Colloidal Change and Mitosis.

By

L. V. Heilbrunn,

University of Michigan.

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The evidence is slowly accumulating that most vital phenomena are related to colloidal changes in the protoplasm. This is indeed a view that has had the support of many physiologists in the past. The great difficulty has always been to discover some method of determining colloidal change. Many colloids can undergo sudden and sharp changes in their fluidity. A colloid with relatively low viscosity, one capable of flow, may in a few minutes become so viscous that it will scarcely flow at all. It then becomes a jelly or a gel or a coagulum. Doubtless similar changes in fluidity occur in protoplasm. But how to determine them? The older physiologists made various attempts to guess at the fluidity of protoplasm, they looked at cells and decided whether the protoplasm was fluid or not.

The first clear-cut evidence of a great decrease in fluidity (i. e. increase in viscosity) of active protoplasm was given in a paper by *Heilbrunn* (1915). He introduced the centrifuge method as a means of testing the relative viscosity of animal cells. His results have been generally confirmed by various investigators and it is now an established fact that after fertilization the viscosity of the protoplasm of the sea-urchin egg increases sharply. Of all methods of determining the protoplasmic viscosity of animal cells the centrifuge method is certainly the most reliable. There is no injury to the cell by the introduction of a dissecting needle, nor is there as much chance for guess work as there must be in the estimation of protoplasmic viscosity by the manipulation of a needle rigidly held by screws. Everyone who has attempted the determination of the fluidity of protoplasm admits that the centrifuge method is more accurate than any other method which has ever been used on animal cells. In *Weber*'s review of the methods of determining protoplasmic viscosity, published in Abderhalden's Handbuch der biologischen Arbeitsmethoden, the centrifuge method is the only method used on animal cells that is classed as a measurement method. Microdissection is regarded as a „Schätzungsmethode". The details of the centrifuge method, the reasons for its reliability, etc, have been fully described (*Heilbrunn*, 1921). Concerning the results obtained with it, *Weber* says: „Als Paradigma sei ausführlicher geschildert die Versuchsanstellung *Heilbrunn*'s bei seinen neuesten Studien der Viscositätsveränderungen des *Cumingia*-Eies während der Mitose. Es gehören diese Bestimmungen jedenfalls zu den exaktesten, die bisher durchgeführt worden sind".

And yet in a recent article, *Spek* repeatedly casts doubt on the results that have been obtained with the centrifuge method. The reasons for these doubts are quite obvious. *Heilbrunn* had claimed that all agents that incite sea-urchin eggs to divide cause a great increase in viscosity, that is to say a coagulation or gelation within the cell. This work was not known to *Spek*, and

in 1920 he found that various salts wich he regards as liquefying or swelling agents increase the division rate of paramecium. *Spek* therefore proposed a theory to the effect that the cell is stimulated to divide by agents which cause the liquefaction or swelling of the protoplasmic colloids. Obviously there is a conflict between the views of *Heilbrunn* and *Spek*, and this has been commented on by *Kornfeld* (1922).

But as far as the facts in the case go, there is no conflict at all. Spek found that LiCl and several other salts increase the division rate of paramecium, and that $CaCl_2$ tends to decrease the division rate. LiCl causes a pronounced increase in the volume of the cell and *Spek* assumed that this increase in volume indicated a liquefaction. This is a logical enough assumption, but it is by no means a demonstration. In the sea-urchin egg the rapid expansion or cytolysis of the cell was always regarded as a liquefaction until it was shown by *Heilbrunn* in 1915 that the expansion of the cell is accompanied by a coagulation of the protoplasm. There can be no question of this, for in cytolyzed eggs the granules refuse to move through the egg even after prolonged centrifugal treatment.

As a matter of fact even in protozoa it has been shown (*Heilbrunn* 1923) that LiCl, which *Spek* thought caused liquefaction, really causes a coagulation of the cell protoplasm. $CaCl_2$, which he assumed to cause coagulation, really tends to liquefy. There is absolutely no antagonism between the actual data of *Heilbrunn* and *Spek*. Indeed *Spek*'s data, when properly interpreted, furnish valuable support to *Heilbrunn*'s theory.

Nor need there be such a sharp difference in theoretical interpretation. *Heilbrunn* attempts to explain the formation of the mitotic spindle as a result of the gelation or coagulation of the interior protoplasm. *Spek* believes that a liquefaction is necessary in order that the cell may divide in two. That a relatively fluid condition of the outer envelope of the cell favors its division was emphasized by *Heilbrunn* in 1915 (see p. 183). Later (1920b) this point was even more clearly shown for the egg of *Cumingia*. This egg when shed has its maturation spindle fully formed. But the maturation divisions can not proceed until the stiff vitelline membrane of the egg is either softened or removed. A conception of this sort might easily be made to fit in with some of *Spek*'s ideas.

In view of the essential agreement in fact and the possibility of some agreement in theory, it is unfortunate that *Spek* should recently have launched such a violent attack on *Heilbrunn*'s work. In his eagerness to discredit, he is guilty of frequent misunderstanding and of occasional misquotation.

Spek is particularly aroused by the fact that ether and other anesthetics which prevent the formation of the mitotic figure, were found to have a liquefying effect on protoplasm. This is really an essential point. Not only is it true that all agents which provoke cell-division cause gelation, but it is only necessary to prevent such gelation in order to prevent the formation of the mitotic spindle. *Spek* in 1920 thought that the anesthetics which prevent spindle formation in the sea-urchin egg produce this effect by decreasing oxidations, although *Warburg* (1910) and *Loeb* and *Wasteneys* (1913) had already pointed out that such anesthetics had little or no effect on the egg oxidations. Nor can one suppose an influence of the anesthetic in decreasing the permeability of the egg to dissolved substances, for the very opposite effect has been claimed for the sea-urchin egg by *Harvey*. Actual measurement of protoplasmic viscosity shows that various anesthetics have a liquefying action on protoplasm (*Heilbrunn* 1920a). In the experiments which prove this point careful controls were kept in every single instance. Similar controls are to be found for all

Heilbrunn's experiments. Whenever eggs treated in any fashion were centrifuged, normal untreated eggs were centrifuged with them. There is no justification in the slightest for *Spek*'s slurring statement that „Die Versuche *Heilbrunn*'s sind in den meisten Fällen nach keiner Richtung durch Kontrollen gedeckt".

In spite of all *Spek*'s assumptions to the contrary, anesthetics actually do prevent gelation in the protoplasm of the sea-urchin egg. Moreover, although this is not an essential point in *Heilbrunn*'s work and was never stressed, anesthetics do also reverse gelation in protoplasm, once such a gelation has occurred. This is shown in an experiment recorded on page 225 of *Heilbrunn*'s 1920a paper[1]). This experiment was overlooked by *Spek* who states emphatically that no such experiment was recorded. *Spek* is also in error when he states that *Heilbrunn* ascribed an antigelatinizing effect to potassium cyanide. This is quite the opposite of the truth.

It is hard for *Spek* to understand how if concentrated ether solutions cause coagulation, more dilute solutions may have the opposite effect. Perhaps it does sound improbable, but it is nevertheless true. It seems to be a general truth that dilute ether solutions make protoplasm more fluid, while solutions of higher concentration coagulate. At any rate *Weber* in 1921 confirmed *Heilbrunn*'s results for the protoplasm of spirogyra. So too, although it seems incomprehensible to *Spek* that cold should have a liquefying action on protoplasm, this point has also been confirmed by an independent observer (*Heilbronn*, 1922).

There are many other instances in which *Spek* scoffs at the results of actual measurements. When the viscosity determinations agree with *Spek*'s speculations, he accepts them, otherwise he finds them at fault.

In spite of all *Spek*'s argumentation and in spite of his various assumptions, he has presented not a single experimental fact to contradict the direct evidence of *Heilbrunn* which has shown (1) that all agents which stimulate egg cells to segment cause a gelation or coagulation and (2) that all agents which prevent such gelation prevent the division of the egg.

In a final page of criticism *Spek* leaves the subject of the discussion completely and seeks to discredit *Heilbrunn*'s theory of membrane elevation in the sea-urchin egg. It may be well to answer his arguments briefly.

He says first that if *Heilbrunn*'s theory is correct hypotonic solutions should cause membrane elevation, and that nothing of the sort has ever been described. Membrane elevation by hypotonic solutions has been described by *Schücking* (1903), by *Loeb* (1909), and by *Konopacki* (1918). He then asks why when the surface tension is lowered the whole egg does not expand instead of just the membrane. The answer is that it usually does, and it is difficult to find the exact concentration of the reagent or length of exposure which will cause membrane elevation rather than the expansion of the egg or cytolysis. Thirdly, he asks why cells other than egg cells do not have their osmotic equilibrium disturbed by substances which lower surface tension. The answer is that they do. All isolated cells expand when substances of low surface tension are added. When blood cells are placed in solutions of low surface tension they increase in volume and undergo hemolysis. There is a large literature on this subject. Protozoa also swell up in solutions of low surface tension, or they may even throw off a membrane just as egg cells do (*Bresslau*, 1921). Fourthly, *Spek* states that surface tension could have no effect on a

[1]) It has also repeatedly been shown in more recent unpublished experiments.

gel. But *Quincke* (1902) has described surface tension effects on gels and on precipitation membranes.

All these points of course have no relation to the main question. They merely cloud the issue. The essential point of difference between the theories of *Spek* and *Heilbrunn* is that the former assumed that the agents which incite cells to division cause a liquefaction of the protoplasm, whereas *Heilbrunn* showed by an actual measurement of the fluidity that the reverse is true.

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