Biochemistry. — *Behaviour of microscopic bodies consisting of biocolloid systems and suspended in an aqueous medium. VI. Composition of degenerated hollow-spheres, formed from complex coacervate drops (gelatine-gum arabic).* By H. G. BUNGENBERG DE JONG and E. G. HOSKAM. (Communicated by Prof. H. R. KRUYT.)

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Introduction.

In Communication IV of this series we described the vacuolization phenomena of complex coacervate drops, when they are brought in contact with dist. water [1]). It appeared that when the coacervate drops are originally charged negatively, the primary vacuolization passes into foam formation and finally results in hollow spheres. It is supposed that abnormal osmosis is the cause of the formation of this foam structure and of the hollow spheres. Further investigation gives strong support to this supposition [2]). Nevertheless we felt the necessity of knowing something about the composition of the coacervate skin which forms the wall of the hollow spheres, three questions especially requiring a solution:

1. Does the wall of the hollow spheres still consist of complex coacervate, or, owing to the removal of one of the colloid components (gum arabic), does it consist of the second colloid component only (gelatine?)

2. Is it possible to foresee any changes in composition which may arise with regard to the original composition?

3. Is the composition of the liquid wall such as we can expect for a complex coacervate of still negative charge?

In what follows it will be seen that the results of the chemical analysis support the views concerning the mechanism of the formation of hollow spheres, developed previously. In these theories we had started from the supposition that the wall of the hollow spheres still consists of a coacervate with a negative charge.

Experimental.

On account of their large vacuoles the hollow spheres themselves are no suitable objects for analysis, at least if we wish to find out the water percentage of the wall besides the gelatine-gum arabic proportion.

So we must content ourselves with analyzing the coacervate drops free from vacuoles formed in consequence of spontaneous degeneration. The circumstances determine the length of existence of the hollow spheres, in the experiments described here they lasted at most 20—30 minutes. So long as the hollow spheres are typical, i.e. so long as they have a very thin wall, they will settle only very slowly in a sedimentation tube. As the vacuole volume decreases they settle more rapidly. The sedimentation layer forming in a sedimentation tube when it is left undisturbed, consists therefore mainly of coacervate drops free from vacuoles or containing a little vacuole only. Here another difficulty arises: whereas the ordinary coacervate drops easily coalesce on sedimentation to a clear coacervate layer, this is not the case with "degenerated" hollow spheres. Although they too are liquid internally, their surface is apparently in a particular condition, owing to

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

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

which coalescence practically does not or hardly takes place. But they flatten each other considerably in the sediment layer [1]), so that only little of the medium liquid is enclosed.

In consequence of this difficulty, analysis can at most give a slightly too high figure for the water percentage of the coacervate, but this will have no practical effect on the proportion of gelatine and gum arabic as the small quantity of medium liquid enclosed contains relatively few colloids.

For the calculation of the composition of coacervate and the above liquid it was necessary to determine the dryweight and the nitrogen percentage. The dryweight was determined as follows: a weighed quantity of the sample was placed for one hour in a nickle box on a boiling water bath and then for one hour in an electric drying stove at 120° C.

The nitrogen was determined by DEKKER's method [2]). With the aid of the dry weights determined thus and the N-percentages of the dry substance and using the N-percentages of the gelatine (determined at 16.65 %) and gum arabic (= 0.33 %) the gelatine and gum arabic percentage is calculated [3]).

Results.

We started from a system which is known to give excellent hollow spheres. For this isohydric solutions were prepared (pH 3.67) of gelatine and gum arabic from the corresponding stock solutions.

Stock solutions: 22 g air-dry gelatine resp. gum arabic are dissolved in 380 g dist. water.

I. Isohydric gelatine solution: 40 ccm stock sol. + 13 ccm 0.1 N HCl + 47 ccm H_2O (dryweight determination 1.985 %).

II. Isohydric solution of gum arabic: 40 ccm stock solution + 4.5 ccm 0.1 N HCl + 55.5 ccm H_2O (dryweight determination 2.06 %).

In each of 4 sedimentation tubes with a contents of 250 ccm we then placed: 84 ccm sol. I (isohydric gelatine) and 166 ccm sol. II (isohydric gum arabic).

We always worked at a temperature of 40° C. After sufficient sedimentation 2 tubes were placed for ca. 10 minutes in cold water and after gelatination of the coacervate the upper layer was poured off and replaced by 250 ccm of an isohydric HCl solution [4]).

These two tubes were again placed in the thermostat and after the contents had reached the right temperature they were well shaken. Typical hollow spheres then formed which slowly sank. The other two tubes did not undergo this treatment. Dry weight and N percentage were then determined of each of the 4 tubes of the upper layer as well as of the coacervate, resp. of the layer of degenerated hollow spheres. With the aid of these values and with the dryweight and nitrogen percentage of solutions I and II we then calculated the gelatine and gum arabic percentages in each of the layers.

In the following table are given the analysis data and the results calculated from them for the original coacervated system (left) and for the system after passing the stage of the hollow spheres (right).

1) When the sediment tube is placed in cold water the complex coacervate gelatinises in a short time (within 20 minutes). In the case of an ordinary complex coacervate the gelatinized sediment layer forms a cohering mass (turbid owing to vacuolization). In the case of a sediment layer of degenerated hollow spheres this layer can be separated by vigorous shaking to a suspension of separate polygonal bodies. (The coacervate drops mentioned before, they are flattened by contact with each other, but have not coalesced.)

2) W. A. L. DEKKER, Handleiding voor het klinisch chemisch onderzoek, 3e dr. Leiden 1940.

3) See Kolloid Beihefte **43**, 215 (1936).

4) Prepared by adding the calculated quantity of HCl to distilled water.

TABLE.

	Original system		After passing the hollow spheres stage	
	Coacervate layer	Equilibrium liquid	Sedimentation layer	Upper layer
Dryweight %	12.93	0.795	17.16	0.617
N-percentage of dry substance	7.21	3.59	7.66	5.21
Gum arabic (A) %	7.48	0.64	9.45	0.43
Gelatine (G) %	5.45	0.16	7.71	0.18
A/G	1.37	4.0	1.23	2.38

Discussion.

The results enable us to answer the questions asked in the introduction:

1. The degenerated hollow spheres do indeed contain gum arabic besides gelatine. So the wall of the hollow spheres does not exist exclusively of gelatine, but of a typical complex coacervate.

2. The composition of the degenerated hollow spheres is changed in two respects with regard to the original composition:

a. The water percentage is smaller (dryweight of 12.93 % has increased to 17.16 %).

b. The proportion of the two colloids has shifted in favour of the gelatine, which also applies to the upper layer (see lowest horizontal row in the table).

As regards *a.* this change is to be expected from the removal of neutral salt ($CaCl_2$) formed from the counter ions of the two colloids (Ca from gum arabic, Cl' from the gelatine). On complex coacervation the two colloid ions + water combine in principle to the coacervate, the remaining neutral salt dividing itself over the two liquid layers. Neutral salts increase the waterpercentage of the complex coacervates and consequently the removal of the upper layer and its substitution by an isohydric HCl solution results in the decrease of the waterpercentage of the complex coacervate. It is also the cause of the primary vacuolization, which on sufficiently negative coacervates passes secondarily into a foam structure and the formation of hollow spheres.

As regards *b.* we should remember that the mixing proportion chosen of the sols is such that a negatively charged complex coacervate is formed. With the pH given there is then an excess of gum arabic (A) in the total system from the point of view of the mutual charge compensation of the two colloids of opposite charges. This excess of gum arabic is divided over coacervate and equilibrium liquid in such a way that A/G in the coacervate is smaller than in the total system, while A/G in the equilibrium liquid is greater than in the total system [1].

Of a coacervate with positive charge the reverse is true while on charge compensation these proportions become mutually equal:

Coacerv. neg. (A/G) coac. $<$ (A/G) total $<$ (A/G) equil. liquid.
Uncharged coac. (A/G) coac. $=$ (A/G) total $=$ (A/G) equil. liquid.
Coac. pos. (A/G) coac. $>$ (A/G) total $>$ (A/G) equil. liquid.

[1]) H. G. BUNGENBERG DE JONG, Kolloid Beihefte **43**, 213 (1936). C.f. fig. 11, p. 234, loc. cit., where this appears from the curves for $\frac{A}{A+G}$, which applies therefore also for $\frac{A}{G}$.

That this is indeed true of the original coacervate is seen when A/G in the total system is calculated. From the dryweight of the two stocksols (G = 1.985 %; A = 2.06 % and the mixing proportion (84 cc gelatine sol + 166 cc gum arabic sol) we calculate a percentage of 0.667 % gelatine and 1.368 % gum arabic, i.e. for the total system A/G = 2.05. This figure lies indeed between the two values for A/G, viz. 1.37 and 4.0.

When in preparing the hollow spheres we remove the original equilibrium liquid (which has a comparatively high gum arabic percentage), replacing it by an isohydric HCl solution, the gum arabic still present in the coacervate will again divide over the two layers, the consequence of which will be a decrease of the A/G proportion in the coacervate, which is indeed proved by the table (1.37 → 1.23).

3. The question if the complex coacervate forming the wall of the hollow spheres has the composition typical of a negative coacervate can now at once be answered in view of what has been said above. This is already indicated by the fact that A/G of the upper layer (2.38) is greater than A/G of the sedimentation layer (1.23). Moreover we can see if A/G in the total system, as it has been formed by the removal of the upper layer, lies indeed between these two values. Auxiliary determinations on a smaller scale showed that the original coacervate volume was 22.4 ccm (1.12 cc for 12.5 cc final volume, this amount was here taken 20 times).

By removing the upper layer = 250 — 22.4 = 227.6 cc we subtracted from the total system: 227.6 × 0.0064 = 1.46 g gum arabic and 227.6 × 0.0016 = 0.36 g gelatine, while originally there was 250 × 0.01368 = 3.42 g gum arabic and 250 × 0.00667 = 1.67 g gelatine. So in the system there was left 1.96 g gum arabic and 1.31 g gelatine, from which it follows that for the total system A/G = 1.50, which value is indeed between the A/G values of 1.23 and 2.38 in the way characteristic of a negative complex coacervate.

Summary.

1. The composition of degenerated hollow spheres formed from the complex coacervate gelatine-gum arabic is investigated.

2. Besides gelatine they contain gum arabic and are therefore still complex coacervates.

3. Their water percentage is lower than that of the original coacervate and they contain relatively less gum arabic.

4. The modifications in 3 can be foreseen from the treatment the original coacervate has undergone.

5. From the analysis figures it can be concluded that the degenerated hollow spheres are complex coacervates with negative charge.

6. What is said in 5 is in accordance with the views concerning the mechanism of the formation of hollow spheres published elsewhere.

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