one detail however we are not sure if the analogy discussed in *b* is also applicable here, namely the question of the complete embedding of the nucleus in the cytoplasm, i.e. that the nucleus though it may be pressed against the vacuole, must yet be separated from the vacuole liquid by a thin layer of cytoplasm, even if this layer is so thin as to be invisible. We have not yet been able to observe such a covering of the coacervate of high nucleinate percentage by a visible film of the coacervate of high arabinate percentage. So there are here two possibilities:

a. There is here nevertheless an invisible layer of coacervate of high arabinate percentage (I), between the coacervate of high nucleinate percentage (II) and the equilibrium liquid (see scheme fig. 2 A),

b. part of the surface of the coacervate of high nucleinate percentage lies immediately against the equilibrium liquid (see scheme fig. 2 B).

This alternative means that either there is "three phase contact" (B) between the two

coëxisting coacervates and the equilibrium liquid, or there is not (A). But complications may have arisen owing to the facts that we made the experiments in the celloidin membrane at a comparatively low temperature, and that in consequence of their approaching

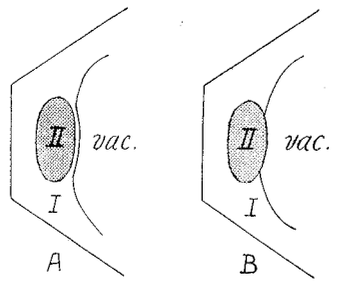

Fig. 2.

a condition of gelation the coacervates at least the coacervate of high nucleinate percentage was in a highly viscous intermediate stage.

We have therefore verified if an unmistakable answer to the alternative may be obtained at higher temperature and apart from possible complications of enclosure in the celloidin membrane.

When at 40° coacervation is caused by adding to 10 cc of a stock solution of colloids (3 G + 1 A + 1 N + 100 H_2O) 20 cc of the in 3) mentioned buffer and when the composite drops are viewed through the microscope on a starched object glass at 40°, the pictures of these drops are usually as shown in fig. 3 A the coacervate of high nucleinate percentage is surrounded on all sides by the coacervate of high arabinate percentage. But when the coacervated system is seen in a cuvette through a microscope turned horizontally, it appears that again the coacervate of high nucleinate percentage is pressed against the boundary of the coacervate of high arabinate percentage and the surrounding equilibrium liquid (fig. 3 B). Owing to the fact that the coacervate of high nucleinate percentage has greater specific gravity than the coacervate of high arabinate percentage, the centre of gravity of the composite drop will be below the point of application of the upward pressure and so the composite drops viewed horizontally must take up the

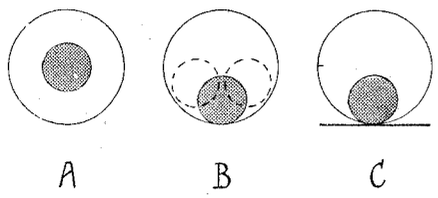

Fig. 3.

position of fig. 3 B in the equilibrium liquid (or at least they will have to fluctuate round the position of fig. 3 B owing to convection currents in the equilibrium liquid). When such composite drops sink from the surface of a starched object glass (starching prevents the coacervate from running over the glass surface), they will lie on it as in fig. 3 C, which corresponds with the picture of fig. 3 A when the drops are viewed vertically.

From the above it is clear that also apart from possible complications owing to the celloidin membrane and at temperature at which the coacervates are sufficiently liquid, the location of the two coëxisting coacervates and of the equilibrium liquid will be again such that we are confronted with the same question; is there "three phase contact" or

does an extremely thin layer (or film) belonging to the coacervate of high arabinate percentage yet separate the coacervate of high nucleinate percentage from the equilibrium liquid. We do not dispose of further data which enable us to answer this question either positively or negatively.

Summary.

1. A suitable selected mixture of gelatine, gum arabic and Na-yeast nucleinate sols is enclosed in the cells of a celloidin membrane and coacervation is caused by conducting past the membrane a liquid of suitable pH.

2. The positions with regard to each other of the two demixing coëxisting complex coacervates and the equilibrium liquid is observed. In principle we find here analogy with the morphology of the mature plant cell. The coacervate of high arabinate percentage (analogous with the cytoplasm) becomes parietal and surrounds the equilibrium liquid (analogous with the central vacuole) while the coacervate of high nucleinate percentage (analogous with the nucleus) is embedded in the coacervate of high arabinate percentage.

3. The coacervate of high nucleinate percentage is pressed against the boundary of equilibrium liquid and coacervate of high arabinate percentage, as more or less rounded bodies. It is uncertain whether there is here "three phase contact".

4. Owing to the fact that the celloidin membrane is not sufficiently impermeable and gradually allows nucleinate to diffuse, the coacervate of high nucleinate percentage in course of time disappears.

Leiden, Laboratory for Medical Chemistry.