also wegen (1) und (6)

$$S_{ik}^{(\varkappa)} = \varkappa \left\{ \frac{\partial a_{ik1}}{\partial x_2} - \frac{\partial a_{ik2}}{\partial x_1} + \tfrac{1}{2} \alpha^*_{\underline{pq}} \left( \frac{\partial Q^*_{kpq}}{\partial x_i} + \frac{\partial Q^*_{ipq}}{\partial x_k} \right) \right\} +$$

$$+ \left\{ \frac{\partial Q^*_{ik1}}{\partial x_2} - \frac{\partial Q^*_{ik2}}{\partial x_1} + \tfrac{1}{2} \alpha^*_{\underline{pq}} \left( \frac{\partial a_{kpq}}{\partial x_i} + \frac{\partial a_{ipq}}{\partial x_k} \right) \right\}$$

oder

$$S_{ik}^{(\varkappa)} = \varkappa\, n_{ik} + u_{ik} \qquad (\varkappa^2 \neq 1) \quad \ldots\ldots \quad (11)$$

Für $\varkappa^2 = 1$ wird die Operation $N$ sinnlos, da dann $\triangle_\varkappa$, $R_\varkappa$ und $Q_\varkappa$ gleich Null werden. Man findet jedoch durch Addition und Subtraktion von (8) und (9) zwei nicht verschwindende Kovarianten:

$$S_{ik}^{(1)} = \frac{\partial (a_{ik1} + Q^*_{ik1})}{\partial x_2} - \frac{\partial (a_{ik2} + Q^*_{ik2})}{\partial x_1} +$$

$$+ \tfrac{1}{2} \alpha^*_{\underline{pq}} \left\{ \frac{\partial (a_{kpq} + Q^*_{kpq})}{\partial x_i} + \frac{\partial (a_{ipq} + Q^*_{ipq})}{\partial x_k} \right\}$$

$$S_{ik}^{(-1)} = \frac{\partial (-a_{ik1} + Q^*_{ik1})}{\delta x_2} - \frac{\partial (-a_{ik2} + Q^*_{ik2})}{\partial x_1} -$$

$$- \tfrac{1}{2} \alpha^*_{\underline{pq}} \left\{ \frac{\partial (-a_{kpq} + Q^*_{kpq})}{\partial x_i} + \frac{\partial (-a_{ipq} + Q^*_{ipq})}{\partial x_k} \right\}.$$

Setzt man noch

$$f + Q^* = v_x^3 \qquad -f + Q^* = w_x^3$$

worin $v_x^3$ und $w_x^3$ reine Kuben sind [3]), so bekommen diese Kovarianten die neue Gestalt:

$$S_{ik}^{(1)} = \frac{\partial v_{ik1}}{\partial x_2} - \frac{\partial v_{ik2}}{\partial x_1} + \tfrac{1}{2} \alpha^*_{\underline{pq}} \left( \frac{\partial v_{kpq}}{\partial x_i} + \frac{\partial v_{ipq}}{\partial x_k} \right)$$

$$S_{ik}^{(-1)} = \frac{\partial w_{ik1}}{\partial x_2} - \frac{\partial w_{ik2}}{\partial x_1} - \tfrac{1}{2} \alpha^*_{\underline{pq}} \left( \frac{\partial w_{kpq}}{\partial x_i} + \frac{\partial w_{ipq}}{\partial x_k} \right)$$

Unterwirft man diese Kovarianten der Transformation, welche $f$ überführt in die kanonische Gestalt (10), so wird

$$S^{(1)} = 2 \frac{\partial a_{111}}{\partial x_2} dx'^2 \quad \text{und} \quad S^{(-1)} = 2 \frac{\partial a_{222}}{\partial x_1} dx''^2.$$

Ihre Diskriminanten sind Null.

*$S_{ik}^{(1)}$ und $S_{ik}^{(-1)}$ sind also die entarteten Exemplare des Büschels $S_{ik}^{(\varkappa)} = \varkappa\, n_{ik} + u_{ik}$. Die Differentialkovarianten erster Ordnung der binären kubischen Differentialform führen zurück auf $n_{ik}$ und ihre Ueberschiebung mit $\alpha^*_{ik}$.*

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**Biochemistry.** — *Behaviour of Microscopic Bodies consisting of Biocolloid Systems and suspended in an Aequeous Medium. I. Pulsating Vacuoles in Coacervate Drops.* By H. G. BUNGENBERG DE JONG. (Communicated by Prof. J. VAN DER HOEVE.)

(Communicated at the meeting of May 28, 1938.)

1. *General introduction.*

Experimental cytology studies the behaviour of microscopically small systems from biocolloids and it is faced by a remarkable difficulty in the interpretation of the morphological changes brought about under the influence of external or internal factors. It is tried to find some connection with the results of colloid-chemical researches, e.g. on sols and gels. However, the dimensions of the latter objects of research are usually such that their properties are practically determined only by those of the three-dimensional content of these systems and the influence of the bordering surfaces is not expressed in it.

With the objects of cytology, however, the proportion of bordering surface and content is totally different and the morphological changes observed by it consist of changes of the biocolloid systems, in which both, surface and content, are simultaneously contained and influence each other mutually.

For this reason we are inclined to think that, if colloid chemistry wishes to be of use to cytology, it will have to occupy itself with the study of microscopically small colloid bodies; by bodies is meant here: colloid systems surrounded by an external surface.

In the study of the coacervation phenomena the present writer already applied this method, in so far as not only the properties of the three-dimensional coacervates were examined but also those of microscopical coacervate drops. The significance of these systems in biology, which consequently may be regarded as fluid biocolloid bodies, has been stated elsewhere [1]).

In so far as the study of these fluid and other kinds of biocolloid bodies

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[1]) Summarizing articles on coacervation:
H. G. BUNGENBERG DE JONG, Die Koazervation und ihre Bedeutung für die Biologie, Protoplasma, **15**, 110 (1932).
H. G. BUNGENBERG DE JONG, La Coacervation et son importance en Biologie. Tome I et II. Hermann et Cie; Paris 1936.
H. G. BUNGENBERG DE JONG, Koazervation, Kolloid Z., **79**, 223, 334 (1937), **80**, 221, 350 (1937).

yields new results, which in cytology may be important as models, they will be briefly mentioned in the present and following communications.

2. *Coacervation of gum arabic sol with toluidine blue.*

If on an object glass at a small distance from each other are placed a drop of a concentrated toluidine blue solution and gum arabic solution (2 to 3 %) and both are covered by a cover glass, we observe under the microscope that in the mixing-zone of the two liquids coacervation of the gum arabic takes place. This coacervation belongs to the type of auto-complex coacervation and takes place with the negative gum arabic sol and many basic stains [1].

By means of the above-mentioned method a zone of optimal coacervation is found, which is *not* situated where the colour is just no longer perceptible, but at some distance to the side of the stain solution. Here the largest coacervate drops are formed, which are opaque, owing to the stain they contain, while they lie in a liquid zone which is hardly stained.

This zone of optimal coacervation is explained by the fact that here gum arabic and stain are present in the most favourable mixing-ratio. On either side of this zone the coacervation decreases and the colour of the liquid containing the coacervate drops grows stronger. Towards the side of the stain solution the colour is typically that of an aequeous tiluidine blue solution, towards the side of the gum arabic solution the colour has shifted more to red ("meachromasia"). This shifting of the tint may be regarded as a result of the adsorption of the stain cation to the arabinate colloid anion. Since the ratio stain, gum arabic grows gradually smaller towards the side of the gum arabic solution, the coacervation soon stops here.

3. *Pulsating vacuoles.*

The coacervate drops sink on the object and stick to it, so that, when afterwards either of its own accord or by tilting or straining through with a filtering-paper the zone of contact of the gum arabic and stain solution is shifted, the coacervate drops will become surrounded by a medium with which they are no longer in equilibrium. They disappear at last, when they are surrounded by the gum arabic solution.

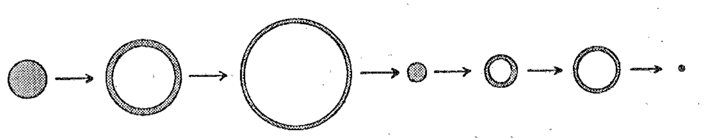

Fig. 1.

In the latter case, however, unexpected phenomena occur (cf. fig. 1). The coacervate drops, instead of being dissolved by exclusively peripheral

[1]) H. G. BUNGENBERG DE JONG and J. LENS, Biochem. Z., **254**, 15 (1932).



action of the medium, besides absorb liquid from the medium, which is separated in the coacervate drops under formation of a vacuole.

The phenomena may be best observed when the zone of contact of the two liquids is slowly shifted. It is then seen that originally opaque coacervate drops increase in volume and in the centre grow transparent. They consist now of a still strongly stained, soon fairly thin spherical skin of coacervate enclosing a centrally situated vacuole which increases in volume. At a certain moment they burst and change into a small coacervate drop, which is again opaque. In its turn the latter can swell, forming a central vacuole, and burst again. As a rule there is not enough coacervate left then for the phenomenon to be repeated once more.

The phenomena taking place here are interesting from a biological point of view, since they remind us of the functioning of pulsating vacuoles. Meanwhile there is this difference that in the case of the coacervate drops the pulsating system is destroyed after a few pulsations. Nevertheless, a closer examination of the mechanism of these phenomena seems of importance to biology and in due time this will be discussed further. Incidentally it may be remarked that in principle the same phenomena were observed with some other basic stains, e.g. brilliant cresyl blue, but so far they were realized most beautifully with toluidine blue.

*Summary.*

1. The importance to cytology is discussed of the study of microscopical bodies consisting of biocolloid systems.

2. In coacervate drops, originated from gum arabic sol + toluidine blue, under certain conditions when they are no longer in equilibrium with their medium, pulsating vacuoles are formed.

3. Although this pulsating system is destroyed after a few pulsations, these phenomena may be of importance in biology.

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