Resumen por el autor, Lewis V. Heilbrunn.

Cambios de la viscosidad del protoplasma durante la mitosis.

En estudios precedentes el autor ha observado los cambios de la viscosidad del protoplasma durante la mitosis. En el presente trabajo ha intentado determinar la magnitud de estos cambios y su exacta relación temporal con el proceso de la mitosis. El metodo de la centrifugacion ha sido empleado para medir la viscosidad del protoplasma. La mayor parte de las medidas fueron llevadas a cabo en el ovulo del lamelibranquio Cumingia. Los ovulos fueron centrifugados con cortos intervalos durante el tiempo transcurrido entre la fecundacion y segmentacion. La velocidad del movimiento granular bajo la influencia de una fuerza centrifuga determinada han sido considerada como la medida de la viscosidad protoplasmica. Tanto durante las divisiones de maduracion como en la segmentacion, la aparición del huso esta precedida por una aumento brusco en la viscosidad, seguido de una brusca disminucion de la misma. Al final de la mitosis, inmediatamente antes de dividirse la celula, la viscosidad aumenta marcadamente. Las medidas cuantitativas han sido obtenidas y los resultados han sido representados graficamente. Durante las divisiones de maduración y la segmentacion del ovulo del anelido Nereis tienen lugar cambios de viscosidad semejantes a los de Cumingia.

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PROTOPLASMIC VISCOSITY CHANGES DURING MITOSIS¹

L. V. HEILBRUNN

ONE CHART

THE PROBLEM

Earlier work showed that the segmentation of the sea-urchin egg and the appearance of the mitotic spindle in this egg depend upon a viscosity increase in the cytoplasm (Heilbrunn, '15, '17, '20). During mitosis protoplasmic viscosity changes were demonstrated. It is of interest now to inquire whether similar viscosity changes occur in the dividing eggs of other forms. If the mitotic mechanism depends upon viscosity changes, then such changes should be present in all dividing cells. Furthermore, it has been thought advisable to determine more accurately the magnitude of these changes.

Most of the work was done with the *Cumingia* egg, although some data from the sea-urchin and *Nereis* eggs is included for the sake of comparison. The *Cumingia* egg is immature at fertilization and therefore offers an interesting comparison to the egg of the sea-urchin. In *Cumingia*, three cell divisions occur in a short space of time between fertilization and the first cleavage. Do viscosity changes accompany these divisions and do they show the same time relation to the mitotic process that was previously demonstrated for the sea-urchin egg?

At Woods Hole *Cumingia tellinoides* is fairly common, and during the summer of 1920 good material was generally available throughout July and August. My thanks are due to Prof. F. R. Lillie, director of the Marine Biological Laboratory, for the privilege of carrying on the work there.

¹ Contribution from the Zoological Laboratory of the University of Michigan.

THE METHOD

In general the viscosity of a fluid may be determined in any one of three ways. The usual method is to measure the rate of flow of the fluid through a tube. One can also obtain a measure of the viscosity by determining the rate at which solid substances pass through the fluid, or one can measure viscosity by determining the force necessary to push solid materials through the fluid. All three methods are used by physicists, but only the second and third can be applied to the living cell. When the protoplasm is not very viscous and contains large granules of relatively high specific gravity, these granules may be seen to move under the influence of gravity. The time of fall can then be used as a measure of the viscosity of the protoplasm. This method has been applied to some plant cells by Heilbronn ('14). In animal cells generally the granules do not appear to be affected by gravity, and a stronger force is necessary to move them. Such a force can be obtained with the centrifuge, and the centrifugal method of measuring viscosity is doubtless capable of wide application. With the centrifuge both the second and third methods of measuring viscosity can be used. One can either determine the speed attained by protoplasmic granules under the influence of a given centrifugal force or one can determine the amount of force necessary to produce a certain degree of movement. To some extent both methods were used in earlier work.

At this point it might be well to consider the theory of viscosity measurements obtained by observing the movements of solids through liquids. This method has been used comparatively rarely by physicists and chemists. Arndt ('07) used it to determine the viscosity of fused salts. A general discussion of the subject with references to literature is given by Ladenburg ('07). When fairly small particles are made to move through viscous liquids, this movement is found to occur at a uniform rate. For such a movement in the case of spherical particles, Stokes ('50) derived a formula often used by physicists. In a suitable form it is:

$$V = \frac{2g(\sigma - \rho)a^2}{9\mu}$$

in which V is the velocity of movement, g the gravity constant, σ the specific gravity of the particle, ρ the specific gravity of the liquid, a the radius of the particle, and μ the viscosity of the liquid.

In the mathematical derivation of Stokes' formula various assumptions are made. Arnold ('11) has considered the significance of all these assumptions. He has shown that for small particles dropping through viscous liquids, Stokes' law holds. When the particles drop through comparatively narrow tubes of the liquid, then a correction must be made for the effect of the walls of the tube. For comparative tests of viscosity in which the same tube is used throughout, this correction disappears.

Stokes' law applies to the action of gravity. In order to make it apply to centrifugal force, one must insert a factor c for the centrifugal force in terms of gravity. The formula then becomes:

$$V = \frac{2cg(\sigma - \rho)a^2}{9\mu}$$

Centrifugal force may be many times gravity. It might be thought that with such a powerful force, the velocity of the particles might become so great that Stokes' law would no longer hold. In the present experiments no such high velocity was ever attained. The speed of the granules ranged approximately from 0.0002 to 0.0018 cm. per second. This is a very low rate of speed.

We are reasonably safe, therefore, in assuming that Stokes' law can be applied to the movement of cytoplasmic granules under the influence of centrifugal force. With a given centrifugal force, the speed with which the granules move is accordingly inversely proportional to the viscosity of the fluid through which they move, the cytoplasm. But this speed also depends on two other factors, the radius of the particle, and the difference in specific gravity between the particle and the fluid of the cytoplasm. If the simple formula is to be used to measure viscosity

it is important that these factors remain constant. During mitosis in the *Cumingia* egg the granules do not change appreciably in size. This constancy is shown in the drawings of Morgan ('10).² Perhaps the specific gravity of the granules changes. In the egg there are two sets of granules, one set lighter, the other heavier than the cytoplasm. When the eggs are centrifuged, the lighter granules go to one pole, the heavier granules to the opposite pole. It has been found that whenever the lighter granules move more rapidly, the heavier granules also move more rapidly, and a drop in the speed of the lighter granules is always accompanied by a drop in the speed of the heavier granules. If the change in speed were in any case due to a change in specific gravity of the granules, we would have to assume that an increase in the specific gravity of the lighter granules was in every case accompanied by a corresponding decrease in the specific gravity of the heavier granules; and vice versa. At first sight this appears impossible, but it is not. An entrance of water into the granules would increase the specific gravity of the lighter granules and decrease the specific gravity of the heavier granules, and an exit of water would have the opposite effect. But such a passage of water would of necessity produce very decided changes in the diameter of the granules and these changes, as we have already noted, do not occur.

Since, therefore, the diameter of the granules and their specific gravity can be taken as constant, the velocity of the granules affords a measure of the viscosity of the cytoplasm. In measuring changes in protoplasmic viscosity, one can either determine the variation in the speed of the granules with a certain amount of centrifugal force or one can determine the amount of force necessary to produce a certain velocity of the granules. In actual practice it is easier to use the first method, especially if a hand centrifuge is employed. With such a centrifuge one acquires the knack of turning very regularly at a given speed but it is somewhat more difficult to change from one speed to another.

² It was also shown by careful measurement of granules in living eggs.

In all of the measurements made in 1920 a new model Bausch & Lomb centrifuge with haematocrit attachment was used. With this instrument one turn of the high-speed handle produces 130 turns of the tubes. The distance between the ends of the tubes is about 14.6 cm. The centrifugal force in dynes is given by the formula of Huyghens:

$$C = 4\pi^2 n^2 r m.$$

in which C is the centrifugal force, n is the number of turns per second, r is the radius of the circle described by the ends of the tubes, and m the mass. The force of gravity is 980.36 dynes (at Boston) times m . In order to express the centrifugal force in terms of gravity, we must therefore divide by 980.36 m . Thus:

$$C' = \frac{4\pi^2 n^2 r m}{gm} = \frac{4\pi^2 n^2 r}{g}$$

in which C' is the centrifugal force in terms of gravity, and g is the gravity constant. With the hand centrifuge it was found most convenient to maintain a speed of one turn of the high-speed handle per second. This represents a force 4968 times gravity.

When the *Cumingia* egg is centrifuged, the oil globules pass to one pole, the yolk and pigment granules to the opposite pole. The egg then appears banded and three zones show, a dark zone contains the oil globules, a pigmented zone contains the yolk granules and the pink pigment, and between the two is a clear and transparent zone. In referring to these zones they will be spoken of as the dark zone, the pigment zone, and the hyaline zone. The appearance of these zones is beautifully shown in the illustrations of Morgan.

THE VISCOSITY CHANGES IN THE CUMINGIA EGG

The protoplasm of the unfertilized *Cumingia* egg has a relatively low viscosity. A number of tests were made during the season. At a speed of one turn per second of the high-speed

handle of the centrifuge, usually two or three seconds elapsed before the first indication of zones appeared. In one case four seconds were required, but this was exceptional. In another case a single turn was sufficient to show zones, but these eggs of unusually low viscosity were not absolutely normal and at segmentation they showed poorly indented cleavage furrows. Apparently the viscosity varies with the temperature, decreasing slightly as the temperature rises from 19° to 25°. More data are necessary, however, in order to establish this point.

The viscosity changes during mitosis are shown in the following tables. Eggs were centrifuged at rapid intervals between fertilization and cleavage. One series of tests was made in which the centrifuge handle was turned three times in three seconds, another series in which it was turned four times in four seconds, and so on. In all of the tests recorded here, a uniform speed of one turn per second was maintained. This was continued for from two to twenty seconds in the various tests. Because of the difficulty in starting, it is not easy to turn the handle twice in exactly two seconds. After a turn or two has been made, however, it is a simple matter to keep the speed uniform.

In the tables the first column shows the number of seconds elapsed after fertilization at the time a given test was made. In working with various sets of eggs on different days, the length of time between fertilization and cleavage varies considerably. In order to compare one set of eggs with another, it is necessary to reduce all the time records to some standard basis. The average time between fertilization and cleavage is about fifty minutes. Fifty minutes was therefore taken as a standard time interval, and by a simple calculation it was determined in each case how much time would have elapsed if the total time until cleavage had been fifty minutes. These figures are given in the second column and are referred to as 'standard time.' There is always some variation in the time of cleavage of individual eggs of any one batch. The time of first cleavage was taken as the time when 50 per cent of the eggs had completely divided. In order to obtain this time, rapid counts had to be made at frequent intervals. These counts are recorded in the third

column in the form of fractions. The numerator represents the number of eggs completely segmented, the denominator the total number of eggs counted.

In the third column also are recorded the observations made during the progress of the tests. It must be pointed out that these observations were necessarily very hasty, and are therefore not as accurate as they might have been if the observer had had time to proceed leisurely. Usually the tests were made at very frequent intervals, and all observations had to be completed in the shortest possible time.

TABLE 1
Three-second tests. Temperature, 24°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
5	4.9	Dark zone distinct in some eggs
7½	7.3	3 zones appear in some eggs; others lack zones, these have polar body.
10	9.7	No zones visible. All eggs have polar body.
12½	12.1	No zones.
15	14.5	No zones.
17½	17.0	A few eggs show zones (perhaps 10 to 20 per cent). Most eggs lack zones.
20	19.4	Dark zone present, often it is not very sharply defined.
22½	21.8	No zones.
25	24.3	No zones.
27½	26.7	No zones.
30	29.1	No zones. The two pronuclei appear large, but they are not yet apposed.
32½	31.6	No zones.
35	34.0	No zones. The pronuclei are apposed.
37½	36.4	No zones. The pronuclei have fused.
40	38.8	Most eggs lack zones. A few show the dark zone, but it is not very sharply marked off.
43	41.7	Eggs generally lack zones. 1 or 2 perhaps show dark zone.
46	44.7	No zones.
48½	47.1	No zones. Many eggs in mitotic elongation stage. Some are beginning to segment.
50-51		12/100 segmented.
51½		23/50 segmented. This taken as the time of segmentation.
52½		37/50 segmented.

TABLE 2

Four-second tests. Eggs fertilized at 4.30 p.m. Temperature at 4.29 p.m., 25.6°; at 5.15 p.m., 25.8°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
2	2.4	Three zones show plainly.
4	4.8	In some eggs zones appear faintly. Most eggs lack zones. A few polar bodies observed (5 min. after fertilization).
6	7.2	No zones. Polar bodies generally present.
8	9.6	Most eggs lack zones. A few eggs have zones indicated.
10	12.0	Three zones show plainly.
12	14.5	Zones indicated in many eggs, never plain.
14	16.9	Zones indicated in many eggs, never plain.
16	19.3	No zones generally (a few eggs have zones faintly indicated).
18	21.7	No zones generally. The second polar body is forming (or has formed).
20	24.1	No zones.
22	26.5	No zones.
24	28.9	No zones.
26	31.3	No zones. Pronuclei large, not apposed.
28	33.7	Zones show faintly in some eggs. Pronuclei large, apposed.
30	36.1	Zones appear; usually they are not very sharply marked off. Pronuclei large, apposed.
32	38.6	Zones show fairly plainly. Pronuclei usually broken down.
34	41.0	Zones show fairly plainly.
36	43.4	Zones usually show fairly plainly. In some eggs they appear indistinct.
38	45.8	Some eggs show zones. Most eggs lack zones. A few eggs are in the mitotic elongation stage.
40	48.2	No zones generally. Eggs have elongated and some have begun to segment.
41½		8/25 segmented.
41¾		15/25 segmented. 41½ minutes taken as the time of segmentation.
42½		21/25 segmented.

TABLE 3

Five-second tests. Eggs fertilized at 12.15 p.m. Temperature at 12.18 p.m., 22.0°; at 1.10 p.m., 23.6°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
2½	2.2	Three zones show plainly.
5½	4.8	Most eggs lack zones. Some eggs show them.
8	7.0	Eggs generally lack zones.
11½	10.1	No zones.
14	12.3	No zones.
16½	14.5	No zones.
18½	16.2	No zones.
21	18.4	Zones appear in many eggs, others lack them.
23½	20.6	Zones appear generally.
26	22.8	Zones in some eggs, others lack them.
28½	25.0	No zones.
30½	26.8	No zones.
33	29.0	No zones.
36	31.6	No zones.
39½	34.6	No zones. Pronuclei large.
42½	37.3	No zones show clearly. Some show zones indistinctly.
45	39.5	Eggs generally show zones.
47½	41.9	Eggs generally show zones.
50½	44.3	No zones.
53	46.5	No zones. Eggs generally in mitotic elongation stage.
56		13/40 segmented.
58		21/31 segmented. 57 minutes taken as the time of segmentation.
59		83/100 segmented.

TABLE 4

Six second tests. Eggs fertilized at 11.00 a.m. Temperature at 10.59 a.m., 23.4°; at 11.52 a.m., 26.4°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
2½	2.6	Three zones show plainly.
5	5.3	Zones when present are indistinct and not sharply marked off.
7½	7.9	No zones. Eggs have polar bodies.
10	10.5	No zones.
12	12.6	Zones indicated. The dark zone is often plain but the pigment zone does not show.
14	14.7	Zones show generally. The pigment zone is not always distinct.
16	16.8	Three zones show plainly.
18	18.9	Three zones show plainly.
20	21.1	Dark zone sharply defined. Pigment zone usually not sharply defined.
22	23.2	No zones.
24	25.3	No zones.
26	27.4	No zones generally (a few exceptions).
28	29.5	No zones generally (a few exceptions).
30	31.6	No zones.
32½	34.2	No zones.
35	36.8	Zones appear. They are not very sharply defined.
37	38.9	Three zones show plainly.
39	41.1	Three zones show plainly.
41	43.2	Three zones show plainly.
43	45.3	Many eggs show zones; in these the zones are not as sharply defined as in last test. Some eggs lack zones.
45	47.4	Some eggs with zones. Most eggs in mitotic elongation stage or with cleavage furrow beginning. These lack zones.
47¼		7/25 segmented.
47¾		13/25 segmented. 47½ minutes taken as the time of segmentation.
49		19/25 segmented.
50		23/25 segmented.

TABLE 5

Seven-second tests. Eggs fertilized at 5.20 p.m. Temperature at 5.19 p.m., 22.3°; at 6.17 p.m., 24.°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
2	1.9	Three zones show plainly.
4	3.7	Three zones show, but not as plainly as in previous test.
6	5.6	Most eggs show three zones. In some eggs these zones are indistinct.
8	7.4	Generally no zones. Polar body beginning to appear in one egg.
10	9.3	No zones, polar bodies general.
12	11.1	No zones.
14	13.0	No zones (perhaps they are indicated in a few eggs).
16	14.8	Many eggs show zones; these are not very distinct.
18	16.7	Three zones show plainly in most eggs.
20	18.5	Three zones show plainly.
22	20.4	Three zones show plainly.
24	22.2	Some eggs show zones, others lack zones or show them indistinctly.
26	24.1	No zones generally. Many eggs are elongated, indicating mitotic elongation for second maturation division.
28	25.9	No zones generally (a few exceptions).
30	27.8	No zones generally (a few exceptions).
32	29.6	No zones generally.
34	31.5	No zones generally. Pronuclei fairly large, not apposed.
36	33.3	No zones generally. Pronuclei large, apposed or nearly apposed.
38	35.2	Zones appear indistinctly in most eggs; in a few eggs they are distinct. Pronuclei large, apposed.
40	37.0	The three zones show quite distinctly. Pronuclei are still present.
42	38.9	Three zones show plainly. Pronuclei have broken down in most eggs.
44	40.7	Three zones show plainly.
46	42.6	Three zones show plainly, they appear sharper and clearer than at any previous test.
48	44.4	In most eggs, three zones distinct; in some, no zones.
50	46.3	A few eggs show three zones, the majority lack zones or show them indistinctly. Mitotic elongation is beginning in some eggs.
52	48.1	No zones. Eggs generally show mitotic elongation.
53½		3/25 segmented.
54		10/25 segmented. This taken as time of segmentation.
54¼		19/25 segmented.
55		23/25 segmented.

TABLE 6
Eight-second tests. Temperature, 24.2°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
2	1.8	Zones show.
5	4.6	Dark zone shows, but pigment zone does not.
7½	6.9	No zones. Polar bodies just beginning to be lifted off.
10	9.2	No zones.
12	11.0	No zones.
14	12.8	Most eggs lack zones, a very few show dark zone.
16	14.7	Three zones show plainly.
18	16.5	Three zones show plainly.
20	18.3	Dark zone plain, pigment zone not so distinct.
22½	20.6	Most eggs with zones not very distinct. A few eggs lack zones completely.
26	23.9	No zones.
28	25.7	No zones.
30	27.5	No zones.
32½	29.8	No zones.
35	32.1	No zones. Pronuclei large.
37½	34.4	No zones. Pronuclei large and close to each other.
40	36.7	In many eggs zones show quite distinctly. These apparently have pronuclei broken down. Some eggs lack zones.
42¾	39.2	Three zones show plainly.
44¾	41.1	Three zones show plainly.
47½	43.6	Three zones show plainly.
50½	46.3	No zones.
53½		3/50 segmented.
53¾		7/50 segmented.
54½		9/50 segmented.
54¾		14/25 segmented. 54½ minutes taken as the time of segmentation.
55½		21/25 segmented.

TABLE 7

Ten-second tests. Eggs fertilized at 6.45 p. m. Temperature at 6.57 p.m., 27.4°; at 7.28 p.m., 27.4°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
2	2.5	Three zones show plainly.
4	4.9	In most eggs three zones show plainly, in some they are not so plain.
7	8.6	Most eggs lack zones, these have first polar body. A few with zones lack polar body.
10	12.3	Three zones show plainly in most eggs.
13	16.0	Three zones show plainly.
15	18.5	Three zones show plainly in some eggs. Other eggs lack zones.
17½	21.6	Zones are indicated, but are not sharply defined.
20½	25.3	No zones.
23	28.4	No zones.
25½	31.4	No zones.
28	34.5	Most eggs lack zones, a few show them. Pronuclei large.
30½	37.7	Three zones show plainly.
32½	40.1	Three zones show plainly.
34½	42.6	Three zones show plainly.
37	45.7	Three zones show plainly in many eggs. Some eggs are in mitotic elongation stage and these lack zones.
39	48.1	No zones. Segmentation beginning in some.
40½		10/25 segmented. This taken as the time of segmentation.
41		17/25 segmented.
41½		22/25 segmented.
42½		23/25 segmented.

TABLE 8

Twelve-second tests. Eggs fertilized at 3.55 p.m. Temperature at 3.51 p.m., 25.6°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
2	2.2	Three zones show plainly.
5	5.6	Some eggs with zones distinct, others with dark zone fairly sharp, but with pigment zone not well marked off.
8½	9.6	No zones. Polar bodies generally present.
11½	12.9	Three zones generally present. Pigment zone not always sharply defined.
14	15.7	Three zones show plainly.
16½	18.5	Three zones show plainly.
19	21.3	Three zones show plainly in some eggs. In others only the dark zone is clearly defined.
24	27.0	No zones (a few exceptions show indications of a dark zone).
26½	29.8	No zones.
29	32.6	Most eggs without zones. Zones are indicated in a few. Pronuclei large.
31½	35.4	Zones appear, but they are not very sharply delimited. The pronuclei are large and apposed.
34	38.2	Three zones show plainly.
36½	41.0	Three zones show plainly.
38½	43.3	Three zones show plainly.
41	46.1	Majority lack zones. Some have zones indicated.
43	48.3	No zones. Cleavage furrows beginning.
44½		10/25 segmented. This taken as the time of segmentation.
45		20/25 segmented.
45½		23/25 segmented.
45¾		25/25 segmented.

TABLE 9
Fourteen-second tests. Eggs fertilized at 10.50 a.m. Temperature at 10.49 a.m., 19.9°; at 12.06 p.m., 22.8°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
2	1.4	Three zones show plainly.
4½	3.2	Three zones show plainly (in some they are not as sharply defined as in previous test).
7	4.9	Three zones show generally, but the pigment zone is not sharply marked off.
10	7.0	Zones are either not present or if present they are indicated rather than sharply defined.
13	9.1	No zones. Polar bodies generally present.
15½	10.9	No zones.
18	12.7	Zones indicated in some eggs.
20½	14.4	Three zones appear plainly in some eggs. In others only the dark zone appears.
23	16.2	Three zones show plainly.
25½	18.0	Three zones show plainly.
28	19.7	Three zones show plainly.
30½	21.5	Three zones show plainly in most eggs, in some they are not sharply defined.
33	23.3	Most eggs lack zones, a few show them. Eggs have one, but not two polar bodies.
35	24.6	No zones. A few eggs were examined carefully and showed second polar body forming.
38	26.8	Zones appear in many eggs. They are not sharply defined.
40½	28.5	Zones do not show plainly. Sometimes the dark zone appears, but the pigment zone does not.
44	31.0	No zones.
46½	32.8	No zones.
49	34.5	Zones indicated, never distinct.
51½	36.3	Zones appear, they are not very distinct. The pronuclei are very large.
54	38.0	Three zones show plainly.
56½	39.8	Three zones show plainly.
59	41.5	Three zones show plainly.
61½	43.3	Three zones generally show plainly (a few exceptions in which zones were not very sharply marked off).
63½	44.7	Three zones generally show plainly (a few exceptions in which zones were not very sharply marked off).
66	46.5	A few eggs show zones plainly. Most eggs are in mitotic elongation stage and lack zones.
68½	48.2	No zones. Eggs generally in mitotic elongation stage.
70		3/25 segmented.
70½		7/25 segmented.
71		15/25 segmented. This taken as the time of segmentation.
71½		19/25 segmented.
72½		22/25 segmented.
73½		23/25 segmented.

TABLE 10
Sixteen-second tests. Eggs fertilized at 11.20 a.m. Temperature at 11.18 a.m., 19.0°; at 12.34 p.m., 23.4°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
2	1.5	Three zones show plainly.
7½	5.5	Three zones show plainly in most eggs. A few exceptions.
10	7.4	Zones show, but are generally indistinct.
12½	9.2	Zones not distinct. Dark zone indicated, no pigment zone.
15½	11.4	Zones show indistinctly.
18	13.2	Three zones show plainly.
20	14.7	Three zones show plainly.
22½	16.5	Three zones show plainly.
25	18.4	Three zones show plainly.
27½	20.2	Three zones show plainly.
30	22.1	Zones appear, but are not as sharply marked off as in previous test.
33	24.3	Elongated eggs (i.e., those giving off second polar body) lack zones or have them barely indicated. Spherical eggs show zones.
36½	26.8	Zones are indicated, but do not show plainly. The dark zone is more distinct than the pigment zone.
39	28.7	Zones not distinct, but often they can be made out. The dark zone is more plainly indicated than the pigment zone.
41½	30.5	Zones indicated, especially the dark zone.
44½	32.7	No zones generally, a few exceptions.
47½	34.9	Zones indicated generally. Pronuclei large.
50	36.8	Three zones appear distinctly, although they are often not very sharply marked off. Pronuclei large.
52½	38.6	Three zones show plainly. Pronuclei large, their boundaries fading.
55	40.5	Three zones show plainly. Pronuclei not large or prominent; they have probably broken down.
57½	42.3	Three zones show plainly.
60	44.1	Three zones show plainly.
62½	46.0	Some eggs show zones. Many eggs in mitotic elongation stage and lack zones.
65	47.8	Most eggs in mitotic elongation stage or beginning segmentation. They lack zones.
67		5/25 segmented.
67½		8/25 segmented.
67¾		13/25 segmented. 68 min. taken as time of segmentation.
68½		18/25 segmented.
69		20/25
70		22/25
71		23/25

TABLE 11

Eighteen-second tests. Eggs fertilized at 12 m. Temperature at 12.07 p.m., 23.0°; at 1.00 p.m., 24.5°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
2	1.7	Zones show very plainly.
5	4.2	Three zones show very plainly.
8	6.7	Three zones show very plainly.
11	9.2	Three zones show plainly, but eggs with polar body have hyaline zone clouded over with granules.
14	11.8	Three zones show plainly, hyaline zone clouded with granules.
17	14.3	Three zones show plainly.
19½	16.4	Three zones show plainly.
22	18.5	Three zones show plainly.
24½	20.6	Three zones show plainly. In some eggs hyaline zone is clouded with granules.
27	22.7	Three zones show plainly. In some (elongated eggs), hyaline zone is somewhat cloudy.
30	25.2	Three zones show plainly. A few with hyaline zone cloudy.
33	27.7	Three zones show. Hyaline zone usually cloudy.
36	30.3	Three zones show. Hyaline zone clouded with granules. Pronuclei fairly large.
39	32.8	Three zones show. Hyaline zone somewhat clouded. Pronuclei large, apposed.
42	35.3	Three zones show plainly, hyaline zone not clouded. Pronuclei large, apposed.
45	37.8	Three zones show plainly. In some eggs pronuclei are present, in others they have disappeared.
48	40.3	Three zones show plainly. In most eggs pronuclei have disappeared.
50½	42.4	Three zones show plainly.
53	44.5	Three zones show plainly in most eggs. A few are in mitotic elongation stage and have zones indicated.
55½	46.6	Elongated eggs and dividing eggs have zones indicated. They are not distinct, but can be made out.
59		7/25 segmented.
59½		10/25 segmented. This taken as the time of segmentation.
60		18/25 segmented.
62		17/25 segmented. Thus only about 70 per cent segmented.

TABLE 12

Eighteen-second tests. Eggs fertilized at 11.40 a.m. Temperature at 11.42 a.m., 24.3°; at 12.30 a.m., 24.0°

ACTUAL TIME AFTER FERTILIZATION	STANDARD TIME	OBSERVATIONS
<i>minutes</i>	<i>minutes</i>	
3	3.1	Three zones show plainly.
6	6.2	Many eggs show three zones plainly. In some eggs hyaline zone is clouded with granules and sometimes the pigment zone is not sharply marked off.
9	9.3	Dark zone and pigment zone show plainly, hyaline zone clouded somewhat.
14	14.4	Three zones show plainly.
22	22.7	Zones appear, but are not always sharply marked off.
25	25.8	Dark zone sharply defined, pigment zone not sharply marked off.
29	29.9	Zones do not show distinctly. They are indicated rather than sharply marked off. Dark zone plainer than pigment zone.
32½	33.5	Zones show more plainly than in previous test. Pro-nuclei apposed, apparently fused.
36	37.1	Three zones show plainly.
39	40.2	Three zones show plainly.
41	42.3	Three zones show plainly.
43	44.3	In most eggs, three zones show plainly. A few eggs are elongated and in these there is no distinct pigment zone; the dark zone appears, but is not well marked off.
45⅝	47.2	Most of the eggs are elongated. These show no zones. Other eggs show zones.
48½		24/50 segmented. This taken as the time of segmentation.
49½		43/50 segmented.

The tables show that the viscosity of the protoplasm undergoes marked changes. With the same centrifugal speed sometimes two seconds are sufficient to produce zones, sometimes sixteen seconds must elapse before zones appear. In the latter case obviously the velocity of the granules is only one-eighth as great. Since the viscosity varies inversely as the velocity (see previous discussion) this indicates an eight-fold increase in viscosity. The number of seconds necessary to cause the appearance of zones is thus an inverse measure of the velocity of granular

movement and a direct measure of the viscosity. As shown previously it requires on the average two turns of the centrifuge handle (in two seconds) to produce zones in unfertilized eggs. An arbitrary scale of viscosity may be established and the viscosity of the protoplasm of unfertilized eggs can be taken as 2. At a stage when sixteen turns are required to show zones the viscosity in terms of the arbitrary unit is 16.

From the data in the tables a viscosity curve may be plotted. This is not, however, a simple matter. In general it is not possible to obtain direct readings of viscosity. Usually each test indicates that the viscosity of the protoplasm is above or below a certain value, but it does not show how much above or below. Moreover, there is often some variation in the eggs of a given lot, especially at times of transition. By noting for each test whether the viscosity is above or below a certain value a curve may be plotted by drawing a line above the one set of points and below the other set.

The following symbols were used in marking out points:

- ∇ = Zones show plainly in all eggs. Viscosity above value at apex of *V*.
- ^ = No zones appear in any eggs. Viscosity below value at apex of inverted *V*.
- ◊ = Zones appear but indistinctly (usually dark zone plainer than pigment zone). Viscosity about equal to value at center of circle.

The above three symbols were combined in a variety of ways.

- × = Eggs with zones and without zones about equal in number.
- × = Most eggs with zones, a few without.
- × = Most eggs without zones, a few with.

In those cases where zones appeared indistinctly, the arc of a circle was added to the upper or lower portion of any of the last three symbols.

In the curve the abscissa represents time in minutes after fertilization. Of course for purposes of comparison the so-called 'standard time' had to be employed. The ordinates represent comparative viscosity values on the basis of the arbitrary scale mentioned above.

Not all of the available data were included in the curve. All of the tests in table 12 were omitted. Introduction of these figures would not have changed the curve. One test is included which is not given in the tables. This is a twenty-second test made when 5 per cent of the eggs had segmented, hence about a minute before 50 per cent had segmented.

Curve and tables show that there are three times when the viscosity is high. These are just before the first polar body is given off, just before the second polar body is given off, and just before the completion of the first cleavage division. Thus in every case immediately before the division of the cell, there is an increase in viscosity. There is also a high viscosity in the early stages of each mitosis. Generally the completion of one division is bound up with the preparation for the next. As the first polar body is given off, the second maturation spindle begins to form,³ and the viscosity increase at this time is probably causally related to the appearance of the spindle. As the second polar body is given off there is again a viscosity increase. After the completion of the second maturation division the viscosity decreases slightly. It soon rises again in preparation for the cleavage spindle. As the cleavage spindle forms the viscosity drops and remains low until immediately before cleavage, when there is a sharp increase. In general the viscosity increase at the conclusion of each mitosis is directly related to the appearance of the spindle in the mitosis which follows. The concluding phases of one division are bound up with the initial phases of the next. The same sort of phenomenon was previously found to occur in successive cleavages in the sea-urchin egg.

COMPARISONS WITH OTHER EGGS

One point emphasized by the curve is the sudden increase in viscosity just before division. This increase preceded division by a very short interval. The curve shows the interval to be about two minutes or a trifle more. It must be remembered,

³ The second maturation spindle is a new structure and does not result from a transformation of the first maturation spindle. This is shown by the observations of Griffin ('99), Lillie ('01), and various other observers.

however, that the time of cleavage is taken as the time of completed segmentation. The interval between viscosity increase and the first constriction of the cell is somewhat less. Obviously it is not much more than a minute.

In the sea-urchin egg a similar viscosity increase occurs just prior to division. This was indicated by some of my earlier data (cf., for example, the second table on page 216 of my 1920 paper). In earlier experiments, however, I was primarily interested in the viscosity increase which precedes the appearance of the spindle, and I did not study carefully the viscosity at the conclusion of mitosis. The intervals between tests were too large and, moreover, I took as the time of segmentation the time when the first eggs began to divide. It is much more exact to consider as the time of segmentation the moment when 50 per cent of the eggs have divided.

In *Arbacia* there is a rise in viscosity before division. This is shown conclusively by some tests made in 1919. In these tests an old-model Bausch & Lomb centrifuge was used. It differs from the new model in having a shorter distance between the ends of the tubes, the radius of turn being 6 cm.

June 30, 1919. Temperature 18.0° at 10.58 A.M., 19.0° at 11.42 A.M. *Arbacia* eggs were fertilized at five-minute intervals. In finger-bowl *A* eggs were fertilized at 10.55 A.M., in *B* at 11.00 A.M., and in *C* at 11.05 A.M. At 12.10 $\frac{1}{2}$ P.M. less than 1 per cent of the eggs in *A* had begun segmentation. At 12.10 $\frac{3}{4}$ P.M. eggs from *A* and *B* were centrifuged simultaneously in separate tubes, the high-speed handle being turned 30 times in 25 seconds. On examination of centrifuged eggs, the great majority of eggs from *A* showed no hyaline zone, only about 10 to 20 per cent showing such a zone. The centrifuged eggs from *B* all showed a hyaline zone plainly extending one-third of the distance through the egg.

At 12.17 $\frac{1}{4}$ P.M. about 5 per cent of the eggs in *B* were beginning to segment. Starting at 12.17 $\frac{1}{2}$ P.M., eggs from *B* and *C* were centrifuged simultaneously, the high-speed handle being turned 30 times in 28 seconds. When eggs from *B* were examined, there was no hyaline zone in any of the eggs. Eggs from *C* all showed a hyaline zone, sometimes indistinct, sometimes extending a third of the distance through the egg.

Other tests gave similar results. Evidently just before division in *Arbacia* as in *Cumingia* there is a sudden increase in

viscosity. This increase is of considerable importance and can of course be studied experimentally in the same manner that the viscosity increase in early prophase has been studied. This I propose to do in the near future.

The viscosity changes in *Arbacia* and *Cumingia* are absolutely parallel. In each case the appearance of a spindle is preceded by an increase in viscosity and followed by a decrease in viscosity. And in both eggs division of the cell is immediately preceded by a viscosity increase.

Besides the two eggs already mentioned the egg of *Nereis* was also studied. The *Heteronereis* form of *Nereis limbata* is common at Woods Hole. Its early development is well known through the studies of E. B. Wilson and of F. R. Lillie. The egg is immature at fertilization and three complete mitoses occur between fertilization and cleavage. In *Nereis* viscosity changes are not as sharply marked as in *Cumingia* or *Arbacia* and the egg is therefore more difficult to study. The *Nereis* egg is much larger than the eggs of the other two forms studied and it contains a considerable amount of yolk material. In proportion to the size of the egg, the spindle is small. We might expect that the viscosity changes associated with the appearance of the spindle would be masked in an egg when the spindle constitutes but a small fraction of the material of the egg.

A number of tests were made on the *Nereis* egg, but only one series is included here, these are given in table 13. All tests were made at a uniform centrifugal speed. The new-model Bausch & Lomb centrifuge was used and the handle was turned five times in five seconds.

The table shows that in the immature *Nereis* egg the protoplasm is very stiff. Soon after fertilization and the disappearance of the germinal vesicle the viscosity decreases. Following this there are four times of viscosity increase, at 20, 35, 47, and 65 minutes after fertilization. These viscosity increases presumably correspond with, 1) early prophase of first maturation division; 2) conclusion of first and early prophase of second maturation division; 3) conclusion of second maturation division and early prophase or cleavage mitosis; 4) conclusion of

TABLE 13

Five-second tests. Nereis. Eggs fertilized at 10.55 a.m. Temperature at 11.11 a. m., 23.7°; at 12.20 p.m. 24.9°

TIME AFTER FERTILIZATION	OBSERVATIONS
2	No hyaline zone. Germinal vesicle present.
5	No hyaline zone. Germinal vesicle present.
8	No hyaline zone. Germinal vesicle nearly faded out.
11	Hyaline zone beginning to appear. No germinal vesicle.
14	Hyaline zone appears plainly; it extends through about one-fifth of egg.
17	Hyaline zone not pronounced. In some eggs it is absent; in others, if present, it is indicated rather than shown plainly.
20	Hyaline zone is absent generally (perhaps it is indicated in a few).
23	Hyaline zone plain. About one-fifth.
26	Hyaline zone appears plainly.
29	Hyaline zone can be seen, but in most cases it is indistinct.
32	Hyaline zone can be seen, it is generally narrow and not distinct.
35	Generally no hyaline zone.
38	Hyaline zone present. It is generally narrow, but distinct and clear.
41	Hyaline zone plain, extending through one-fifth of egg.
44	Hyaline zone narrow if present at all.
47	No hyaline zone.
50	No hyaline zone.
53	No hyaline zone.
56	In many eggs hyaline zone is wide, clear, plain. It extends through one-fourth of egg, and its inner margin is almost at the equator.
62	Hyaline zone wide and clear as in previous test.
65	No hyaline zone in any eggs.
67	Segmentation beginning generally.
67½	5/50 segmented completely.
68	8/25 segmented completely.
68½	17/25 segmented completely.
69	21/25 segmented completely.
70	49/50 segmented completely.

cleavage division.⁴ Of the first three periods of increased viscosity the third, as might be expected, is the longest. It corresponds to the second period of increased viscosity in the *Cumingia* egg.

⁴ According to Wilson ('92), the first polar body is formed thirty-five to forty minutes after fertilization, the second polar body ten to twelve minutes later, and the egg divides after about seventy-five minutes. Evidently Wilson worked at about the temperature of the above experiment. At lower temperatures the times are of course longer.

DISCUSSION

In the last few years the importance of protoplasmic viscosity changes has been increasingly realized. Of all the properties of protoplasm the fact that it is a viscous fluid is perhaps its most general characteristic. That a viscous colloidal fluid like protoplasm would probably undergo marked viscosity changes was obvious to biologists long ago. They tried to associate many activities of protoplasm with changes in its consistency. In spite of the numerous theories that were proposed, actual attempts at measuring viscosity have until recently been almost totally lacking. Without doubt, if marked changes in protoplasmic viscosity occur, they must play an important part in the mechanics of many vital processes. There is now actual evidence to show that great viscosity changes do occur in protoplasm.

In addition to the results obtained on marine ova, Seifriz ('18, '20) and Leblond ('19) have shown viscosity changes in plant protoplasm and Bayliss ('20) has shown similar changes in *Amoeba*. Various methods have been used. The gravity method was employed by Heilbronn ('14) and later by F. and G. Weber ('17). Leblond and Bayliss used the presence of Brownian movement as a criterion of viscosity.

All of the methods are not equally reliable. To my mind the microdissection method particularly is open to question. Kite ('13) was the originator of the method. According to Kite, all egg protoplasm was a highly viscous gel which scarcely varied its consistency. After my demonstration of the fluidity of unfertilized sea-urchin egg protoplasm and the change to a gel after fertilization ('15), Chambers was able to confirm these facts with microdissection. At first I was inclined to consider Chambers' work as a valuable support to my views of protoplasmic viscosity change. In my 1920 paper this point of view is brought out. But the recent publications of the microdissectionists have made me doubt the value of the method. Compare, for example, recent statements of Chambers and Seifriz. Chambers ('19) says: "The time of appearance of the amphiaster until completion of cleavage lasts from 10 to

15 minutes. The *increased*⁵ viscosity of the egg during this amphiaster stage could be more easily demonstrated by the needle in the eggs of *Echinarachnius* and *Cerebratulus* than in those of *Arbacia*.

"After completion of the cleavage process, there are indications that the firmness of the cytoplasm persists in the two blastomeres while they are still more or less spherical."

These remarks of Chambers apparently apply to the sea-urchin egg. Working on the same egg apparently without any knowledge of Chambers' 1919 paper, to which he does not refer, Seifriz ('20) finds: "With the first appearance of the amphiasters there is a pronounced *decrease*⁵ in viscosity of the central region of the cell, and this condition is maintained throughout the intermediate stages of divisions (from middle prophase to late anaphase)." Later he says, "with the completion of division we have in each daughter cell of the embryo a general protoplasmic consistency identical with that of the egg before fertilization."

Thus Chambers considers the amphiaster stage to involve increased viscosity and Seifriz regards it as involving a pronounced decrease in viscosity (at least of the central region). And whereas, after the first cleavage, Chambers finds high viscosity, Seifriz finds a viscosity identical with that of the unfertilized egg. It might be thought that the difference is due to a difference in the capability of the two investigators, but this does not appear to be the case. For whereas Seifriz is wholly wrong in stating that the viscosity just after cleavage is no greater than in the unfertilized egg, Chambers is equally wrong in stating that the amphiaster stage is a stage of high viscosity. Evidently the difficulty lies primarily with the method. No doubt it is too subjective. Apparently all evidence as to protoplasmic viscosity gained from microdissection should for the present at least be regarded as merely suggestive.

In this paper it has been shown that during mitosis viscosity changes occur in *Cumingia* and *Nereis* which are similar to those

⁵ Italics mine.

occurring in the sea-urchin egg. The latter perhaps presents a somewhat simpler case, for the first cleavage division does not immediately follow the maturation divisions. The viscosity changes that occur in the sea-urchin egg are probably typical of mitosis in general. There is a marked viscosity increase in early prophase, then a decrease, and finally an increase just before the cell divides. This final viscosity increase in the sea-urchin egg continues as the early prophase viscosity increase of the second cleavage division. In *Cumingia* and *Nereis* the maturation divisions are related to each other and to the first cleavage division as the first cleavage division is related to the second in the sea-urchin egg. Apparently whenever two mitoses follow each other in rapid succession, the viscosity increase at the conclusion of the first provides the essential viscosity increase for the beginning of the second.

In the various eggs studied the viscosity changes are apparently not of the same magnitude. In *Nereis* they are not as great as in the other eggs. This is readily understood in the light of the fact that the *Nereis* egg is considerably larger and its mitotic spindle forms a much smaller fraction of the material of the egg.

The magnitude of the changes has been determined only for *Cumingia*. In this egg the viscosity rises from an arbitrary value of 2 or 3 to a value of 16, and then drops to about its original value. These changes are very rapid and the increase in viscosity just mentioned occurs within a minute or two. Such a sharp and sudden viscosity increase is almost conclusive evidence that a gelation or coagulation has occurred within the egg. This does not mean that the entire protoplasm has gelled. Presumably only a part is affected. It seems likely that were the entire protoplasmic mass of the egg to gel, the viscosity increase would be greater. It is more logical to assume, however, that only that portion of the cytoplasm which is concerned with spindle formation undergoes gelation. This is borne out by the fact that in the *Nereis* egg, in which the spindle forms a smaller fraction of the egg material, the viscosity change is not so great.

The mechanism of viscosity increase and decrease or gelation and solation within the cell is not yet understood. It might be

thought that gelation was due to an abstraction of water and solation to an addition of water. In an earlier paper some evidence for the first view was presented. If solation were due to an addition of water, it would naturally be expected that the breakdown of the nucleus during mitosis would be the primary cause of solation. This is, however, not the case in *Cumingia*. In the curve shown in figure 1, after the second polar body has formed the viscosity remains high for the formation of the cleavage spindle. The viscosity then drops, but this drop occurs not after the breakdown of the pronuclei, but before the pronuclei have shown any visible indication of breakdown. Thus the return of the protoplasm to a more fluid state is apparently not due to a passage of water from nucleus to cytoplasm. As far as can be judged from the published figures of Morgan ('10), Jordan ('10), the time of liquefaction is approximately simultaneous with the appearance of the fully formed cleavage spindle. The above authors show the spindle appearing at a time when the pronuclei have grown to a large size. It is at this time that the drop in viscosity occurs. Further evidence that the liquefaction of the protoplasm is not due to a breakdown of the nucleus is indicated in *Cumingia* by the fact that in this egg the protoplasm becomes more fluid immediately after the second maturation spindle is formed. In this case no nuclear breakdown occurs.

It appears that whenever a spindle forms there is a return to a fluid condition. It is as though the spindle were coagulated out of the cytoplasm. Thus the curve of viscosity at the time of spindle formation is like the viscosity curve of a coagulating albumen solution. As coagulation occurs in such a solution the viscosity suddenly increases greatly, after coagulation has been completed there is a sharp drop in viscosity. The first part of the *Cumingia* viscosity curve resembles the curve of a coagulating albumen solution given in Dunstan and Thole's 'Viscosity of liquids' (fig. 9). Of course, this resemblance may be superficial. At present, however, the idea that the spindle coagulates out probably offers the best interpretation of the viscosity changes which precede and follow spindle formation.

SUMMARY

1. The centrifuge method of measuring protoplasmic viscosity is a perfectly sound method from a physical standpoint. With it quantitative data may be obtained.

2. The velocity of granular movement under the influence of centrifugal force was taken as a measure of the viscosity.

3. The viscosity of *Cumingia* egg protoplasm was plotted for the period between fertilization and first cleavage. The data may be presented in the form of a curve (fig. 1).

4. In both maturation divisions and cleavage, appearance of the spindle is always preceded by a sharp viscosity increase and followed by a sharp viscosity decrease.

5. At the conclusion of mitosis, within a minute or two before the division of the cell is completed, a sharp increase in viscosity occurs.

6. When two mitoses follow each other, the concluding viscosity increase of the first mitosis becomes the initial viscosity increase of the second.

7. In *Nereis*, the viscosity changes between fertilization and cleavage are similar to those found in *Cumingia*. Apparently these changes are not as marked as in *Cumingia*, a fact which is correlated with the larger size of the *Nereis* egg and the relatively small size of its spindle.

8. The discussion includes a critique of the microdissection method of viscosity measurement as well as certain interpretative considerations.

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