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Estudio experimental de la división celular.

La viscosidad del citoplasma de los óvulos de erizo de mar en vias de división puede comprobarse por el método de la centrifugación. En relación con la mitosis tienen lugar cambios marcados de viscosidad; a un aumento inicial de viscosidad sigue una disminución de la misma. El considerable aumento de viscosidad puede expresarse como una gelificación, que alcanza su máximo precisamente antes de la aparición del huso. Los cambios de viscosidad determinan aparentemente la aparición del anfiaster o huso. Cuando se suprime la gelificación, no se forma figura mitósica, aun cuando los óvulos pueden permanecer ilesos. Tal supresión de la formación de la gelatina fué producida por el autor usando varios anestésicos y tambien el frío. Aunque su efecto final es el mismo, el frío y el éter son mutuamente antagónicos. Las soluciones hipertónicas aumentan la viscosidad del citoplasma de los óvulos en vías de division, y tienden a suprimir la reversión normal de la gelificación. El cianuro potásico y la cloretona producen un efecto semejante. Existen algunas pruebas en favor de la idea que considera a la gelificación mitósica como el producto de la extración de agua.

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AN EXPERIMENTAL STUDY OF CELL-DIVISION

I. THE PHYSICAL CONDITIONS WHICH DETERMINE THE APPEARANCE OF THE SPINDLE IN SEA-URCHIN EGGS

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INTRODUCTION

In my work on artificial parthenogenesis, I showed that all substances which incite the sea-urchin egg to divide mitotically produce a marked increase in the viscosity of the cytoplasm. A similar viscosity increase was also found to occur after the normal stimulus of fertilization. Accordingly, I held the view that some sort of 'solidification' was the essential factor which initiated mitosis. For purposes of clearness, I shall refer to this stiffening as a gelation. In order to prove my view correct, I have now attempted to show that if this gelation is prevented, no stimulus to mitosis can occur. If the cytoplasm of a fertilized egg is kept in the fluid condition, then no mitotic figure forms, and the egg remains undivided, although it is not necessarily injured by the treatment. Here, then, is additional evidence in favor of the view that the formation of the amphiaster is a direct consequence of cytoplasmic gelation.

It can readily be seen that such a study of mitosis leads in two directions. In the first place, it throws light on the genesis of the mitotic figure; secondly, it affords a physical interpretation of stimulation and anesthesia in the egg. In this contribution I propose to emphasize the first of these problems, reserving the discussion of anesthesia for a later paper.

The experimental work which furnishes the basis for this paper was done at Woods Hole during the summer of 1916. The form used was Arbacia punctulata (Gray).

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VISCOSITY CHANGES DURING MITOSIS

During the process of mitosis the cytoplasm of the sea-urchin egg undergoes marked physical changes. I have already shown (Heilbrunn, '15) that a gelation occurs soon after mitosis, so that the early stages of the mitotic process are associated with a gradual stiffening of the egg protoplasm. In later stages this gelation is reversed, so that in the anaphase the egg has again returned to its original fluid condition.

In order to follow closely the physical changes which occur in the cytoplasm, viscosity measurements were made by the centrifuge method. An accurate measure of the cytoplasmic viscosity can be obtained by a determination of the ease with which granules move through it. When the cytoplasm is quite fluid, a moderate centrifugal force is sufficient to throw all the granules into one half of the egg, so that a clear 'hyaline zone' extends through the other half (except for a small polar accumulation of lipoids, the 'gray cap'). As the viscosity of the cytoplasm increases, the granules cannot respond so readily to centrifugal force, and the hyaline zone, instead of extending through half of the egg, only appears in a limited portion. With still greater increase in viscosity, the same or even a stronger centrifugal force causes no perceptible movement of the granules. The width of the hyaline zone is not so easy to determine in the fertilized egg as in the unfertilized egg, for soon after fertilization the pigment granules characteristic of the Arbacia egg all migrate to the cortex, and since they are not so readily moved when in this position, they remain as an outer coat completely surrounding and partially obscuring the interior cytoplasm. But with a little practice this difficulty is easily overcome by proper focusing, and it becomes a simple matter to estimate the width of the hyaline zone. So great are the changes in its extent that it is sufficient to express its width approximately in terms of the egg diameter. In this way rapid determinations can be made and viscosity tests can be completed in a very short time. Thus, in a number of experiments I was able to determine the egg viscosity at intervals of about four minutes.

The centrifuge used was a Bausch & Lomb hand centrifuge, equipped with two speeds. In the following experiments, however, the high speed was always employed. This necessitated a use of the hematocrit attachment, the eggs being placed in small glass tubes. Because of the difficulty with which such small tubes are cleaned, it was found advisable to use new tubes for each centrifuge test. A pair of tubes can be made in less time than it takes to thoroughly cleanse them.

In comparing the viscosity of eggs at one stage with the viscosity at a later stage, two methods of procedure are possible. In the first place, the eggs can be fertilized at two different times, and then eggs from both lots centrifuged simultaneously. In this way, the experimenter can assure the same treatment to both sets of eggs. Earlier tests were made in this way, and it was possible to demonstrate that after fertilization the egg viscosity first increased and then decreased. But a closer study of the process demanded viscosity tests at frequent intervals. In order to accomplish this conveniently, only a single lot of eggs can be studied. The experimenter must therefore learn to handle the centrifuge with such precision that the centrifugal force generated in each of the tests is constant. The handle of the centrifuge has to be turned a certain number of times in a certain number of seconds, and great care must be taken that the turning is uniform, for the centrifugal force of course depends on the speed. With a little practice, one may acquire the knack of turning the handle with machine-like precision.

In the accompanying tables, the amount of centrifugal force exerted on the eggs is given precisely by recording the number of times the handle was turned in a certain time interval. Each turn of the handle resulted in 130 revolutions of the tubes. The distance between the ends of the tubes was 12 cm. The centrifugal force is given by the formula $C = \frac{mv^2}{r}$. In this formula, the mass m is unknown, but presumably constant, the velocity $v = 2\pi r \times$ the number of turns per second, r the radius is always 6. Thus, when the handle is turned at different speeds, the amount of centrifugal force generated varies directly as the square of the number of turns per second.

In order to make the tables as brief and compact as possible, many less important observations have been omitted. In the third column, in indicating the number of times the centrifuge handle was turned and the number of seconds consumed in the process, the number of turns is given first and this is followed by the number of seconds. Thus "30 in 25" indicates that the handle was turned thirty times in twenty-five seconds. In each case the turning is assumed to have been uniform unless otherwise stated. In the fourth column, the extent of the hyaline zone, and, in many cases, the degree of distinctness with which it appeared, is recorded. In referring to the hyaline zone, the abbreviation 'H.Z.' is sometimes employed; more generally, however, it is omitted, the remarks being then understood to pertain to the hyaline zone. The various fractions in the fourth column are indices of the extent of the hyaline zone. A line passing perpendicularly to the zones of the centrifuged eggs and through the egg center is regarded as the axis of stratification. The fraction of this line which is included by the hyaline zone is taken as an index of the extent of this zone.

The temperature of the water containing eggs was recorded both at the beginning and at the end of each experiment. As every one knows, the length of time necessary for cleavage varies with the temperature. In the second column is recorded the time when cleavage began. Of course, there is always some variation among the eggs of a given batch. Usually a very few eggs start ahead of the others. A minute or two later, 5 to 10 per cent of the eggs can be observed segmenting, and in another minute about 40 per cent will be dividing. The cleavage time indicated in the tables is the time when about 5 to 10 per cent of the eggs have begun to divide.

In order to correlate the viscosity tests with the mitotic changes occurring within the egg during the tests, the eggs were observed as closely as possible. The Arbacia egg is unfortunately rather opaque, and comparatively little cytological detail can be observed in the living egg. After fertilization, the first indication of mitosis is a long, clear streak of hyaline material. This streak is probably the profile view of the disc which Fol described in 1877,

and named the 'amas sarcodique.' It later becomes converted into the spindle, its end forming the material for the asters. I shall, for convenience, refer to it as the 'pre-spindle.'

Of course, with centrifuge tests every four minutes, it was impossible to keep the eggs under constant observation. Accordingly, I could not record the exact minute when the pre-spindle first appeared. Nevertheless, in some of the experiments, I was able to fix the time of its appearance within narrow limits. And this is all that could be hoped for, anyway.

These observations and measurements of the living egg might well be supplemented by observations and measurements of eggs fixed after being centrifuged. I hope to make such a study at some time in the future.

I pass now to the consideration of individual experiments:

Eggs fertilized at 10:35 A.M.
Temperature { 22.3° at 10:35 A.M.
23.3° at 11:30 A.M.

MINUTES AFTER FERTILIZATION	OBSERVATIONS ON NORMAL (i.e., UNCENTRIFUGED) EGGS	CENTRIFUGAL TREATMENT	HYALINE ZONE
6		50 in 30	$\frac{1}{2}$
10		50 in 30	$\frac{1}{2}$ not distinct
14		50 in 29	H.Z. not sharply marked off
19	No spindles	50 in 30	H.Z. indicated toward lighter pole, but not very distinctly.
$24\frac{1}{3}$		50 in 31	Barely indicated, at most $\frac{1}{5}$ to $\frac{1}{4}$
$26\frac{1}{2}$	No spindles		
$27\frac{1}{2}$	Pre-spindles beginning to appear		
$28\frac{1}{3}$		50 in 30	$\frac{1}{4}$ to $\frac{1}{3}$
33		50 in 31	$\frac{1}{4}$ shows plainly
37		50 in 31	$\frac{1}{2}$ shows plainly
43		50 in 31	$\frac{1}{2}$ shows plainly
46	Cleavage beginning		

September 2nd

Eggs fertilized at 10:20 A.M.

Temperature {21.0° at 10:20 A.M.
21.8° at 10:28 A.M.

MINUTES AFTER FERTILIZATION	OBSERVATIONS ON NORMAL (i.e., UNCENTRIFUGED) EGGS	CENTRIFUGAL TREATMENT	HYALINE ZONE
6		50 in 30	$\frac{1}{2}$
10		50 in 30	In some eggs, H.Z. shows plainly and extends $\frac{1}{2}$ way, in other eggs, it is indistinct
14		50 in 31	Usually does not show, in some eggs, $\frac{1}{6}$
18	No spindles		
20		50 in 32	$\frac{1}{4}$ in eggs best stratified.
22	Pre-spindles first noted		
24		50 in 31	$\frac{1}{4}$ to $\frac{1}{3}$ in best eggs
28		50 in 32	$\frac{1}{3}$ in best eggs
32		50 in 31	$\frac{1}{3}$ to $\frac{1}{2}$
36		50 in 31	$\frac{1}{2}$
40		50 in 31	$\frac{1}{2}$
45		50 in 31	$\frac{1}{2}$
48	Cleavage beginning about 10 per cent		

September 4th

Eggs fertilized at 11:55 A.M.

Temperature {31.0° at 11:54 A.M.
22.8° at 12:47 P.M.

6		30 in 22	$\frac{1}{2}$ distinct
10		30 in $22\frac{1}{2}$	H.Z. indistinct toward lighter pole
14		30 in $22\frac{1}{2}$	No trace of a H.Z.
18		30 in 22	No trace of a H.Z.
19	No spindles		
21	Pre-spindles show plainly		
22		30 in 23	In a few eggs, H.Z. may be indicated, but this is doubtful
26		30 in 22	$\frac{1}{3}$ in a few eggs
30		30 in 22	$\frac{1}{3}$ to $\frac{1}{2}$, but not very distinct
34		30 in $22\frac{1}{2}$	Nearly $\frac{1}{2}$, but it does not show very plainly
38		30 in $22\frac{1}{2}$	$\frac{1}{3}$ to $\frac{1}{2}$, shows more plainly than in previous test
42		30 in 22	$\frac{1}{2}$, shows very plainly
46		30 in 23	Most eggs show no H.Z. In some, however, $\frac{1}{3}$ to $\frac{1}{2}$.
$48\frac{1}{2}$	About 5 per cent segmenting		

September 6th

Eggs fertilized at 11:50 A.M.

Temperature { 19.8° at 11:50 A.M.
21.0° at 12:44 P.M.

MINUTES AFTER FERTILIZATION	OBSERVATIONS ON NORMAL (i.e., UNCENTRIFUGED) EGGS	CENTRIFUGAL TREATMENT	HYALINE ZONE
2		30 in 22	$\frac{1}{3}$
7		30 in 23	$\frac{1}{3}$ to nearly $\frac{1}{2}$, eggs appear to be better stratified than in previous test
11		30 in $23\frac{1}{2}$	Usually H.Z. does not show. Rarely $\frac{1}{3}$
15		30 in 22	No H.Z.
18	No spindles		
19		30 in 22	No H.Z.
20	No spindles		
$21\frac{1}{2}$	Pre-spindles present		
23		30 in 22 (turns not quite regular)	Faintly indicated in a few eggs
27		30 in 23	Barely indicated in a few eggs
31		30 in 22	$\frac{1}{2}$, but not all distinct
35		30 in 22	$\frac{1}{2}$, but not very distinct
39		30 in 22	$\frac{1}{2}$, much more distinct
45		30 in 22	$\frac{1}{2}$, shows plainly
51	About 10 per cent segmenting		

September 9th

Egg fertilized at 1:45 P.M.

Temperature { 19.8° at 1:46 P.M.
20.8° at 3:50 P.M.

MINUTES AFTER FERTILIZATION	OBSERVATIONS ON NORMAL (i. e., UNCENTRIFUGED) EGGS	CENTRIFUGAL TREATMENT	HYALINE ZONE
4		30 in 25	$\frac{1}{3}$
9		30 in 25	$\frac{1}{2}$
13		30 in 25	Usually does not show, rarely as much as $\frac{1}{3}$
18		30 in 25	No H.Z.
22	No spindles		
23		30 in 25	No H.Z.
26	Pre-spindles present		
28		30 in 25	No H.Z.
32		30 in 25	Faintly indicated in some eggs
36		30 in 25	H.Z. indicated toward one pole
40		30 in 26	$\frac{1}{3}$ or slightly more
$44\frac{1}{2}$		20 in 20	$\frac{1}{3}$
51	See 4th column	30 in 25	$\frac{1}{3}$. Some eggs show cleavage furrows beginning
55		30 in 25	No eggs show H.Z. or any trace of stratification
59		30 in 24	No H.Z.
63		30 in 24	No H.Z.
67		30 in 24	Indicated in a few eggs
71		30 in 25	Indicated in some eggs
76		30 in 25	Extends as much as $\frac{1}{3}$ way
82		30 in 25	Nearly $\frac{1}{2}$
$86\frac{1}{2}$		30 in 25	In eggs still in 2-cell stage H.Z. shows, but those which are undergoing the 2nd cleavage do not show it

September 11th

Eggs fertilized at 4:40 P.M.

Temperature { 21.0° at 4:30 P.M.
18.6° at 6:02 P.M.

MINUTES AFTER FERTILIZATION	OBSERVATIONS ON NORMAL (i.e., UNCENTRIFUGED) EGGS	CENTRIFUGAL TREATMENT	HYALINE ZONE
3		30 in 25	$\frac{1}{3}$
7		30 in 25½	$\frac{1}{4}$
12		30 in 25	No H.Z.
16		30 in 25	No H.Z.
19½	No spindles		
20		30 in 23	H.Z. indicated by a light spot at one pole. (Nucleus?)
21	No spindles		
23	Pre-spindles present		
24		30 in 25	H.Z. indicated at one pole
28		30 in 25	$\frac{1}{5}$ to $\frac{1}{4}$
32		30 in 25	$\frac{1}{3}$, usually indistinct
36		30 in 25	$\frac{1}{3}$, fairly distinct
40		30 in 25	$\frac{1}{3}$ to nearly $\frac{1}{2}$, distinct
44		30 in 25	$\frac{1}{3}$ to nearly $\frac{1}{2}$, distinct
47		30 in 25	$\frac{1}{3}$ to $\frac{1}{2}$, indistinct
50		30 in 25	$\frac{1}{3}$ to $\frac{1}{2}$, indistinct
52⅙		30 in 25	$\frac{1}{3}$ to $\frac{1}{2}$, indistinct
55		30 in 25	H.Z. appears indistinctly and consists largely of the wide anaphase spindle
59	About 5 per cent segmenting		
61		40 in 30	No H.Z.
65		40 in 30	No H.Z. except in a few cases when one end of cells is slightly paler than the opposite end
69		40 in 30	When the axis of stratification is parallel to the 1st cleavage plane, the H.Z. shows indistinctly $\frac{1}{3}$ way.
73		40 in 30	When the axis of stratification is parallel to the 1st cleavage plane, the H.Z. extends nearly $\frac{1}{2}$ way. It also shows readily in other positions
78		40 in 30	Same as in previous test
95	About 5 to 10 per cent have undergone second cleavage		

All of the above tables tell the same story. During the mitotic process there is a marked increase in viscosity, which is later followed by a decrease. These changes in viscosity are very considerable. Soon after fertilization and again before the first cleavage, a hyaline zone can be made to appear distinctly through a large part of the egg, if the centrifuge handle is turned thirty times in twenty-five seconds. On the other hand, at about twenty minutes after fertilization, even if the centrifuge handle is turned fifty times in thirty seconds, the hyaline zone either does not appear at all or shows in only a small region of the egg. In the latter case the force exerted is approximately twice as great. The increase in viscosity is at least twofold and is almost certainly far more than this. Such a marked viscosity increase is beyond much doubt due to a gelation with the cytoplasm.

As the tables show the gelation reaches its height slightly prior to the time that the pre-spindle first becomes visible. The appearance of the spindle is then followed by a decrease in viscosity. These facts suggest a definite time relation between the viscosity changes and the formation of the spindle.

During the second mitotic division of the egg, there is a parallel series of viscosity changes. As the first cleavage occurs, and apparently even before the cells are fully separated, there is a renewed stiffening of the cytoplasm. This is in preparation for the second mitosis. This gelation lasts ten or fifteen minutes, and then the cytoplasm becomes more fluid again. No attempt was made to follow similar changes in later divisions of the egg.

The fact that gelation occurs during mitosis is indicated by various other observations. In his book on the cell, Flemming pointed out that during the process of division the refractive index of the cell rises. With this fact he correlated the increased staining capacity of fixed preparations of dividing cells, as first observed by van Beneden ('75). Flemming ('82) apparently believed that these observations indicated a stiffening of the cytoplasm. In a later paper ('91) he states that the refractive index of a dividing connective-tissue cell increases, and then in the anaphase begins to decrease again. Similar observations have been made by Levi ('16), apparently without any knowledge of Flemming's work.

An observation of Spek ('18) on Nematode eggs is also of interest. In these eggs, as is well known, the cytoplasm near the surface is in continual movement. But at the moment when the spindle becomes visible all this movement ceases completely. Spek finds "alle amöboide Bewegungen an der Oberfläche der Eizelle . . . . ganz plötzlich—in dem Augenblick, wo der Spindel sichtbar wird—völlig aufhören." Probably this cessation of movement is due to a cytoplasmic stiffening. This stiffening, however, does not extend to the center of the egg, for in the central region the spindle oscillates from side to side.

In sea-urchin eggs Albrecht ('98) found that there was an increase in viscosity after fertilization. This was more conclusively shown by myself in 1915.

But the most remarkable work is that of Chambers ('17). By microdissection he was able to demonstrate in the dividing eggs first a gelation and later a return to a more fluid condition. My findings agree absolutely with his. Moreover, Chambers was able to study the morphological aspect of the gelation. In the astral radiations only the granular material is solidified. The hyaline rays which lie between the granular radiations are fluid.

The solidified materials of the cytoplasm apparently extend out to the cortex of the egg and are attached there. This is indicated by the shape of the egg in the centrifuge tests. When they are centrifuged soon after fertilization, the contour of the egg remains spherical. However, as soon as gelation begins, the eggs assume irregular shapes after centrifugal treatment. This distortion of the eggs always occurs at this time and is quite striking. Oftentimes various regions of the eggs are flattened, and frequently parts of the egg surface are indented, indicating a strong pull on certain regions. In the later stages of mitosis, when the cytoplasm is again more fluid, the eggs nevertheless assume irregular shapes when centrifuged. Apparently, there are still some gelatinous strands, perhaps more slender, which retain their connection to the egg surface, and pull upon it when the egg is centrifuged. This view is supported by the fact that when such eggs are centrifuged more vigorously, there is less tendency for irregularity in contour. In this case, very probably

the strands are torn from their attachment and the shape of the egg can remain spherical.

The conception of astral radiations or gelatinous strands of some kind attached to the periphery of the cell is of importance in any theoretical interpretation of cell division. That there is actually such an attachment is also indicated by a direct observation of the egg during the mitotic process. On a number of occasions I have noticed that the first appearance of the amphiaster in the egg is followed by a change in the outline of the surface contour. The hyaline membrane, at first perfectly smooth, now appears slightly crenate. This crenate appearance is more evident when the egg is looked at from one pole of the mitotic spindle, and is apparently due to the pull of gelatinous strands which radiate from the centrosphere. I have not been able to observe such a crenate appearance in all eggs.

INHIBITION OF SPINDLE FORMATION

The measurements of cytoplasmic viscosity recorded in the previous section afford evidence that the appearance of the mitotic figure is closely bound up with gelation phenomena. Much more convincing evidence can be obtained experimentally. My earlier work showed that artificial stimulation to mitosis, no matter what the reagent, always involves a preliminary gelation of the cytoplasm. These observations indicated that there is a definite causal sequence, but there was nothing to show that gelation was more than a secondary phenomenon, having no direct relation to the formation of the mitotic figure. In order to show that the appearance of the spindle depends absolutely on a preliminary cytoplasmic gelation, it is necessary to show that suppression of the gelation in every case results in a suppression of the spindle. This I have been able to do.

In searching for an antigelatinizing agent, I was guided by the earlier observations of Hertwig ('90) and Wilson ('01). The former discovered that cold effectively prevented the appearance of the amphiaster, the latter found that ether acted in a similar manner.

In the course of my work I have been able to discover a large number of substances which prevent the normal gelation of the egg or reverse it after it has occurred.

If the fertilized eggs are treated with antigelatinizing agents before the spindle has appeared, spindle formation is inhibited and cell division does not occur. This suppression of cell division need not involve injury to the egg, and in most cases when the eggs are returned to normal sea-water they resume their development.

The following substances were found, in proper concentration, to prevent gelation: ether, chloroform, acetone, paraldehyde, propyl alcohol, isoamyl alcohol, ethyl butyrate, ethyl nitrate, acetonitrile, nitromethane, chloral hydrate, phenyl and ethyl urethanes.

In studying the effect of these substances on the egg, the usual procedure was as follows: Soon after fertilization, eggs were placed in a series of concentrations of the desired reagent. Usually about six different concentrations were employed, and they were kept in tightly stoppered vials. Then, as soon as possible, the cytoplasmic viscosity of the eggs was tested. As my studies progressed, I was able to predict the fate of the various groups of eggs. A certain degree of antigelatinizing action always prevented the formation of the spindle. The weaker solutions, unable to produce the requisite effect on cytoplasmic viscosity, did not prevent the division of the cell. On the other hand, too concentrated solutions produced intense gelation and killed the eggs. Intermediate concentrations gave the desired results. In these cytoplasmic gelation was inhibited or reversed, and viscosity determinations showed the cytoplasm to have a liquid consistency. In such eggs with fluid cytoplasm, no mitotic figure ever formed. Nevertheless, the eggs were not all killed by the reagent, as some were able to resume their development on return to sea-water.

The results obtained with the fourteen substances enumerated above were all similar. Individual differences occurred, but in every case the general scheme was the same. It will scarcely be necessary, therefore, to record all of the experiments, and I shall content myself with presenting only some representative

ones. Moreover, in the tabulation of these experiments, I shall attempt to reduce the data to the briefest possible form, omitting many of the less essential observations.

In order to compare the cytoplasmic viscosity of the treated eggs with that of the normal controls, normal and treated eggs were centrifuged simultaneously in separate tubes of the centrifuge. These comparison tests had to be made before the control eggs segmented, and preferably at a time when the cytoplasmic viscosity of the eggs was high. Thus only a limited period was available, and it was impossible to test the viscosity of the eggs in all the vials. Oftentimes it was necessary to repeat experiments. It was always found that when a particular concentration of a reagent completely prohibited gelation, the eggs subjected to this concentration never formed a spindle. This relation always held true.

In the following description, frequent reference is made to per cent solutions. In most cases, solutions of liquids in liquids are referred to, and in such instances, per cents by volume are understood. By this I mean cc. of solute per 100 cc. of solution. In the few cases when solid substances were dissolved, the per cent indicated is a weight per cent. All solutions were of course made up in sea-water.

Wilson ('01) found that 2.5 per cent ether prevented the appearance of astral rays or spindles. I began my experiments by determining the effect of such a concentration of ether on the fertilized egg.

June 22nd. Some eggs were fertilized at 9:42 A.M. and put into 2.5 per cent ether at 9:52 A.M. At 10:01 A.M. normal and etherized eggs were centrifuged simultaneously, the high-speed handle being turned 30 times in 28 seconds. The normal eggs remained unstratified, the etherized eggs showed a very evident stratification. Thus $2\frac{1}{2}$ per cent ether has an antigelatinizing action. This concentration of ether prevents spindle formation, although it does not otherwise harm the cell. Higher concentrations of ether are injurious. Thus $4\frac{1}{2}$ per cent and 5 per cent ether produce a rapid coagulation, and $3\frac{1}{2}$ per cent and 4 per cent ether, although they at first tend to liquefy the cytoplasm, after an hour or so coagulate it.

Chloroform has a similar effect, as is borne out by the following experiment:

July 10th. Eggs fertilized at 10:16 A.M. were placed in a 0.13 per cent solution of chloroform at 10:26½ A.M. At 10:33 A.M. normal fertilized eggs and some of those in the chloroform solution were centrifuged simultaneously, the handle being turned 50 times in 29 seconds. The normal eggs had a hyaline zone barely indicated, whereas the chloroformed eggs showed a hyaline zone extending halfway along the axis of stratification. The eggs treated with chloroform did not segment

Various experiments with paraldehyde showed that a 4 per cent solution was very effective in reversing gelation.

July 10th. Eggs fertilized at 11:51 A.M. were transferred to 4 per cent paraldehyde at 12:02 P.M. At 12:09 P.M. normal eggs and those exposed to paraldehyde were centrifuged simultaneously. In the normal eggs the hyaline zone was barely indicated, in the treated eggs it extended through half of the egg. The concentration of paraldehyde used was sufficient to prevent segmentation.

The following experiments deal with the effects of various other substances which act like ether and chloroform.

July 17th. Chloral hydrate. At 12:05½ to 12:06 P.M., eggs fertilized 24 minutes previously (at 11:42 A.M.) were subjected to seven different concentrations of chloral hydrate, varying from $\frac{1}{6}\frac{1}{0}$ per cent to 1 per cent. Of these concentrations, 1 per cent of the reagent produced coagulation, but concentrations of ¼ per cent to $\frac{1}{1}\frac{1}{2}$ per cent had the opposite effect and produced reversal of the normal gelation. These concentrations prevent segmentation, but the inhibition is only temporary, and the eggs segment (although often somewhat irregularly) upon return to sea-water.

In the above description many of the less important details of of the experiment were omitted. The following experiment is reported more fully:

July 22nd. Amyl alcohol. At 11:44 A.M., eggs fertilized five minutes previously were put into vials containing various concentrations of isoamyl alcohol (isobutyl carbinol). Six vials were used. *A* contained 2 per cent of the alcohol, *B* 1.33 per cent, *C* 1 per cent, *D* 0.67 per cent, *E* 0.33 per cent, and *F* 0.17 per cent.

At 11:51 A.M., eggs in *A* and normal eggs were centrifuged simultaneously, the handle of the centrifuge being turned 50 times in 29 seconds. The *A* eggs were evidently coagulated, for they showed no stratification; the normal eggs had the hyaline zone barely indicated. At 12:02½ P.M., eggs in *B* and normal eggs were centrifuged simultaneously at the same speed as in the previous test. They, too, gave no evidence of stratification. At 12:12½ P.M., the eggs in *D* and normal eggs were

centrifuged, the handle being turned 50 times in 30 seconds. The *D* eggs had a hyaline zone extending one-third or more of the distance along the stratification axis. In these eggs the gray cap was apparently lacking. In the control normal eggs the original gelation was beginning its normal reversal, and the hyaline zone extended about one-fourth of the stratification axis. At 12:23½ P.M. eggs in *C* and normal eggs were centrifuged simultaneously, the handle being turned 50 times in 30 seconds. Of the *C* eggs almost all were cytolyzed and showed no stratification. A few, however, showed a hyaline zone one-half to two-thirds of the distance along the axis of stratification. Such a marked liquefaction is often the preliminary of coagulation. In the normal eggs at this time, segmentation was beginning, and the hyaline zone was poorly shown.

The eggs in *A*, *B*, *C*, *D*, did not segment. Those in *E* had numerous small cells cut off from the margin. This is an appearance typically found in all cases where the concentration of the anesthetic is not quite sufficient to stop segmentation completely. In *F*, the cleavage was much more nearly normal. These observations were made at about 1:30 P.M. At 12:36 to 12:37 P.M. some eggs from each of the dishes *A* to *F* had been transferred to Stender dishes containing fresh sea-water. Those from *A* were put into *a*, those from *B* into *b*, etc. These dishes were then examined at 1:45 P.M. No segmentation occurred in *a*, *b*, *c*, and in *d* only abnormal evidences of the segmentation process were found. But in *e* and *f* normal segmentation occurred, and motile blastulae were later found in these dishes.

July 24th. Acetonitrile (methyl cyanide). At 11:35½ A.M. eggs were fertilized. Ten minutes later they were placed in vials containing solutions of acetonitrile in sea-water. Vial *A* contained 5 per cent, *B* 4 per cent, *C* 2½ per cent, *D* 2 per cent, *E* 1 per cent, *F* ½ per cent, *G* ¼ per cent. At 11:52 A.M., eggs in *B* and normal eggs were centrifuged simultaneously the handle being turned 50 times in 28 seconds. The *B* eggs had a hyaline zone one-third to one-half the extent of the egg axis, whereas the normal controls showed barely a trace of hyaline zone in a few cases. At 11:59 A.M., eggs in *A* and normal eggs were centrifuged at the same speed as in the previous test. A few of the *A* eggs showed a hyaline zone extending along one-third of the egg axis, but in most cases the eggs were coagulated and showed no stratification. The control of normal eggs exhibited no stratification, except in a few cases which showed trace of a hyaline zone.

At 12:05 to 12:07½ P.M. some eggs were transferred out of *A*, *B*, *C*, *D*, *E*, *F*, *G*, into normal sea-water in Stender dishes *a*, *b*, *c*, *d*, *e*, *f*, *g*, respectively. At 2:15 to 2:30 P.M. eggs in *A* to *G* and *a* to *g* were examined. In *A* and *B*, no segmentation of any kind had occurred. In *C* and *D*, there was no normal segmentation, but in many of the eggs small cell-like masses had apparently been cut off from the cell periphery. In *E*, 10 per cent of the eggs had segmented in more or less normal fashion, others had segmented abnormally. In *F* and *G*, normal segmentation had occurred generally. In *a*, 20 per cent of the

eggs had segmented, some of these had stopped at the two-cell stage, but others had gone on. In *b*, 98 per cent had segmented, and almost all were normal. In *c* to *g* normal segmentation of course occurred.

At about 7 P.M., *a* to *g* were examined for blastulae. In *a*, about 2 per cent of the eggs had developed motile blastulae. In *b* to *g*, practically all the eggs had developed to motile forms, only immature eggs remaining on the bottom of the dish. The eggs in *A* to *G* were then examined. No motile blastulae were found in *A* to *E*, in *F* and *G*, motile blastulae were abundant.

July 28th. Ethyl nitrate. Eggs were fertilized at 11:50 A.M. and at 12 M they were placed in vials *A* to *F*. Vial *A* contained 0.5 per cent ethyl nitrate in sea-water, *B* 0.4 per cent, *C* 0.3 per cent, *D* 0.2 per cent, *E* 0.1 per cent, and *F* 0.05 per cent.

At 12:06½ P.M., eggs in *C* and normal fertilized control eggs were centrifuged simultaneously, the handle being turned 50 times in 30 seconds. The eggs in *C* had a hyaline zone extending through half the egg, in the normal eggs the hyaline zone was not distinct, but was indicated in a fourth of the egg. At 12:19 P.M., eggs in *A* and normal eggs were centrifuged simultaneously, the handle being turned 50 times in 30 seconds. The eggs in *A* showed a hyaline zone extending halfway. In the normal eggs, the cytoplasmic gelation had reversed, and the hyaline zone extended one-third of the distance along the axis of stratification.

At 12:30 P.M., some eggs were removed from each of the vials *A* to *F* and transferred to normal sea-water in Stender dishes *a* to *f*, respectively. In *b* to *f* normal segmentation occurred and motile blastulae were produced. In *a*, some eggs segmented normally, others abnormally; a few motile blastulae resulted. Of the eggs which remained in *A* to *F*, those in *A*, *B*, *C*, did not segment, those in *D*, *E*, *F*, segmented abnormally. No blastulae were produced in *A* to *F*.

It is scarcely necessary to mention other experiments of the same sort which were performed with the various substances previously enumerated. All fourteen substances, in suitable concentration, prevent the appearance of the mitotic figure without otherwise injuring the egg. Those concentrations which act in this way are the very ones which inhibit gelation and preserve the fluid condition of the cytoplasm.

Perhaps the action of these substances depends upon their lipoid-solvent action. This action does not appear to be exerted on the plasma-membrane, as many students of anesthesia have thought, for the vitelline membrane, which I have shown ('15) to be the plasma membrane of the unfertilized egg, is, as far as I can determine, morphologically unaffected. Similarly, the hyaline layer, which becomes the plasma membrane soon after

fertilization, shows no signs of alteration. On the other hand, the lipoids of the egg interior are oftentimes visibly changed. This is easily seen after the egg is centrifuged, for the cytoplasmic lipoids then become aggregated at one pole of the egg, forming there a small polar accumulation known as the gray cap. After the egg had been treated with one of the substances used in the above experiments, it was often noted that the gray cap appeared pale and indistinct. Sometimes the gray cap was apparently absent. Thus these substances which prevent gelation possibly produce their effect by acting on the lipoids of the egg.

Even before Wilson had shown that ether prevented the appearance of asters and spindle, O. Hertwig had made the observation that this effect could be produced by low temperatures ($-2°$ to $-3°$) without otherwise injuring the egg. My views, therefore, demanded that such low temperatures have a liquefying effect on the cytoplasm. This was fully borne out by experiment.

June 24th. At 4:36 P.M., eggs fertilized sixteen minutes previously were exposed to a temperature of $-3°$. Fifteen minutes later (at 4:51 P.M.), the eggs were removed from the cold, and after an interval of two minutes they were centrifuged simultaneously with control eggs, also fertilized at 4:36 P.M., but not exposed to cold. The handle was turned 40 times in 30 seconds. On examination, the untreated control eggs showed no stratification whatever, whereas the eggs exposed to cold showed the various stratification zones plainly.

Not only does cold exert an antigelatinizing action on fertilized eggs, but it has a similar liquefying effect upon the cytoplasm of unfertilized eggs.

June 24th. Some unfertilized eggs were exposed to a temperature of $-3°$, and after ten minutes they were centrifuged simultaneously with normal eggs, the centrifuge handle being turned 21 times in 15 seconds. Both sets of eggs showed stratification. In the normal eggs, however, the granular zone was not distinct from the pigment zone, whereas in the cold-treated eggs the pigment granules had migrated more completely, thus effecting a separation between granular and pigment zones.

The question now arises as to how this antigelatinizing effect of cold is produced. Obviously it cannot act act as a lipoid solvent. The idea suggested itself, however, that cold might pro-

duce the same effect that fat solvents do, but in quite a different way. Possibly low temperatures tend to precipitate the fat globules out of the cytoplasmic emulsion. Such a precipitation might produce an effect comparable to that of the lipoid solvents, for either precipitation or solution would tend to remove the lipoids from their emulsified state. If cold and lipoid solvents both produce their effect by acting on the lipoids of the cell, it is evident that these effects, instead of being complementary, would be antagonistic. Actual experiment demonstrated an antagonism between cold and ether. Eggs treated both with cold and ether showed less antigelatinizing effect than when treated with cold alone or with ether alone.

EFFECT OF INCREASED GELATION

The preceding discussion was concerned with the effects of various antigelatinizing agents upon the cytoplasm. It might be interesting to mention briefly some other experiments with substances which tend to intensify the normal gelation. Many authors have investigated the effect of hypertonic solutions on dividing eggs. Loeb ('92) and Norman ('96) found that if the hypertonic solution was sufficiently strong, cleavage was stopped. Oftentimes, nuclear division without cytoplasmic division resulted.

The effect of hypertonic solutions was investigated by the centrifuge method, and in all cases an intensified gelation could be demonstrated. This is apparently especially marked in the cortex of the egg, and it is probable that such a cortical gelation is the main factor which inhibits division of the cell, even when the nucleus is still able to divide mitotically.

July 5th. Eggs were fertilized at 10:43½ A.M. At 11:08 A.M. some of these eggs were placed in Stender dishes *A*, *B*, *C*, *D*, *E*.

A contained 40 cc. sea-water plus 2 cc. 2½ N NaCl
B contained 40 cc. sea-water plus 4 cc. 2½ N NaCl
C contained 40 cc. sea-water plus 6 cc. 2½ N NaCl
D contained 40 cc. sea-water plus 8 cc. 2½ N NaCl
E contained 40 cc. sea-water plus 10 cc. 2½ N NaCl

At 11:17 A.M., eggs from *C* were centrifuged simultaneously with a control lot of untreated fertilized eggs, the handle being turned 50 times in 29 seconds. Whereas the normal eggs had the hyaline zone well indicated, the eggs from *C* showed not a trace of stratification.

At 11:25½ A.M., eggs from *B* were compared with normal control eggs. The centrifuge handle was turned 50 times in 29 seconds. The normal eggs showed a hyaline zone extending a third of the distance along the axis of stratification. The *B* eggs showed not a trace of a hyaline zone.

At 11:36½ A.M., eggs from *A* were compared with normal eggs. The centrifuge handle was turned 50 times in 28 seconds. The normal eggs showed a prominent hyaline zone, extending at least a third of the distance along the axis of stratification. In the *A* eggs, the hyaline zone was barely indicated.

At 11:53 A.M., the eggs from *D* and from *E* were centrifuged, the handle being turned 50 times in 30 seconds. Neither eggs from *D* nor those from *E* showed any trace of stratification.

Thus it is evident that the addition of hypertonic NaCl to sea-water, has the effect of intensifying the gelation of the egg cytoplasm.

At 12 M, the control of untreated fertilized eggs contained eggs in the two-celled stage, but there was no segmentation in *A*, *B*, *C*, *D*, *E*. At 2 P.M., these dishes were again examined. At this time cleavage was occurring in *A*. In *B* there was nuclear division without cytoplasmic division. In *C*, *D*, and *E* there was neither nuclear nor cytoplasmic division.

At 2:24 P.M., eggs from *B* and from *C* were centrifuged, the handle being turned 50 times in 28 seconds. In the *C* eggs the hyaline zone was indicated in some eggs, but not very clearly. In the *B* eggs the hyaline zone was prominent, extending through about one-third of the egg. But it was not very transparent, for a cortical zone of granules covered it. This indicates a cortical gelation. It will be remembered that the *B* eggs are those in which nuclear division without cytoplasmic division was found to take place.

The effect of potassium cyanide is worth recording. Even in very dilute concentrations, the cyanide inhibits cell division. But, curiously enough, the early phases of mitosis are able to continue in such concentrations. Likewise, the final stages of the process can go on. The explanation that I would offer is a very simple one. Potassium cyanide intensifies gelation and the normal gelation is rendered irreversible. This can be shown true by viscosity measurements.[1]

[1] Moreover, it is directly in line with my previous observation that cyanide prevents swelling of the gel which forms the vitelline membrane of the egg.

In order to determine the concentration of KCN necessary to suppress division, I made a 0.004 per cent solution[2] in sea-water by diluting a 1 per cent solution (in distilled water) with 249 parts of sea-water. This 0.004 per cent solution was successively diluted with equal parts of sea-water until nine solutions were obtained, each half the concentration of the preceding one. Eggs were subjected to all these solutions, and it was found that a concentration of 0.000625 per cent sufficed to check segmentation. In concentrations of 0.0000313 per cent and 0.0000156 per cent, segmentation proceeded to about the four-cell stage, but then went no further.

The following experiment shows that the early stages of mitosis can occur in concentrations much above those which inhibit the entire process:

June 25th. Eggs were fertilized at 5:18½ P.M. In these eggs the spindle first began to be visible twenty minutes later (at 5:39 to 5:40 P.M.). At 5:23 P.M., 5:27 P.M., 5:32 P.M., 5:38 P.M., some of the fertilized eggs were transferred into Stender dishes *A*, *B*, *C*, *D*, respectively. Each of these dishes contained 0.0025 per cent KCN, prepared by diluting 1 per cent KCN (in distilled water) with 399 parts of sea-water.

The eggs in *A* were observed at 5:47 P.M. Instead of showing a small nucleus, they showed a large pale spot with a vague border. This spot was often elongated, and probably represented an abnormal spindle. When the eggs in *B* were examined at 5:58 P.M., they showed a pre-spindle plainly, and they appeared much like the normal eggs. None of the eggs in *A*, *B*, *C*, *D*, proceeded to develop any further than the spindle stage, and observation at 10:40 P.M. showed them all with spindles, but unsegmented.

It might be thought that the above experiment owes its explanation to the fact that the cyanide penetrates the eggs slowly, and that only after a time is its influence felt. However the eggs in *D* appeared to be checked almost immediately. Moreover, a later experiment showed that this interpretation could not be the correct one. In this case the fertilized eggs were put into a 0.004 per cent solution of KCN in sea-water five minutes after fertilization. The eggs in the KCN solution were kept in a test-

[2] Of course the actual concentration of KCN is not referred to, as there is a reaction between KCN and the salts of sea-water.

tube tightly sealed with a rubber stopper, and the test-tube was then exposed to a temperature which varied from 10° to 12°. No spindle appeared while the eggs were in the cold, but when the test-tube was removed from the cold after an exposure of two hours and thirty-eight minutes and warmed with the hand, then spindles soon made their appearance. Thirteen minutes later they could be observed plainly. During their two and one-half hours' stay in the cold, the eggs must have been thoroughly penetrated by the KCN solution, and yet as soon as they were placed in a warmer temperature, development proceeded as far as the spindle stage. Of course no segmentation occurred in these eggs.

The following experiment shows that in a 0.005 per cent solution of KCN, the final stages of mitosis can proceed:

August 23rd. Some eggs were fertilized at 8:23 P.M. and they first began to segment forty-two minutes later. At 30, 33, 35, 38, 40, 42 minutes after fertilization these eggs were removed to stoppered test-tubes *A*, *B*, *C*, *D*, *E*, *F*, respectively, each of which contained 0.004 per cent KCN in sea-water. Counts of the segmenting eggs showed in *F*, $\frac{96}{100}$; in *E*, $\frac{94}{100}$; in *D*, $\frac{88}{100}$; in *C*, $\frac{96}{100}$; in *B*, $\frac{94}{100}$; in *A*, $\frac{59}{100}$; normal control eggs, $\frac{94}{100}$. In these fractions the numerator represents the number of segmenting eggs counted, the denominator, the total number of eggs observed. Thus it is apparent that for the last twelve minutes the cleavage process is able to continue in the presence of 0.004 per cent KCN.

In order to explain this curious action of KCN on one particular stage of mitosis, I have already suggested that the cyanide renders irreversible the normal gelation. Even before the above experiments were performed, I had evidence supporting this view.

June 25th. Some eggs fertilized at 2:54 P.M. were at 3:14 P.M. subjected to the action of 0.005 per cent and 0.0025 per cent KCN. At 3:34 P.M. eggs in 0.005 per cent KCN were centrifuged simultaneously with normal eggs, the handle of the centrifuge being turned 45 times in 30 seconds. The eggs exposed to the cyanide showed no stratification whatsoever, whereas the normal eggs, as expected, showed a very distinct stratification. A later test of the eggs in 0.0025 per cent KCN gave similar results. Thus the KCN prevents the normal reversal of gelation.

The results with KCN lend additional support to the views already expressed on the relation of the mitotic process to the colloidal changes in the cytoplasm. Cyanide acts by intensifying gelation. Hence, as is to be expected, it does not (in moderate concentration) prevent the early stages of mitosis, and development proceeds as far as the spindle stage. But that particular stage of mitosis which is associated with a reversal of gelation cannot take place in the presence of the cyanide.

Chloretone acts somewhat like KCN. A 0.08 per cent solution checks segmentation, although it does not markedly injure the eggs. Such a solution intensifies the normal gelation.

THE NATURE OF THE NORMAL MITOTIC GELATION

Earlier in this paper I have attempted to show that the appearance of the mitotic figure is necessarily preceded by a cytoplasmic gelation. Such a gelation can be artificially produced in unfertilized eggs by various reagents, the best of which apparently is a hypertonic solution. The question now arises as to how the gelation occurs normally. The fact that its artificial production appears to be best imitated by hypertonic solutions leads to the suggestion that, similarly in the developing egg, gelation and consequent formation of astral rays and spindle is initiated by the abstraction of water from the cytoplasm by the growing pronuclei. Some of the early students of artificial parthenogenesis thought that the essential step in the initiation of development was the abstraction of water from the cytoplasm.

If the normal gelation is produced by an excessive salt concentration, then it should be possible to show that gelation produced artificially by hypertonic solutions behaves like the normal gelation. To a certain extent this has been done. When the cytoplasm of the unfertilized egg is gelatinized by hypertonic solutions, such a gelation can be reversed by ether. On the other hand, ether has no effect in reversing or antagonizing the gelatinizing (or coagulating effect) of acids or of distilled water. Hence of these three types of gelation, that produced by hypertonic solutions behaves most nearly like the normal.

June 28th. At 9:38 A.M., 8 cc. of $2\frac{1}{2}$ N NaCl were added to 50 cc. of sea-water containing unfertilized eggs. At 10:39 A.M., some of these eggs were removed to a solution containing 3 per cent ether. This solution was made up by adding to $43\frac{1}{2}$ cc. of sea-water, 5 cc. of $2\frac{1}{2}$ N NaCl, and $1\frac{1}{2}$ cc. of ether.

At $10:49\frac{1}{2}$, the cytoplasmic viscosity of the eggs in both solutions was compared by a simultaneous centrifuge test, by which it was found that the eggs in the solution containing ether had a more fluid cytoplasm. The centrifuge was turned 45 times in 28 seconds. After this treatment, the eggs in the hypertonic solution without ether showed no sign of stratification, whereas the eggs which had for ten minutes been exposed to ether (although still in a hypertonic solution) showed the beginnings of stratification. In them, the pigment granules had shifted somewhat, the gray cap and hyaline zone were beginning to appear.

That ether exerts an antagonistic action toward hypertonic solutions was shown in still another way. As has been previously pointed out, $2\frac{1}{2}$ per cent ether reverses the normal gelation and thus prevents the appearance of the mitotic figure. However, when $2\frac{1}{2}$ M NaCl was added, although the concentration of ether remained the same, the ether was no longer able to repress the formation of spindles and asters.

In several experiments I tried to discover if ether would prevent the gelation of the egg cytoplasm by distilled water or by acid. Rather than a decrease, the addition of ether apparently produced a slight increase in the gelatinizing power of dilute acid solutions. The following experiment serves as a sample:

July 20th. At 5:20 P.M., unfertilized eggs were placed into Stender dish *A*, which contained 50 cc. of sea-water plus 1.3 cc. $\frac{N}{10}$ HCl, and also into Stender dish *B*, which contained 50 cc. of 2 per cent ether dissolved in sea-water + 1.3 cc. $\frac{N}{10}$ HCl. At 5:25 P.M. the cytoplasmic viscosity of the eggs in *A* and *B* was simultaneously tested with the centrifuge, the handle being turned 35 times in 30 seconds. Many of the eggs from *A* were injured. The intact eggs showed a hyaline zone extending one-fourth of the distance along the axis of stratification. The eggs from *B* were coagulated and showed not a trace of stratification. At 5:35 P.M., eggs from *A* and *B* were again centrifuged, the handle being turned 35 times in 28 seconds. This time both sets of eggs were coagulated, and in neither case was any stratification visible.

The following experiment, although perhaps not conclusive, indicates that ether does not prevent gelation of egg cytoplasm by distilled water:

June 29th. At 11:38 A.M., some unfertilized eggs were dropped into distilled water, and one minute later they were transferred out of the distilled water into *A*, which contained pure sea-water, and *B*, which contained $2\frac{1}{2}$ per cent ether in sea-water.

At 11:50, the eggs in *A* and *B* were centrifuged simultaneously, the handle being turned 50 times in 30 seconds. When the centrifuged eggs from *A* and *B* were compared, both lots appeared the same. In both cases most of the eggs showed stratification, with a wide and distinct hyaline zone. In both cases, a considerable number of the eggs were cytolyzed and showed not a trace of stratification.

If the normal gelation is due to an abstraction of water, then it should be possible to show an antagonism between cold and hypertonic solutions, whch would be comparable to the effect of cold on the normal gelation. So far, my results in this direction have not been completely successful. Although I have been able to show that cold retards the gelatinizing effect of hypertonic solutions on the unfertilized egg, I have not yet demonstrated that cold can cause a reversal of gelation when once this has been produced by hypertonic solutions. But only a single experiment has been tried, and perhaps further observation will also show this to be true. The following experiments shows that cold tends to prevent gelation of the cytoplasm by a hypertonic salt solution:

August 30th. A hypertonic solution was prepared by adding 8 cc. of $2\frac{1}{2}$ n NaCl to 50 cc. of sea-water. This solution was then divided into two portions, of which *A* remained at room temperature, and *B* was kept at a temperature which varied from $-1.3°$ to $+1°$. At 10:58 A.M., unfertilized eggs were placed in *A*. At 11:08 A.M., some of the eggs in *A* were removed to *B*. At 12:31 P.M., eggs in *A* and *B* were centrifuged simultaneously, the handle being turned 50 times in 30 seconds. The eggs in *B* showed a gray cap and a hyaline zone extending about one-third of the distance along the axis of stratification. The eggs in *A* showed not a trace of stratification.

If the gelation which occurs normally is due to water abstraction, it might be also expected that when the egg is made to take up water, this gelation could be reversed. This is in fact true, and hypotonic solutions effectually cause a reversal of gelation in the fertilized egg. This was shown clearly by centrifuge tests. Because of this antigelatinizing effect, hypotonic solutions act like ether and prevent segmentation without otherwise injuring the egg.

June 28th. Eggs fertilized at 11:31 A.M., were at 11:46½ A.M., dropped into Stender dish *B*, which contained 40 cc. sea-water plus 10 cc. distilled water. The untreated fertilized eggs remained in Stender dish *A*. At 11:51½ A.M., eggs in *A* and *B* were centrifuged simultaneously, the handle being turned 45 times in 26 seconds. The eggs in *A* showed just the beginnings of a hyaline zone. The eggs in *B* were markedly more stratified, they showed gray cap and hyaline zone plainly. In this experiment the sea-water was not sufficiently dilute to prevent segmentation. In another experiment it was found that a solution made up of equal parts of sea-water and distilled water was the most favorable for the reversible prevention of cleavage. In such a solution, eggs remained unsegmented, and yet after a three-hour exposure, they were able to resume their development when returned to normal sea-water.

SUMMARY

1. During the period between fertilization and the first cleavage of the sea-urchin egg, the viscosity of the cytoplasm rises until it reaches a maximum, then it decreases again.

2. Similar viscosity changes occur in relation to the second cleavage.

3. The changes in viscosity are very marked and indicate the occurrence of a gelation in the cytoplasm.

4. This gel-formation reaches its height just before the spindle appears. Later the cytoplasm becomes more fluid again.

5. That gelation is not secondary, but is a predetermining factor in spindle or aster formation, is indicated by the fact that when gelation is suppressed, the mitotic figure does not form, although the eggs may be otherwise uninjured.

6. Such suppression of gel formation was produced by fourteen different substances, all lipoid solvents.

7. It can also be produced by cold.

8. Although they produce the same effect, the action of cold and of lipoid solvents is mutually antagonistic.

9. The effect of hypertonic solutions on dividing eggs can be interpreted on the basis of the fact that they increase the cytoplasmic viscosity. Potassium cyanide and chloretone also act in this way.

10. The cytoplasmic gelation which occurs in relation to mitosis is apparently due to an abstraction of water, for—

a. It can be most closely imitated by an abstraction of water. Cytoplasmic gels produced by hypertonic solutions on unfertilized eggs behave toward cold and ether much like the normal gel of the fertilized egg. Cytoplasmic gels produced by acid or by distilled water do not exhibit this resemblance.

b. Entrance of water into the fertilized egg reverses the normal cytoplasmic gelation.

LITERATURE CITED

ALBRECHT, E. 1898 Untersuchungen zur Struktur des Seeigeleies. Sitzungsber. d. Ges. f. Morph. u. Physiol. in München, 1898, S. 133–141.

VAN BENEDEN, E. 1875 La maturation de l'oeuf, la fécondation et les premières phases du développement embryonnaire des Mammifères, d'après des recherches faites chez le Lapin. Bull. de l'Acad. Roy. de Belgique, 2me ser., T. 40.

CHAMBERS, R. 1917 Microdissection studies. II. The cell aster; a reversible gelation phenomenon. Jour. Exp. Zoöl., vol. 23, pp. 483–505.

FLEMMING, W. 1882 Zellsubstanz, Kern- und Zelltheilung. Leipzig 1882 (see especially pp. 206–208).

1891 Über Theilung und Kernformen bei Leukocyten, und über deren Attraktionsphären. Arch. f. mik. Anat., Bd. 37; S. 249–298.

FOL, H. 1879 Recherches sur la fécondation et le commencement de l'hénogénie chez divers animaux. Mem. d. l. Soc. de Phys. et d'Hist. Nat. de Geneve, T. 26.

HEILBRUNN L. V. 1915 Studies in artificial parthenogenesis. II. Physical changes in the egg of Arbacia. Biol. Bull., vol. 29, pp. 149–203.

1917 An experimental study of cell division. Proc. Amer. Soc. Zool., Anat. Rec., vol. 11, pp. 487–489.

HERTWIG, O. 1890 Experimentelle Studien am tierischen Ei, vor, während, and nach der Befruchtung. Jenaische Zeitschr., Bd. 24, S. 268–313.

LEVI, G. 1916 La costituzione del protoplasma studiata su cellule viventi coltivate 'in vitro.' Archivio di Fisiologia, T. 14, pp. 101–112.

LOEB, J. 1892 Investigations in physiological morphology. III. Experiments on cleavage. Jour. Morph., vol. 7, pp. 253–262.

NORMAN, W. W. 1896 Segmentation of the nucleus without segmentation of the cytoplasm. Arch. f. Entwicklungsmechanik, Bd. 3, S. 106–126.

SPEK, J. 1918 Oberflächenspannungsdifferenzen als eine Ursache der Zellteilung. Arch. f. Entwicklungsmechanik, Bd. 44, S. 5–113.

WILSON, E. B. 1901 Experimental studies in cytology. II. Some phenomena of fertilization and cell-division in etherized eggs. III. The effect on cleavage of artificial obliteration of the first cleavage furrow. Arch. f. Entwicklungsmechanik, Bd. 13, S. 353–395.