CELL SIZE AND NUCLEAR SIZE

EDWIN G. CONKLIN

From the Department of Biology, Princeton University

THIRTY-SEVEN FIGURES

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In the development of all organisms considerable difference of size appear, sooner or later, among constituent cells; sometime the blastomeres of the cleaving egg differ in size, in other case these differences appear only later during the blastula, gastrul or larval stages. Endoderm cells are usually larger than thos of the ectoderm, ciliated cells are generally larger than non-ciliate ones, muscle and nerve cells are usually larger than epitheli or mesenchyme cells.

These differences in the size of cells may be due to unequal ce division, to unequal rate of division, or to unequal growth c cells after division, and in some cases all of these factors may b represented in the same egg or embryo. It is frequently assume that unequal cell divisions are caused by the accumulation c metabolic substances, such as yolk at one side of a cell, and th crowding of the protoplasm and nucleus to the opposite sid Such unequal divisions are frequently found in yolk-laden egg and may be artificially produced at will by centrifuging the yol to one pole or the other of a dividing cell. But in many cases th is not the cause of unequal cell division; the yolk may be uniforml distributed with regard to the poles of the spindle and yet th cleavage may be unequal, or unequal division may take place i purely protoplasmic cells, in which the eccentric position of th spindle is not due to pressure. Innumerable cases of this so have been found both in normal and in experimentally altere conditions. Often such unequal divisions are associated wit visible histological differences in the resulting cells. The stud of cell-lineage has shown that in some cases a particular cell distinguished from the time of its formation by its size, prot plasmic structure, rate of division, prospective significance an

potency. In such cases differences in the sizes of cell are associated with some of the earliest differentiations of the developing egg.

But differences in the size of cells may be due, not to unequal cell division, but to unequal rates of division, or to unequal growth of cells subsequent to division. In some instances cells divide rarely and consequently become large, while adjoining cells divide frequently and therefore remain small. The fact that cells are not always of the same size at the time of division is one of capital importance for it shows conclusively that the factors which bring about cell division may be separated from those which cause growth.

In connection with the size of cells as a whole may be considered the sizes of many of their constituent parts, such as nuclei, chromosomes, plasmasomes, centrosomes, etc. The size relations which exist between these parts of the cell and the plasma should throw light upon the interrelation between these cell constituents in other respects than size. The quantitative relations of different cell constituents at various phases of activity should be of significance in the study of many fundamental problems of growth, differentiation and cellular physiology.

Within the past few years several contributions on this subject have appeared, principally from Boveri and R. Hertwig, and their students. In so far as these works have dealt with the development of the egg they have been based on a study of eggs of 'indeterminate cleavage' in which it is not possible to trace individual blastomeres throughout the cleavage period; the results have therefore been mass results, based on averages of cells of a given stage. In the study of vital phenomena it is frequently important to deal with individual rather than with average results; in the following pages I have attempted to apply the method of the quantitative study of cells and of cell constituents to individual blastomeres at various stages of the cleavage.

PART I

CELL SIZE AND NUCLEAR SIZE IN NORMAL DEVELOPMENT

I. Unequal cell divisions

1. The maturation divisions. The most unequal of all cel divisions are those which give rise to the polar bodies. The actual diameter of the first polar body and of the egg, and the relative volumes of the two, are here given for a number of different animals. These measurements were made on eggs which had been fixed, stained, and mounted in balsam.

TABLE 1

Sizes of polar bodies and eggs

SPECIES	DIAMETER FIRST POLAR BODY	DIAMETER OF EGG	RELATIVE VOLUMES
	μ	μ	
Cumingia tellinoides	6	45	1 : 421.8
Amphioxus lanceolatus	6	108	1 : 5832.0
Cynthia partita	9	105	1 : 1560.8
Cerpidula plana	12	136	1 : 1442.8
Crepidula fornicata	12	182	1 : 3443.0
Crepidula convexa	15	280	1 : 6434.8
Crepidula adunca	15	410	1 : 20123.6
Fulgur carica	15	1600	1 : 1211355.0

In many other cases, such as the eggs of selachians, amphibians and birds, the disproportion between the polar body and the egg is much greater than in the cases here measured. The significant thing here is not merely the degree of inequality, but also the relative uniformity in size of the polar bodies as compared with the egg. Although the eggs of different animals vary enormously in size, the polar bodies vary relatively little, and it is safe to conclude, both from observation and experiment, that the polar bodies are in general the smallest cells which can be formed from egg cells by the process of normal cell division.

In spite of this very great inequality of the daughter cells, the mitotic figure in the first maturation division of Crepidula and of many other animals is the largest in the whole life cycle. Whe

first formed this spindle lies near the middle of the egg, and if the division wall were to form while the spindle lies in this position a polar body would be formed whose diameter would be to that of the egg as 1 : 1, 1 : 2, or at the least 1 : 3. Later the spindle moves toward the periphery until one pole comes into contact with the cell membrane. The membrane then protrudes over this pole and into this protrusion the end of the spindle moves; at the same time the spindle itself constantly grows shorter, until finally the spindle is but little more than double the diameter of the polar body, and in the separation of the polar body the division wall passes through the equator of the spindle. In Crepidula plana the first maturation spindle shortens to about half its original length; during the metaphase its maximum length is about 42μ, at the time when the polar body is being separated it is only 24μ long.

By means of centrifugal force it is possible to prevent the spindle from moving from its first position and also from shortening, and under these circumstances giant polar bodies are formed, sometimes quite as large as the remainder of the egg. In all such cases the division wall passes through the equator of the spindle. Evidently the factors which bring about this most unequal of all cell divisions are (1) the eccentricity and (2) the shortening of the maturation spindle.

The second polar body is but slightly smaller than the first, nevertheless the spindle is much smaller, its maximum length in Crepidula plana being 18μ; correspondingly it shortens much less in the anaphase than the first polar spindle, being almost as long when the division wall begins to form as in the metaphase. Though the second polar spindle may appear at some distance from the point at which the first polar body was formed, and although its axis may lie at right angles to that of the first polar spindle, it invariably rotates into the axis of the latter and the whole spindle moves toward the surface until its outer pole comes to lie immediately under the first polar body, and here the second polar body is pushed out. In this case the principal factor which causes the inequality of division is the eccentricity of the spindle. If the spindle is prevented by pressure or cen-

trifugal force from taking this eccentric position the resulting cell division may be nearly equal, or a giant second polar body may be formed (fig. 11).

2. Cleavage. The first cleavage of Crepidula and of Fulgur is approximately equal. The pronuclei lie near the animal pole of the egg, the egg nucleus lying somewhat nearer the polar bodies than the sperm nucleus. The first cleavage spindle is oriented so as to lie at right angles to the egg axis, but it is impossible in these eggs to determine whether the spindle lies in a particular cross axis or not. However in typical cases the spindle invariably lies at right angles to the chief axis with its equator in that axis, and the protoplasm and yolk are divided by the first cleavage plane with strict equality. The same is true of the second cleavage which in all these regards resembles the first.

It has been generally assumed that equal cleavages, alternately at right angles, are due to simple mechanical conditions, such as the greatest diameter of the protoplasmic mass, and that they require no further explanation. As a matter of fact equal cleavages, and successively alternating ones, cannot be explained in so simple a manner. The fact that the first cleavage spindle invariably stands at right angles to the chief axis of the egg and with its equator in that axis shows that there is here some orienting power of the highest significance. It is well known that there is considerable variation in the path which the spermatozoon takes through the egg, and in its manner of meeting with the egg nucleus; there is also much variation in the actual positions of the cleavage centrosomes and in the initial position of the first cleavage spindle, without any corresponding variation in the final position of the spindle or of the cleavage plane. As a result of the study of large numbers of eggs of many different animals, under both normal and experimental conditions, its seems to me necessary to conclude that the same factor which brings about an unequal division of an egg such as that of Unio, operates to cause the equal division of an egg like that of Crepidula; this factor is to be found in the polarity and symmetry of the egg itself. In Unio, where the first cleavage is very unequal,

Lillie ('01) has shown that the spindle oscillates in the cell before coming to rest in its eccentric position; and in many cleavages in Crepidula the spindle may at first lie out of its normal position and may move later into its proper place; and this applies not only to the eccentricity but also to the axial position of the spindle. Something outside of the spindle itself determines the position which it shall take in the cell, and this is as true of equal and alternating cleavages as of unequal and non-alternating ones.

TABLE 2

Sizes of macromeres and micromeres in Crepidula and Fulgur

SPECIES	MACROMERES	DIAMETER	MICROMERES	DIAMETER	RELATIVE VOLUMES
		μ		μ	
C. plana.....	1A–1D	81	1a–1d	30	ca. 19.9 : 1
	2A–2D	80	2a–2d	36	ca. 10.6 : 1
	3A–3D	76	3a–3d	33	ca. 12.1 : 1
	4D	66	4d	38	ca. 4.9 : 1
	4A–4C	60	4a–4c	42	ca. 2.7 : 1
	5A, 5B	75	5a, 5b	60	ca. 1.9 : 1
	5C, 5D	68	5c, 5d	68	ca. 1 : 1
C. convexa....	1A–1D	195	1a–1d	69	ca. 22.0 : 1
	2A–2D	195	2a–2d	50	ca. 59.3 : 1
	3A–3D	195	3a–3d	50	ca. 59.3 : 1
Fulgur carica..	1A–1D	800	1a–1d	80	ca. 1000 : 1
	2A–2D	800	2a–2d	80	ca. 1000 : 1
	3A–3D	800	3a–3d	80	ca. 1000 : 1
	4D	780	4d	130	ca. 216 : 1
	4A–4C	740	4a–4c	370	ca. .8 : 1

The third, fourth, and fifth cleavages of the macromeres of Crepidula and of other gasteropods are successively alternating in direction, and are notably unequal in the size of the daughter cells; while the sixth and all subsequent divisions of the macromeres are more nearly equal than the preceding ones. The diameters of the cells formed by these cleavages and their approximate ratios, in Crepidula and Fulgur, are shown in Table 2.

In the structure of the macromeres there is no visible organization which would explain why the first two cleavages of the egg are equal, the three following ones very unequal and subse-

quent cleavages more nearly equal again, and yet it is certain that some such organization must be present. It is generally believed that the inequality of macromeres and micromeres is due to the quantity of yolk contained in the former and where the quantity of yolk is extremely great, as in Fulgur, this is undoubtedly one of the causes of the great difference in the sizes of the macromeres and micromeres; but that it is not the only cause of the inequality is shown by experiments in which by centrifuging eggs at the first or second cleavage two of the macromeres come to contain no yolk, while the other two contain all of the yolk; in the macromeres which are purely protoplasmic and contain no yolk the subsequent cell divisions are still unequal, protoplasmic micromeres of the usual size being separated from the protoplasmic macromeres, (see p. 81). The study of normal as well as of artificially altered cleavage points unmistakably to the conclusion that the position and axis of each spindle is fixed by the structure of the cell protoplasm, and since the position and axis of the spindles change regularly in successive divisions this protoplasmic structure must change regularly in successive cell generations. Boveri ('05) says that the position of the spindle is not due to a permanent cell structure, but that the constitution of the egg undergoes progressive alterations, which then react on the division centers.

Among the micromeres certain cell divisions are quite unequal, and here there can be no question that this inequality of division is in no way associated with the presence of yolk, since the micromeres are purely protoplasmic. In Crepidula the first and second subdivisions of the first quartet cells (figs. 3, 8), which give rise respectively to the 'turret' cells and the 'apical rosette' cells, are very unequal; as is also the division of the second quartet cells which give rise to the 'tip' cells of the arms of the ectodermal cross. The diameters of the two daughter cells in each of these divisions, and the approximate ratio of one to the other, are as follows in Crepidula plana:

$1a^{1}$–$1d,^{1}$ 25μ, $1a^{2}$–$1d^{2}$, 13μ ..... Ratio 2 : 1
$1a^{1.1}$–$1d^{1.1}$, 30μ, $1a^{1.2}$–$1d^{1.2}$, 18μ ..... Ratio 5 : 3
$2a^{1.1}$–$2d^{1.1}$, 30μ, $2a^{1.2}$–$2d^{1.2}$, 15μ ..... Ratio 2 : 1

In the case of normal eggs it cannot be demonstrated that the inequality may not be due to mutual pressure among the cells, but in certain experiments which will be described in another paper, this factor may be entirely eliminated, isolated blastomeres showing the same inequalities of division as do those in the cell complex. In all of these cases of definite types of cleavage, the position of the spindle, and consequently the direction of division and the relative size of the daughter cells, is determined by some structural peculiarity of the protoplasm and not by the presence of metabolic substances within the cell or by pressure from without.

3. Significance of the yolk lobe. Under normal conditions the line of intersection of the first and second cleavage planes marks the chief axis of the egg, its two ends being the animal and vegetal poles. In eggs in which the cleavages are unequal, the chief axis, thus defined, runs from the animal pole, which is marked by the position of the polar bodies, to a point more or less removed from the diametrically opposite pole.

Is this chief axis predetermined in the egg or is it established by the positions of the first and second cleavage planes? Observation alone affords no positive answer to this question, but the fact that the spindle takes a definite and characteristic position in the egg indicates that something outside the spindle determines its position, and points to the conclusion that the chief axis is already present in the egg, as a structural differentiation before cleavage begins. This conclusion is well supported by experiment, as will be shown later.

In this connection the significance of the so-called 'yolk lobe' is interesting. As is well known this lobe is found in many eggs, especially in those in which the first and second cleavages are unequal. It is present however in minute form in such eggs as those of Crepidula and Fulgur in which the first two cleavages are approximately equal, but in cases in which these cleavages are unequal it is much larger, and in general the size of the yolk lobe is proportional to the inequality of division. In all cases so far as I am aware the yolk lobe lies diametrically opposite the animal pole, and if detached from the egg at the time when it is fully formed, the egg divides into equal blastomeres, as Wilson

('04) found in Dentalium; if it remains attached it fuses, at the close of the cleavage, with one of the cells, which then becomes larger than the other one. In this case the cleavage spindle is not eccentric and the furrow cuts down through the center of the egg until it reaches the yolk lobe when it turns to one side of the lobe leaving it attached to one of the cells. In this way a cleavage which began as an equal one becomes unequal. Where the spindle is eccentric from the start and the furrow does not pass through the center of the egg the yolk lobe is not prominent. In this way inequality of division may arise through the eccentricity of the spindle, or through the formation of a yolk lobe which remains connected with one of the two daughter cells, which would otherwise be equal.

One cannot study the eggs of different animals without being much impressed with the fact that the distribution of yolk to the four macromeres is highly characteristic of different species and orders. Thus among prosobranchs the yolk is distributed either equally to all the macromeres, as in Crepidula, Fulgur, Trochus, etc., or if one of the macromeres is larger than the other three it is the left posterior macromere, *D*, as in Nassa, Urosalpinx, Tritia, etc. Among opisthobranchs, if the macromeres are unequal in size it is one or both of the anterior ones, *A* or *B*, which is the larger. Among pulmonates, so far as I recall, the macromeres are always equal in size.

The fact that there are these characteristic differences in the sizes of the macromeres of different orders indicates that they have some characteristic cause; and the fact that in nearly related species the macromeres may be equal or unequal indicates that in this case the cause is not a very general one. If one considers that the first and second cleavages normally pass through the egg axis, and that their position is determined by this structural feature, the unequal distribution of yolk to the four macromeres may be due to the localization of the yolk in different parts of the ovarian egg,—on the posterior side of the chief axis in prosobranchs, on the anterior side in opisthobranchs; while a larger or smaller yolk lobe would determine the degree of inequality of the macromeres in the different species.

Apart from the relation of the yolk lobe to unequal cleavage Vilson ('04) has shown that it bears some relation to the forma- ion of the pretrochal region in the larva of Dentalium; when the obe was removed the pretrochal organs failed to develop. What he morphogenetic factors are, which are located in the yolk lobe, s not known, but the significance of the lobe can scarcely be for he formation of the pretrochal region, since in animals with no obe or with a very minute one these regions form quite as well s in those with a large lobe.

These explanations refer to the "prospective significance" of he yolk lobe, and I know of no certain evidence as to the cause f its formation. The fact that such a lobe is present in almost ll gasteropod eggs, differing only in size in different species, and hat it is present in the eggs of annelids and a large number of ther animals, indicates that it has some cause of general occur- ence. In 1897 I suggested that the yolk lobe marks the point f attachment of the ovarian egg to the follicular wall. At this oint there is left a little mass of protoplasm on the surface of he egg, and here there is a weak spot in the protoplasmic pellicle which surrounds the egg. If the egg is put under pressure the yolk may be caused to flow out at this point, and in the increased tension which accompanies mitosis a yolk lobe is often pushed ut at this spot.

On the whole then, it seems probable that the yolk lobe rep- resents a temporary extrusion of egg substance during mitotic pressure at the former point of attachment to the ovarian wall, and that as a result of the presence of a large lobe of this kind, the first and second cleavages may be rendered unequal though the intersection of the furrows may lie in the egg axis and in the polar diameter of the egg.

In this connection one recalls the 'Dotterball' and the 'Granula- ball' observed by Hogue ('10) and Boveri ('10) in centrifuged eggs of Ascaris. Boveri comes to the conclusion that these are formed because they lie outside the influence of the asters or spheres: "Man könnte vielleicht sagen:—der von einen Sphäre eingenommene Plasmabezirk sucht sich von allem was ausserhalb dieses Wirkungskreises liegt, abzuschnüren," (p. 123). He sup-

poses that when the spindle, lying at right angles to the egg axis, is pushed far toward the animal or vegetal pole, a 'ball' is formed at the opposite pole. Whether this 'ball' is homologous with the yolk lobe I shall discuss in another paper in which the artificial production of such 'balls' will be considered, but I wish to point out here that although the first and second cleavage spindles in the large eggs of Crepidula and Fulgur lie near the animal pole, and far from the vegetal, the yolk lobe in these forms is very small, whereas in the minute eggs of the oyster and the clam, where the spindles are much nearer the vegetal pole, the yolk lobe is relatively very large. If, as I believe, this lobe is the result of an unsymmetrical distribution of yolk and egg substance with reference to the egg axis, or in the case of Ascaris with reference to the normal division plane, the great size of the lobe in some cases and its minute size in others, in which the area lying outside the "Wirkungskreise" of the spheres is much greater than in the former, would find a ready explanation.

II. Cell size and nuclear size in eggs and blastomeres

Strasburger ('93) was the first to show by detailed measurements that a fairly definite ratio exists between the nuclear size and the cell size in the embryonic cells of any given species of plant. He gives tables of measurements of the sizes of nuclei and cells in some forty different species, the nuclei ranging in diameter from 16μ to 3μ, and the cells from 24μ to 5μ. In general he found that the ratio of nuclear diameter to cell diameter is approximately as 2 to 3; and the ratio which exists in any case, is held to be due in general to the 'working sphere of the nucleus,' i.e., to the extent to which the metabolic interchange between nucleus and cytoplasm can reach.

Gerassimoff ('01, '02) found, in the cell division of Spirogyra, that when both daughter nuclei were caused to remain in one of the daughter cells, that cell grew to a larger size than normal, and he therefore concluded that the nuclear size determines the cell size.

Boveri ('02, '05) found that the size of the nuclei in sea urchin rvae is dependent upon the number of chromosomes which enter to the nuclei; in parthenogenetic or hemikaryotic eggs the nuei are smaller than in fertilized (amphikaryotic) ones, and they e smaller in the latter than in diplokaryotic eggs in which the mber of chromosomes is greater than normal. Furthermore e found that nuclei with a small number of chromosomes are ot only smaller than those containing a larger number but that ie cells in which they lie are also smaller, owing to the occurrence a larger number of cell divisions in cells with small nuclei than cells with large ones.

Boveri's work was based primarily on his studies of echinoderm evelopment and some of his conclusions are not applicable, ithout modification, to the eggs and larvae of other forms, pecially forms in which there are great inequalities of cleavage id in which various cells of the larva differ markedly from one nother in size. Thus his generalization, sometimes mentioned s 'Boveri's Law,' viz., "Die Grösse der Larvenzellen ist eine unktion der in ihnen enthaltenen Chromatinmenge, und zwar t das Zellvolumen der Chromosomenzahl direkt proportional," ould not apply, without modification, to eggs or larvae in which arious cells differ greatly in size without any corresponding ifference in the number of chromosomes. Unequal cell divisions e frequently found in the development of mollusks, annelids nd ascidians, where purely mechanical causes, such as mutual ressure between cells or the pressure of yolk within cells are not nvolved; in such cases the sizes of the nuclei invariably become roportional to that of the plasma, though the number of chroosomes remains the same in every nucleus. Similarly, many ells at first equal in size become unequal through dissimilar rowth, and their nuclei then become unequal also. Finally, n each of the animal groups named, cells at first equal in size may ecome unequal through dissimilar rates of division. In all ich cases the number of chromosomes appears to be, and premably is, the same in every nucleus of a given egg or embryo. vidently in cases of normal development the number of chromoomes does not determine the varying sizes of cells and nuclei.

R. Hertwig ('03, '08) as a result of his earlier work ('89) upon protozoa, has laid especial emphasis upon the fundamental significance of the ratio of nuclear size to cell size. He says ('03, p. 56):

> Wir haben im vorhergehenden sehr komplizirte Wechselwirkung zwischen Kern und Protoplasma kennen gelernt. Verkleinerung der Kernmasse führt zu Verkleinerung der Zellgrösse (Boveri), Vergrösserung der Kernmasse zu einer Vergrösserung der Zelle (Gerassimoff, Boveri). Andererseits kann aber auch Schwund des Plasmas zu einer Reduktion des Kernmaterials Veranlassung werden. Diese Verhältnisse kann man nur erklären, wenn man die oben vertretene Annahme macht, das jeder Zelle normalerweise eine bestimmte Korrelation von Plasma- und Kernmasse zukommt, welche wir kurz die "Kernplasmarelation" nennen wollen.

More recently Hertwig and his students have made many notable contributions to these "new problems of the cell theory," as Hertwig ('08) calls them. It has long been known that large cells have large nuclei, small cells small nuclei:

> Das Neue, welches in der Lehre von der Kernplasma-Relation gegeben ist, ist der Gedanke, dass das Massenverhältnis von Kern zu Protoplasma, der Quotient k/p, d.h. Masse der Kernsubstanz dividiert durch Masse des Protoplasma, ein gesetzmässig regulierter Factor ist, dessen Grösse für alle von Kerne beeinflusten Lebensvorgänge der Zelle, für Assimilation und organisierende Tätigkeit, für Wachstum und Teilung, von fundamentaler Bedeutung ist.

Hertwig calls attention to the fact that the Kernplasma-Relation differs in different phases of cell life, and he chooses for measurement that phase when the cell has come out of division and begins to nourish itself and to grow. This condition is known as the Kernplasma-Norm, and departures from it constitute what he calls Kernplasma-Spannung. This work of Hertwig and his school will be discussed more fully after the presentation of my observations.

1. Cell size and nuclear size in the cleavage of Crepidula plana. In my work on Karyokinesis and Cytokinesis in Crepidula ('02) I showed that the sizes of nuclei, spheres and asters, centrosomes, chromosomes, and plasmasomes are correlated with the quantity of cytoplasm in the cell, and the following pages constitute an

laboration and further extension of that work. The egg of repidula plana is a particularly favorable object for the study f such a subject. The eggs may be stained and mounted entire ı such manner that all of these cell constituents show with great istinctness, and the advantage of seeing whole eggs and nuclei ı making such measurements is sufficiently obvious.

A further advantage of the study of whole eggs is found in the ıct that the exact stage in the cell cycle is more easily determined ı whole eggs than in sections. My work has shown that it is ıost important in comparing the sizes of cell constituents to ɔmpare precisely corresponding stages, and accordingly I have ıosen for measurement stages of the maximum and minimum zes of the nuclei. The growth of the nucleus is more rapid in ıe last stage of the resting period preceding mitosis ('Kernteilngswachstum' of Hertwig) than at any other time in the cell ycle, and in order to find the maximum nuclear size it is necesıry to measure the nuclei just before the nuclear membrane disppears. Such stages are easily selected by looking for eggs in hich part of the nuclei of a certain generation of cells are dividıg while others have not yet begun to divide, as in figs. 1 and 2. .t this stage there is great uniformity in the dimensions of the uclei of particular blastomeres, and as the nuclei at this stage re regular spheres, it is easy to calculate their volumes.

The cell dimensions are more difficult to determine than are ıose of the nucleus. In cells which contain yolk and in cells of regular shape it is not possible to determine the volume of the lasma with accuracy. After the first cleavage the plasma and olk are sufficiently well separated so that the dimensions of the ytoplasm can be fairly well observed; before the first cleavage the lasma is so mixed with the yolk that this can not be done and I ave here had recourse to the method of centrifuging the yolk ut of the egg, leaving only the nucleus and plasma which can ıen be easily measured. Wherever it could be done, I have hosen cells for measurement which were as nearly spherical as ossible, but where the dimensions in different axes differed conderably I have determined the mean diameter, which is the one ecorded.

It is well known that during mitosis the general surface tension of a cell increases, and the cell tends to become spherical in shape. In measuring the maximum cell size, I have usually taken the stage immediately after the nuclear membrane disappears, and when the cell approaches a spherical shape. Similarly the minimum cell size has been determined by measuring the daughter cells during the telophase when they are approximately spherical. I have confidence in the substantial accuracy of my measurements of these maximum and minimum sizes of the purely protoplasmic micromeres. The volume of plasma in the yolk-containing macromeres is merely an approximation.

All measurements were made with Zeiss 1/1 micrometer eyepiece and 3 mm. homogeneous immersion objective. In all cases enough cells and nuclei were measured to give a fair average, though there is relatively little variation in the sizes of particular cells and cell constituents at corresponding stages in the cell cycle. All the eggs studied were fixed, stained and mounted in the same manner, so that alterations due to shrinkage should be approximately the same in all.

It is evident from table 3 that while large cells have larger nuclei than small cells, the relation of nuclear volume to cell volume is not constant. In different blastomeres of the same egg the Kernplasma-Relation, measuring nuclei and cells at their maximum size, varies from 1 : 14.5 to 1 : 0.37; even in purely protoplasmic cells it varies from 1 : 14.5 to 1 : 8.7. In cells containing yolk the ratio of nuclear volume to cell volume (including the yolk) varies from 1 : 89.5 to 1 : 34.8. In the different blastomeres of this egg there is no constant nuclear-plasmic ratio, or Kernplasma-Norm. However in different eggs corresponding blastomeres have the same Kernplasma-Relation, when measured at corresponding stages. The volumes of the protoplasm and of the nucleus show little variation in any given blastomere and the Kernplasma-Relation of each of the blastomeres named in table 3 is practically the same in all eggs. Since many of these blastomeres are peculiar in oöplasmic constitution and prospective significance it is not improbable that the peculiarities in their

TABLE 3

'mum nuclear size and cell size in the blastomeres of Crepidula plana; (measured just before nuclear membrane dissolves)

STAGE ASTOMERES	DIAMETER OF CELL	VOLUME OF CELL	DIAMETER OF PROTOPLASM INCLUDING NUCLEUS	DIAMETER OF NUCLEUS	VOLUME OF NUCLEUS	VOLUME OF PROTOPLASM LESS VOLUME OF NUCLEUS	KERN-PLASMA-RELATION
	μ	*cubic* μ	μ	μ	*cubic* μ	*cubic* μ	
ɜ maturation.	150	1,755,000	ca. 64*	42	32,409	97,131	1 : 3
ɜ first cleav-	142	1,488,910	ca. 65*	♀30+ *♂24=34.5	21,375	121,430	1 : 5.6
'D, before sec- cleavage....	106	619,329	ca. 51*	24	7,238	61,741	1 : 8.5
C, D, before ·d cleavage...	82	286,712	ca. 44*	22	5,775	38,570	1 : 6.6
ɔ), before ɜth cleavage...	81	276,350	ca. 40	21	4,849	28,431	1 : 5.8
, before divi- ı.....			30	14	1,437	12,603	1 : 8.7
D, before fifth ıvage.....	80	266,240	ca. 36	18	3,055	21,196	1 : 7
, before divi- ı.....			36	15	1,767	22,484	1 : 12.7
.d^{1}, before di- ion.....			30	12	905	13,135	1 : 14.5
.d^{2}, before divi- ɲ.....			15	7	180	1,587	1 : 8.8
before sixth ıvage.....	76	228,288	ca. 30	16	2,145	11,895	1 : 5.5
C, before sixth ıvage.....	76	228,288	ca. 22	16	2,145	3,430	1 : 1.6
ı, before divi- ɲ.....			33	14	1,437	19,250	1 : 13.3
ɜd^{1}, before divi- ɲ.....			30	14	1,437	12,603	1 : 8.7
ɜd^{2}, before divi- ɲ.....			30	14	1,437	12,603	1 : 8.7
before seventh ·avage.....	38	28,533	ca. 22	11	697	4,878	1 : 7
:D, before sev- th cleavage.....	60	112,320	ca. 20	18	3,055	1,134	1 : 0.37
ɔ, before sev- th cleavage.....	42	32,409	ca. 14	12	905	532	1 : 0.58

After yolk has been centrifuged out of egg. In normal eggs yolk and protosm are not well segregated at this stage.

Kernplasma-Relation may be the result of differentiations already present in the blastomeres.

In table 3 only maximum nuclear and cell dimensions are given for the different blastomeres. Results would undoubtedly differ greatly if the minimum nuclear and cell dimensions were taken instead of the maximum. Accordingly in table 4 the minimum nuclear and cell dimensions for the various blastomeres of Crepidula plana are given, together with the Kernplasma-Relation of each.

It is well known to cytologists that in cells undergoing regular division the minimum size of the nucleus is reached in the late anaphase, when the individual chromosomes have contracted to their smallest size and when they are most closely crowded together. A little earlier than this stage the chromatic plate is wider and the spaces between individual chromosomes greater; a little later the chromosomes begin to absorb achromatin and to swell up to form the chromosomal vesicles. At this stage of greatest nuclear contraction the chromosomal plate has approximately the form of a disk or short cylinder, and although the polar ends of the chromosomes are closer together than the equatorial ends, the disk being like a truncated cone, rather than a cylinder, we shall not greatly err if we treat this chromosomal disk as a short cylinder, rather than as a truncated cone. In table 4, in the column giving the dimensions of the chromosomal disk the first number is the diameter of the disk, the second its thickness.

Popoff ('08) has found in Frontonia that immediately after cell-division there is a diminution of the nucleus, which is then followed by a slow growth ('Funktionelles Wachstum' of Hertwig), and this by a much more rapid growth of the nucleus preceding division ('Teilungswachstum' of Hertwig). Both the functional growth and the divisional growth occur in the cleavage of Crepidula, but there is no diminution of the nucleus following division as in Frontonia. On the other hand, the minimum nuclear size is reached in the anaphase just before division of the cell body, as has been explained.

In the early telophase the chromosomal plate is drawn close to, and moulded over, the centrosome, and consequently the shape

the chromosomal plate, its degree of curvature and width, is ›endent in part upon the size of the centrosome. I found in)3 that in unequal cell division the centrosomes and asters ome unequal before the cell division is finished, though in the lier stages of mitosis the centrosomes and asters at the two es of the spindle are equal in size; only after the cell division inished do the daughter nuclei become unequal. The present rk has confirmed these earlier conclusions and has shown in lition that the shape of the chromatic plate at the ends of the ndle is influenced by the size of the centrosomes, and hence the equality or inequality of the division. If the centrosome large the chromosomes form a slightly arched plate on its face; if it is small the plate is highly arched. In the former e the plate remains relatively wide and the daughter nuclei en they are formed are disk-shaped; in the latter the plate and › daughter nuclei become more nearly spherical. Therefore, comparing the sizes of chromatic plates it is necessary to meas- › them before this difference in shape appears, i.e., in the late ıphase. But even when all these precautions are taken the)bable error in measuring objects of such small dimensions is ısiderable, but at least these measurements give the relative ler of magnitude of the chromosomal disks in the different stomeres.

The minimum cell dimensions occur in the early telophase, when › daughter cells first separate; at this stage the cells are nearly ıerical in form and it is not difficult to calculate their volumes :h substantial accuracy. While the minimum cell size does t occur at precisely the stage when the nuclei are smallest,)ccurs so soon thereafter that it can make but little difference the determination of the Kernplasma-Relation.

In short the Kernplasma-Relation, when plasma and nuclei › measured at their minimum sizes, varies in different blasto- ›res from 1:29 to 1:285.6. Except in the division of certain ls in the fourth and fifth cleavages (*2A–2D* and *2a–2d*, *2a*^{1}–*2d*^{1} d *2a*^{2}–*2d*^{2}) there is no appearance of a constant ratio between cleus and plasma in these different blastomeres. In general › dimensions of the nuclear plates decrease with every cleav-

TABLE 4

Minimum nuclear size and cell size in the blastomeres of Crepidula plana. (Nuclear plate measured in the late anaphase; cell diameter in early telophase)

STAGE	BLASTOMERES	DIAMETER OF CELL	DIAMETER OF PROTOPLASM INCLUDING NUCLEUS	DIMENSIONS OF NUCLEAR PLATE	VOLUME OF NUCLEAR PLATE	VOLUME OF PROTOPLASM LESS VOLUME OF NUCLEUS	KERNPLASMA-RELATION
		μ	μ	μ	*cubic μ*	*cubic μ*	
2 cells	AB, CD..	105	ca. 46	9 x 3	190.5	30,504	1 : 160.
4 cells	A,B,C,D,..	78	ca. 40	8 x 3	150.6	30,695	1 : 203.8
8 cells	1A–1D....	75	ca. 36	6 x 3	84.6	24,166	1 : 285.6
	1a–1d ...	27	27	6 x 3	84.6	10,235	1 : 120
12 cells	2A–2D....	72	ca. 30	6 x 3	84.6	13,955	1 : 165
	2a–2d.....	30	30	6 x 3	84.6	13,955	1 : 165
16 cells	$1a^1$–$1d^1$ ..	30	30	5 x 3	58.8	13,955	1 : 237
	$1a^2$–$1d^2$ ..	15	15	5 x 3	58.8	1,708	1 : 29
20 cells	3A–3D....	72	ca. 15	4 x 3	37.5	1,729	1 : 46
	3a–3d.....	25	25	4 x 3	37.5	8,087.5	1 : 215.6
24 cells	$2a^1$–$2d^1$ ...	24	24	4 x 3	37.5	7.200	1 : 195
25 cells	4D.....	60		5 x 3	58.8		
	4d	30		5 x 3	58.8		
29 cells	$2a^2$–$2d^2$ ...	24	24	4 x 3	37.5	7,200	1 : 195
	$1a^{1.1}$–$1d^{1.1}$	15	15	4 x 3	37.5	1,729.5	1 : 46
	$1a^{1.2}$–$1d^{1.2}$	24	24	4 x 3	37.5	7,200	1 : 195
32 cells	4A–4C....			4 x 3	37.5		
	4a–4c.....			4 x 3	37.5		

age, while all the cells of the same generation have nuclear plates of about the same size, though their protoplasmic volumes vary widely.

There is a great difference between the maximum and minimum volumes of the same nucleus, ranging from 1:27 to 1:38, while there is a relatively slight difference between the maximum and minimum sizes of the plasma and accordingly the Kernplasma-Relation of any blastomeres varies continuously from the stage of minimum nuclear volume to that of maximum nuclear volume. Hertwig ('08) has chosen as the stage showing the Kernplasma-Norm, "das Verhalten der jugendlichen Zelle, welche eben aus der Teilung hervorgegangen ist und nun anfängt sich

ı neuem zu ernähren, um abermals heranzuwachsen und sich teilen." I have tried to make measurements at the stage cribed by Hertwig, but find that in these segmenting eggs it oo ill defined to be safely used. Between successive divisions nuclei are growing continuously and rapidly and there is no ırly marked pause in the nuclear growth; accordingly slight 'erences in the stages chosen for measurement show relatively ge differences in the sizes of the nuclei.

ın order to take a stage intermediate between the two exmes of nuclear size, and in which the nuclei may be regarded having reached a normal functional condition, not related marily to the preceding or succeeding division, I have chosen ıt stage when the nuclei first become regularly spherical in ıpe. For a considerable part of the resting period the nuclei elongated at right angles to the previous spindle axis, and the plane of the chromatic plate of the previous anaphase; ring the latter part of the resting period they grow very rapidly preparation for the succeeding division ('Kernteilungswachsn' of Hertwig). The stage when the nuclei first become spherl lies somewhere between these two phases and may therefore considered to represent the mean nuclear size. However this ge is not so precisely defined as are the stages of maximum d minimum nuclear size, and therefore the nuclear dimensions likely to be more variable.

[t is obviously more difficult to determine the volume of the avage cells during the resting period, when they are pressed o irregular and polygonal forms, than during mitosis when they proach a spherical shape. However the approximate accuracy the cell dimensions recorded in table 5 may be judged by comring them with the maximum and minimum cell dimensions, ven in tables 3 and 4.

The Kernplasma-Relation varies about as much for mean nensions of the nucleus and cell as for maximum ones, though t as much as for minimum dimensions. In yolk-containing ls it varies from 1:1.1 to 1:27.5 and in purely protoplasmic ls from 1:7 to 1:35.7.

TABLE 5

Mean nuclear size and cell size in the blastomeres of Crepidula plana. (Measured when nuclei first become spherical after division)

STAGE	BLASTOMERES	DIAMETER OF CELL	DIAMETER OF PROTOPLASM INCLUDING NUCLEUS	DIAMETER OF NUCLEUS	VOLUME OF NUCLEUS	VOLUME OF PROTOPLASM LESS VOLUME OF NUCLEUS	KERNPLASMA-RELATION
		μ	μ	μ	*cubic* μ	*cubic* μ	
Before cleavage		136	ca. 60	♀18 ♂12	3,960	109,137	1 : 27.5
2 cells	AB, CD	105	ca. 44	18	3,055	41,240	1 : 13.5
4 cells	A, B, C, D	78	ca. 40	16	2,145	31,135	1 : 14.5
8 cells	1A–1D	75	ca. 36	15	1,767	22,484	1 : 12.7
	1a–1d	30	30	12	905	13,135	1 : 14.5
12 cells	2A–2D	72	ca. 36	15	1,767	22,484	1 : 12.7
	2a–2d	36	36	12	905	23,346	1 : 25.6
16 cells	$1a^1$–$1d^1$	30	30	9	382	13,658	1 : 35.7
	$1a^2$–$1d^2$	15	15	6	113	1,654	1 : 14.6
20 cells	3A–3D	72	ca. 18	14	1,437	1,618	1 : 1.1
	3a–3d	30	30	12	905	13,135	1 : 14.5
24 cells	$2a^1$–$2d^1$	27	27	12	905	9,330	1 : 10.3
	$2a^2$–$2d^2$	27	27	12	905	9,330	1 : 10.3
25 cells	4D	60					
	4D	34		9	382		
29 cells	$1a^{1.1}$–$1d^{1.1}$	15	15	6	113	1,654	1 : 14.6
	$1a^{1.2}$–$1d^{1.2}$	24	24	12	905	6,333	1 : 7
32 cells	4A–4C			15	1,767		
	4a–4c			9	382		

Incidentally the interesting fact appears that nuclei in large cells do not become spherical until they have reached a larger size than their sister nuclei in small cells; for example in the micromeres *1a–1d* and *2a–2d* the nuclei become spherical when they are about 12μ in diameter, in the macromeres *1A–1D* and *2A–2D* their sister nuclei are about 15μ in diameter before they become spherical. Since the same amount of chromatin goes into each of the sister nuclei, the difference in the size of the nuclei when they first become spherical must be found in some other factor. It seems probable that it is due to the shape of the chromosoma disk, which remains flattened, or slightly arched, in large cells

ıd is highly arched in small ones, owing to the greater or smaller ;e of the centrosomes, as explained on p. 19. The flattened ıromosomal plate gives rise to a disk-shaped nucleus, which ıly later becomes spherical, whereas the highly arched plate oner gives rise to a spherical nucleus.

2. Cell-size and nuclear size in the cleavage of Fulgur carica. he eggs of Fulgur carica are the largest gasteropod eggs of which know, while the eggs of Crepidula plana are among the smallest. will be instructive therefore to compare the Kernplasma-elation in these two cases. Table 6 gives the mean nuclear ze and cell size of the blastomeres of Fulgur. The eggs measured ere fixed, stained and mounted entire, as in the case of Crepi-ıla plana. Owing to the great size of these eggs it was necessary

TABLE 6

Mean nuclear size and cell size in the blastomeres of Fulgur carica

	DIAMETER OF CELL	DIAMETER OF PROTOPLASM	DIAMETER OF NUCLEUS	VOLUME OF NUCLEUS	VOLUME OF PROTOPLASM LESS VOLUME OF NUCLEUS	KERNPLASMA-RELATION
	μ	μ	μ	*cubic* μ	*cubic* μ	
Macromeres						
B, CD, before second cleavage	1200	200	40	33,280	4,126,720	1 : 124
, B, C, D, before third cleavage	800	160	32	17,040	2,112,880	1 : 124
A–1D, before fourth cleavage	800	160	32	17,040	2,112,880	1 : 124
A–2D, before fifth cleavage	800	160	32	17,040	2,112,880	1 : 124
D, before sixth cleavage	800	160	32	17,040	2,112,880	1 : 124
A–3C, before sixth cleavage	800	160	48	57,508	2,062,412	1 : 35.8
A–4D, before seventh cleavage	768	160	96	460,063	1,669,857	1 : 3.6
Micromeres						
a–1d		80	16	2,145	264,095	1 : 127.7
a–2d		80	16	2,145	264,095	1 : 127.7
a–3d		80	16	2,145	264,095	1 : 127.7
a^1–$1d^1$		80	16	2,145	264,095	1 : 127.7
a^2–$1d^2$		40	8	266	33,014	1 : 124

to make the measurements under a relatively low power, the 8 mm. apochromat objective and the 1/1 micrometer eyepiece of Zeiss. Owing to the relatively low magnification the probable error is greater than in the measurements of the eggs of C. plana.

With the exception of the cells *3A–3C* and *4A–4D* the Kernplasma-Relation is in this case practically constant, varying only from 1 : 124 to 1 : 127. In view of the fact that I can find no constant Kernplasma-Relation in the blastomeres of Crepidula this result in the case of Fulgur is unexpected. I am sure that my measurements in the case of Fulgur are not so accurate as in Crepidula, the number of eggs measured being relatively small and the magnification used low, so that each interval of the scale stood for 8μ. The uniformity in the measurements of the different blastomeres and nuclei of Fulgur may be due in part to this fact; on the other hand this would only account for the lack of minor variations and would not explain the general uniformity. There is no doubt that the micromeres of Fulgur are more uniform in size, than those of Crepidula; also the whole cleavage process is very much slower, and (with the exception of the macromeres *3A–3C* and *4A–4D*) the divisions are more nearly synchronous in the different cells than in Crepidula. It seems probable that these two facts are connected with the more uniform Kernplasma-Relation of the different blastomeres of Fulgur.

This conclusion is rendered still more probable by a consideration of the two generations of cells in which there is a wide departure from the usual Kernplasma-Relation, viz. *3A–3C* and *4A–4D*. In these cases, as in the same cells in Crepidula the resting stage is particularly long, lasting in the case of *4A–4D* until all the organs of the embryo are outlined and more than one thousand cells are present; consequently the nuclei grow to an enormous size so that the Kernplasma-Relation falls in one case to 1 : 35.8 and in the other to 1 : 3.6. In the corresponding cells in Crepidula the ratio is 1 : 1.6 and 1 : 0.37; the volume of the nucleus in the last named case being about three times that of the plasma. The Kernplasma-Relation of the cells *4a-4c* is 1 : 0.58 in Crepidula, the nuclei being about twice as voluminous as the plasma; in the corresponding cells of Fulgur this ratio cannot be

lily determined since the nuclei undergo several divisions, ıgh the cell body does not divide.

rom these measurements it may be concluded that when cell sion takes place at regular intervals the Kernplasma-Relation ıirly constant; when it takes place at irregular intervals this o is variable. The longer the resting period the larger the leus becomes, and in extremely long resting periods the greater t of the plasma may be taken up into the nucleus.

'hese observations are in full agreement with experiments on eggs of Crepidula which will be described later. They are antagonistic to Boveri's conclusions as to the correlation be- en chromosome number and nuclear size; on the other hand own experiments show that the size of the nucleus is depend- , in part, upon the number of chromosomes which enter into 'ormation. But in normal cells all of which contain the same ıber of chromosomes differences in nuclear size must be due ome other factor.

'he results of my measurements do not indicate that the Kern- ɜma-Relation of Hertwig is either a constant or self regulating o in the blastomeres of these eggs; on the other hand it appears ɔe a result rather than a cause of the rate of cell division, and sequently it is a variable rather than a constant factor. 'thermore the size of the nucleus, in these eggs, is dependent ɔn at least three factors: (1) The initial quantity of chromatin mber of chromosomes) which enter into the formation of the leus (Boveri). (2) The volume of the protoplasm in which nucleus lies. (3) The length of the resting period.

III. Cell size and nuclear size in adult tissue cells

t is generally believed that embryonic cells differ greatly from ılt tissue cells in their "Kernplasma-Relation." In a series of ughtful and suggestive works Minot ('90, '95, '08) has main- ıed that differentiation, senescence and finally death are the ompaniments, if not the results, of an increase of protoplasm compared with nucleus. It is well known that embryonic s of plants are more purely protoplasmic than adult cells,

which are frequently filled with vacuoles and sap so that the size of the cell gives no true idea of the volume of the cytoplasm. Among animals adult tissue cells often become filled with the products of differentiation or metabolism, such as fibers, granules, secretions, oil, etc., which greatly increase the cell dimensions. It is evidently a difficult if not impossible task to determine the quantity of real protoplasm in such cells and thus to discover the true "Kernplasma-Relation." However in certain less highly differentiated cells, especially in epithelial and glandular tissue, the true Kernplasma-Relation may be established with a fair degree of accuracy.

Unquestionably the physiological state of a cell has much to do with its nuclear-plasmic ratio. Hodge ('92) found the nuclei of nerve cells shrunken after extreme stimulation, and it has been long known that the same is true of gland cells. In Crepidula the liver cells, when active, are filled with secretion and are among the largest in the body, but when the secretion has been discharged and they have returned to an inactive condition, the cell body is much smaller and the nucleus larger.

I have measured the cells and nuclei of a number of tissues of Crepidula plana, derived from the three germ layers, and the results are given in table 7. Since these cells vary in shape to a great extent, and in order to facilitate comparison of cell diameter and nuclear diameter, cells were chosen for measurement which were as nearly as possible spherical or cubical in shape In all elongated cells the long axis and one cross axis were measured and it was assumed that the other cross axis was of the same dimensions as the one observed.

It is evident that in these tissue cells of Crepidula plana there is no marked increase of protoplasm over nucleus as compared with the blastomeres of the same species; throughout the cleavage with the exception of the cells *3A–3D* and *4A–4D*, the average Kernplasma-Relation for nuclei and cells of mean size is about 1 : 15, for nuclei and cells of maximum size about 1 : 6; the average ratio in adult tissue cells, which are not filled with metaboli products, is about 1 : 10.5. In the case of the ganglion cells th nuclei are relatively and absolutely larger than in the other tissues

TABLE 7

l size and nuclear size in tissue cells of sexually mature individuals of Crepidula plana

TISSUE CELLS	DIMENSIONS OF CELL	DIAMETER OF NUCLEUS	VOLUME OF NUCLEUS	VOLUME OF CELL LESS VOLUME OF NUCLEUS	KERN-PLASMA RELATION
	μ	μ	*cubic* μ	*cubic* μ	
testinal epithelium....	11 x 11 x 12	6	113	1,339	1 : 11.8
ıstric epithelium.....	10 x 10 x 36	8	68	3,332	1 : 12.4
ver duct epithelium....	10 x 10 x 18	6	113	1,628	1 : 14.4
ver cells (filled with secretion products).....	15 x 15 x 45	6*	113	10,012	1 : 88.6
ver cells (without secretion products).....	14 x 14 x 30	9	382	5,498	1 : 14.4
idney cells (containing secretion products)....	15 x 15 x 15	6	113	3,262	1 : 28.8
ɔtodermal epithelium (near anus).....	5 x 5 x 15	4	33	342	1 : 10.3
ill chamber epithelium..	6 x 6 x 12	4	33	405	1 : 12.2
ill filament epithelium ..	7 x 7 x 9	4	33	408	1 : 12.3
pithelium from foot....	6 x 6 x 15	5	65.4	474.6	1 : 7.1
anglion cell (large).....	17 x 17 x 23	12	905	5,724	1 : 6.3
anglion cell (large).....	10 x 10 x 20	9	382	1,618	1 : 4.2
öcytes I (before yolk formation).....	$12\frac{1}{2}$	7	180	836	1 : 4.6
öcytes I (before yolk formation).....	$11\frac{1}{2}$	7	180	791	1 : 3.4
öcytes I (before yolk formation).....	10	6	113	407	1 : 3.6
öcytes I (before yolk formation).....	8	5	65.4	203	1 : 3.1
öcytes I (before yolk formation).....	$6\frac{1}{2}$	4	33	111	1 : 3.3

*Nucleus shrunken and very irregular in shape.

ıe Kernplasma-Relation being about 1 : 5; however in this ıse the nerve fiber is not added to the cell body and this would ɔubtless greatly increase the volume of the plasma. Muscle ɛlls in Crepidula are long, slender and crooked and I have found impracticable to estimate their volumes with any degree of ɔcuracy. Doubtless the plasma, including the contractile sub-ɜance, is here relatively much more abundant than in embryonic r epithelial cells. In the epithelial and gland cells of adult

Crepidula the embryonic ratio of nucleus to plasma is maintained with little change. In all the oöcytes up to the time that yolk formation begins the nuclei are relatively large, the ratio of nucleus to plasma being about 1 : 3.6, and in the younger and smaller oöcytes the nuclei are relatively larger than in the older and larger ones.

Eycleshymer ('04) found that the volume of the plasma in the striated muscle cells of Necturus increased about ten times as much as the nuclear volume, during development from the 8 mm. embryo to the adult condition. There is, therefore, in these later stages a notable shifting of the Kernplasma-Relation in favor of the plasma. It is probable however that the contractile substance which makes up the larger part of the muscle cell, does not contribute to the growth of the nucleus as does the protoplasm of embryonic cells—that so far as the growth of the nucleus is concerned it acts as does yolk, oil, membranes, fibers and other products of metabolism and differentiation. If only the sarcoplasm of the muscle cell and not its contractile substance is able to contribute to the growth of the nucleus, the small volume of the nuclei as compared with the entire cell would find a ready explanation. There can be no doubt that the plasma is the chief seat of differentiation, as Minot has emphasized, and that highly differentiated cells, such as muscle, nerve, and some kinds of connective tissue, have a larger amount of plasma and its products, relative to the nucleus, than have embryonic cells. In the case of fiber cells, fat cells, and probably muscle cells, the cell body becomes filled with the products of differentiation and metabolism, which like the yolk in egg cells, or the secretion products in liver cells cannot enter the nucleus and consequently do not influence its size. In such tissue cells the cell body is relatively much greater as compared with the nucleus, than in purely protoplasmic cells, but I have been unable to find any evidence that the ratio of protoplasm (using this term in its usual sense) to the nucleus is greater in tissue cells of Crepidula than in the blastomeres.

IV. The inciting causes of cell division

The relative sizes of cells and of nuclei are dependent, in part, on the rate of cell division. Cells which divide infrequently larger, other things being equal, than those which divide often. e turret cells ($1a^2$–$1d^2$) of Crepidula are the smallest cells in entire embryo at the time of their formation (figs. 3, 4); vever they divide but twice during the whole of the cleavage iod, and consequently they grow to be very large; whereas each he apical cells from which they were derived, gives rise during cleavage period to twelve cells the combined volume of which not much greater than that of one full-grown turret cell. idently the factors which bring on or delay cell-division have ch to do, indirectly, with the sizes of cells and nuclei.

Strasburger ('93) supposed that cell division occurred when the io of the cell body to the nucleus increased beyond a certain nt, which might be regarded as marking the limit of the 'work- sphere of the nucleus;' with the division of the cell the normal io was once more restored.

Boveri ('04) sought to find the inciting cause of cell division in chromosomes. He believed that the chromosomes divide en they have reached a size double that which they had at the se of the preceding division. At the same time he showed at the rhythm of the division of the centrosomes may be inde- ident of that of the chromosomes and that division of the cell pends upon the centrosomal rhythm rather than upon the chro- somal rhythm.

That there is a rhythm of division for chromosomes and centro- nes seems to be well established by Boveri's work, but this rthm in the case of the chromosomes is not determined by the ne when they have grown to double their size at the close of the ceding division. Marcus ('06) and Erdmann ('08) have shown at the chromosome size throughout the cleavage of Strongy- entrotus is a constantly decreasing one. Baltzer ('08) admits at the chromosomes do not double in size at each cycle of divi- n; he does not find any great diminution in chromosome size to the 16-cell stage, though the chromosomes in the blastula

stage are undoubtedly smaller than those of early cleavage stages. In Crepidula the chromosomal plate decreases in size in successive cleavages, though by no means uniformly; but at no time during the cleavage period do the chromosomes grow to their original size at the beginning of the cleavage. Boveri's view, therefore, finds no support in the cell-divisions of the cleavage period.

R. Hertwig ('03, '08) finds the inciting cause of division in a 'Kernplasma-Spannung,' due to the unequal growth of nucleus and plasma:

> Die Kernplasma-Relation muss eine Verschiebung erfahren zuungunsten des Kernes, es muss sich eine Kernplasma-Spannung entwickeln, welche allmählich zunimmt, bis schliesschlich ein Grad erreicht wird, den ich früher Kernplasma-Spannung in engeren Sinne genannt habe. In dieser Spannung erblicke ich die Ursache der Teilung. Ich nehme an, dass, wenn ein Höhepunkt der Kernplasma-Spannung erreicht wird, der Kern die Fähigkeit gewinnt, auf Kosten des Protoplasma zu wachsen, und das die hierbei sich vollziehenden Stoffumlagerungen zur Teilung der Zelle führen. Zum funktionellen Wachstum gesellt sich das Teilungswachstum des Kernes, um die Kernplasma-Norm wiederherzustellen." (p. 20)
>
> Relative Zunahme der Kernsubstanz, gleichgültig, ob dieselbe durch Vergrösserung des Kerns bei gleichbleibender Protoplasmamenge oder Verringerung des Protoplasma bei gleichbleibender Kerngrösse herbeigeführt wird, müsste eine Verlangsamung der Teilung und im ersten Fall eine Steigerung der Teilgrösse zur Folge haben; umgekehrt müsste relative Abnahme der Kernmasse den Eintritt der Kernteilung beschleunigen, die Teilgrösse herabsetzen (p. 23).

Hertwig holds that his own work on Infusoria, and that of Gerassimoff on Spirogyra, show that an increase of nuclear mass leads to a slowing of divisions and an increase of the division size of the cell; and that the process of the segmentation of the animal egg shows that a great reduction of nuclear mass leads to a high degree of divisional activity. He says that many external and internal conditions influence the Kernplasma-Relation and he expresses the hope that his theory may not be cast aside because here and there a fact may be found which cannot be brought under it, without further consideration.

As we have seen the Kernplasma-Relation varies widely in certain blastomeres of Crepidula and Fulgur. In these cases wide departures from the Kernplasma-Norm have not brought

ell division, and if Kernplasma-Spannung is a cause of cell-
ion it must be a minor factor in this case. It seems to me
able from my observations and experiments on segmenting
, that the Kernplasma-Relation in these blastomeres is a re-
rather than a cause of the rhythm of cell division, and that
factors which bring on cell division are to be found in some
nsic condition in the nucleus or centrosome, rather than in the
ıtenance of a constant ratio of nuclear volume to cell volume.
ort is lent to this view by the phenomena of oögenesis, for
ıave in the germinal vesicle the largest nucleus in the entire
cycle, following upon the longest resting period, while the
nd maturation division follows immediately upon the first,
ılly before a resting nucleus is formed. The long delay in
appearance of the first maturation division, as well as the
t period intervening between the first and second maturation
ions, must both be attributed, as it seems to me, to intrinsic
litions in the cell, other than 'Kernplasma-Spannung.'

ı the cleavage of the egg the rate of division seems to depend,
art, on the quantity of protoplasm present. As long as a con-
rable quantity of plasma is present in the blastomeres the rate
ivision is rhythmical, but when the macromeres have given off
ost all the plasma in the formation of the three quartets of ecto-
es, a long resting period follows. The first of these macro-
es to divide, giving rise to the fourth quartet, is the one with
largest amount of plasma, viz., *3D*, while the cells *3A–3C*
nally divide much later. However if, by centrifuging at the
t stage, *3C* is caused to contain more plasma than usual it
divide at the same time as *3D*, as shown in fig. 37. The cells
4D, in which the resting period is particularly long, contain
little plasma, and this appears to be absorbed by the nucleus
ost as fast as it is formed. The micromere *1d* is slightly
ller than its fellows, *1a-1c*, and it divides later than the latter.
'turret' cells, $1a^2$-$1d^2$, are the smallest cells in the egg, when
are formed, and they have the longest resting period.

ı spite of this evidence that the quantity of protoplasm has
o with the rate of division, there is other conflicting evidence
ch is hard to harmonise with it; thus, these same 'turret' cells,

which are at first so small and have so long a resting period, become much larger than adjoining cells before they divide. R. Lillie ('10) maintains that "the primary change in the initiation of cell division and development is an increase in the permeability of the plasma membrane." It is well known that the general surface tension of the cell increases during mitosis, and I have found that the tension of the cell membrane is locally reduced at the two poles of the cell before and during division (see Conklin, '02, p. 94; also this paper, p. 82). It is quite possible that this polar reduction in surface tension before mitosis begins may have something to do with initiating division.

On the whole it seems probable that the time of cell division is dependent upon the coincidence of several more or less independent factors. Boveri has shown that the division phases in nucleus and centrosome may be more or less independent of each other, though complete cell division depends upon the coincidence of the two. To these factors may, perhaps, be added the quantity of protoplasm, and thus indirectly the 'Kernplasma-Relation' and perhaps also increased permeability of the cell membrane, and a local reduction of surface tension at the poles of the cell. Gurwitsch ('08) maintains that the blastomeres are ready for division at all times, and that only 'Kernplasma-Koinzidenz' or 'Zustands-Koinzidenz,' is necessary to start division. He suggests that a coincidence of polarity of nucleus and plasma may be necessary, and he concludes from the apparently accidental occurrence of divisions in different parts of an egg or embryo, that several independently variable factors may be concerned, the coincidence of which is necessary to bring on cell division. The latter part of this conclusion seems to me to be justified by the facts which I have presented.

V. Growth of protoplasm during cleavage

It is well known that the egg as a whole does not increase in volume until after the cleavage period. Indeed Godlewski ('08) finds that there is in Echinus and in Strongylocentrotus, no change in the quantity of plasma at the 64-cell stage, as compared with the

gmented egg; however, in the blastula there is an actual loss, otal volume of plasma being about one-third less than in the gmented egg; during this period the nuclear material has ased in volume at the expense of the plasma. Whether the na actually increases during cleavage at the expense of the has not been determined, so far as I am aware, in any case. neans of the centrifuge it is possible to throw the yolk out e egg before cleavage and during the early cleavage stages, ng the plasma which can then be readily measured. In cleavage stages I have not been able to throw the yolk out e small blastomeres by means of the centrifuge; but on the hand the protoplasm and yolk are normally segregated in stages so that it is possible to determine the approximate nsions of both without having recourse to the centrifuge. following table gives the total maximum volumes of all the i, protoplasm and yolk in the eggs of Crepidula plana at us cleavage stages. Following Popoff ('08), I have deter- d the coefficients of growth of the nucleus and of the pro- asm for each stage; these coefficients are obtained by dividing volume of a later stage by that of an earlier one, and they esent the growth in 'times,' or multiples of the initial quantity. ne first half of each column of coefficients the earlier stage is ne before maturation, while in the second half of each column the one before the first cleavage. The coefficient of growth of

TABLE 8

maximum volumes of nuclei, protoplasm and yolk in the eggs and cleavage stages of Crepidula plana

E	VOLUME OF NUCLEI	VOLUME OF PROTOPLASM	VOLUME OF YOLK	TOTAL VOLUME OF EGG	COEFFICIENTS OF NUCLEAR GROWTH		COEFFICIENTS OF PROTOPLASMC GROWTH		TOTAL KERN-PLASMA-RELATION
	cubic μ	*cubic μ*	*cubic μ*	*cubic μ*					
ma- ion..	32,409	97,131	1,625,460	1,755,000	1.0		1.0		1 : 3
first age..	21,375	121,430	1,346,105	1,488,910	0.65	1.0	1.25	1.0	1 : 5.6
.....	14,476	123,482	1,100,700	1,238,658	0.45	0.67	1.27	1.02	1 : 8.5
.....	23,100	154,280	969,468	1,146,848	0.71	1.08	1.58	1.27	1 : 6.6
.....	25,144	164,136	972,320	1,161,600	0.77	1.17	1.68	1.35	1 : 6.5
.....	23,628	233,608	980,156	1,237,392	0.72	1.10	2.45	1.92	1 : 9.8
.....	30,164	258,897	890,727	1,179,788	0.92	1.41	2.66	2.13	1 : 8.6
		(231,000)		(1,151,891)			(2.35)	(1.90)	(1 : 7.7)

any stage, less the coefficient of the initial stage, viz. unity, gives the percentage of growth of that stage, as compared with the initial stage.

Since the ectoderm at the 24-cell stage is a plate of purely protoplasmic cells, nearly square, about 80μ on each side and 36μ thick its volume is about 230,400 cubic μ; subtracting the volumes of the nuclei of the plate, 21,584 cubic μ, leaves 208,816 cubic μ as the volume of the cytoplasm[1] of the ectodermal plate. Adding to this the volume of the protoplasm in the macromeres *3A–3D*, viz. 22,185 cubic μ, we have as the total volume of the protoplasm at the 24-cell stage 231,000 cubic μ. This figure is 27,897 cubic μ less than the volume of protoplasm at the 24-cell stage given in the table, which was calculated from the dimensions of each individual cell, rather than from those of the entire ectodermal plate. It is highly probable that the lower figure is nearer correct than the higher one, since minor errors in the measurements of individual cells are greatly magnified in determining the total volumes of these cells. The same remark applies to the total volume of protoplasm in the 16-cell stage, which is probably actually less than the volume given in the table; and if the total volume of the protoplasm is less than the amount given in the table the total volume of the yolk in these stages is of course increased correspondingly.

But assuming that the smaller number (in brackets) represents the actual volume of the protoplasm in the 24-cell stage of Crepidula plana we must admit that there has been a great growth in the plasma at the expense of the yolk during the cleavage. The coefficient of protoplasmic growth (i.e., the volume of protoplasm of any stage divided by the volume of protoplasm of the stage just before maturation) is given in the next to the last column of the table; and a glance at this shows that the protoplasm at the 24-cell stage is at least $2\frac{1}{3}$ times as voluminous as in the maturation stage, while the yolk is correspondingly less voluminous. The volume of the entire egg, also, is considerably less in the 24-cell stage than at the beginning of development. Indeed there has been a gradual decrease in the volume of the entire egg during

[1] The words 'cytoplasm' and 'protoplasm' are used synonomously throughout this paper.

early cleavages. These results show a general agreement
ı those of Godlewski.

he growth of plasma at the expense of yolk during the matur-
n and the cleavage period, was shown to occur in my studies
he effects of centrifugal force on the eggs of Lymnaea and
sa (Conklin, '10). In the living eggs of these animals the
stances may be stratified by centrifugal force into a gray
ıt) zone, a clear (middle) zone and a yellow (heavy) zone; the
r and clear zones constitute what I have here regarded as proto-
m, while the yellow zone is in large part composed of yolk.
fore the first maturation the yellow substance composes at
t one-half of the entire egg; just before the first cleavage it
poses only about one-eighth of the egg. The clear and gray
stances, which together constitute about one-half of the egg
he earlier period, form seven-eighths of the egg in the later
od," (p. 436).

ı the normal eggs of Lymnaea and Physa, which have not been
rifuged, the clear and yellow substances are easily recog-
ble, and the stages in the transformation of the latter into
former have been studied in the paper mentioned, from which
following summary is quoted:

ı the course of development, from the maturation of the egg to the
rulation, the relative quantities of clear (plasma) and yellow sub-
ce (yolk) are reversed. At the beginning the clear substance is
ll in quantity, and is chiefly visible in the germinal vesicle (though
riments show that some of it is distributed through the yellow sub-
ce) and at this stage the entire cell body is yellow in color. With
establishment of the germinal layers the yellow substance is limited
ıe few cells constituting the endoderm and mesoderm, while all the
of the embryo, by far the larger part, is composed of clear substance.
s change in the relative quantities of these two substances is due in
to their separation and segregation during the course of develop-
t, but in much greater part to the transformation of yellow sub-
ce (yolk) into the clear (plasma). It is a phenomenon of general
ırrence among many animals that the clear protoplasm of the egg
ery small in quantity before the dissolution of the germinal vesicle
that it gradually increases in quantity after that stage. This is
otless due in large part to the dissolving of yolk and its conversion
clear protoplasm, and it is a significant fact that this process takes
e most rapidly after the breaking down of the wall of the germinal
cle and the escape of a large part of the nuclear contents into the
body (p. 423).

There are no eggs wholly without yolk and probably in all of them plasma is formed at the expense of yolk during the cleavage period. This probability is of great significance, for all studies which have had to do with the relative quantities of protoplasmic and nuclear materials during these early stages of development have dealt only with the entire cell contents without attempting to determine what part of this is plasma. In many cases, the great disproportion between cell volume and nuclear volume at the beginning of developemnt is due to the fact that a large part of the cell volume is made up of yolk; if the volume of the plasma only is compared with that of the nucleus it is found that the relative quantity of plasma is actually less at the beginning of development, than in the later cleavages, with the single exception of those blastomeres which have unusually long resting periods. In Crepidula there is no excess of plasma over nuclear material in the early stages, in comparison with the later ones, as Minot and others have assumed, and the process of cleavage is not in this case a method of restoring the Kernplasma-Norm, or of rejuvenating senile cells, by an enormous increase of nuclear material as compared with the plasma. As a matter of fact the plasma increases almost as rapidly as the nuclear material during the cleavage of this egg, and even adult tissue cells have a Kernplasma-Relation but little different from that of the blastomeres, (see p. 25).

VI. Rate of nuclear growth during cleavage

It is well known that during cleavage there is usually no increase in the volume of the egg, but it is generally held that the increase in the nuclear substance is very great. In his book on "Age, Growth and Death" Minot ('08) says: "The nuclei multiply (in cleavage); they multiply at the expense of the protoplasm. They take food from the material which is stored up in the ovum, nourish themselves by it, grow and multiply until they become the dominant part in the structure" (p. 166). He suggests that this nuclear increase during cleavage is a process of rejuvenation, though he admits that the relative increase of nu-

· material as compared with protoplasmic may be prolonged ınd the period of segmentation (p. 167). But although he hasizes the growth of the nuclear material as a whole during cleavage, he specifically recognizes the fact that there is a l reduction in the size of individual nuclei in the early stages 174, 179). Hertwig ('03) also has emphasized this great 'th of the nuclear material during the early stages of develop- t. He says (p. 116):

ıere is an enormous disproportion of nucleus and protoplasm at the ınning of cleavage, and this disproportion is gradually equalized by ›ransformation of cell substance into nuclear substance. The man- ›f this may be imagined by supposing that resting protoplasm con- chromatin and achromatic material and that at every cell division analysed into these constituents serving for the growth of the ›us.

›eb ('09) also has called attention to the doubling of nuclei ıch division, with the consequent increase of nuclear material , geometric ratio, and the resemblance which this bears to katalytic reactions.

he great increase in the nuclear substances during cleavage been commented upon by many writers, and the references l have been chosen rather because of the theories which have ı based upon this phenomenon than because they represent ınusual opinion as to the phenomenon itself. At the time n the following computations of the rate of nuclear growth ng cleavage were made, I was unaware that anyone had made putations of a similar sort. Since my material afforded an ›ually good opportunity for making such computations, I fully measured the diameters of the germinal vesicle, of the and sperm nuclei, of all the nuclei up to the 24-cell stage, of e of the 42-cell stage, and of the 70-cell stage,—every nucleus g measured at its maximum size, so far as possible,—with the lts given in the following tables. These results have been ırprising to me as they are likely to be to any of my readers. fter this work was completed I became acquainted with the k of Godlewski ('08) and Frl. Erdmann ('08) on the sizes of ei and of individual chromosomes of the blastomeres of

Echinus and Strongylocentrotus. Godlewsky found that from the 1-cell to the 64-cell stage the nuclear substance grows nearly in geometric ratio; from the 64-cell stage to the blastula, with about 1256 cells, there is little increase in the nuclear substance, but since he supposes that the number and size of the chromosomes in the later stages remain the same as in the earlier ones, the nuclei must become richer in chromatin in the later stages. He finds that the volume of the plasma in the blastula stage is about one-third less than in the unsegmented egg and he considers that a large part of this lost plasma has been converted into chromatin. Erdmann ('08) has made a careful computation of the volume of the resting nuclei and of individual chromosomes in the early cleavage stages, and in the blastula and gastrula of Strongylocentrotus. She finds that the chromosomes of the pluteus period have only about one-fortieth the volume of those of the first spindle, but though the individual chromosomes grow smaller continually, the total nuclear volume increases at the expense of the plasma up to the late blastula stage.

1. Nuclear growth during the cleavage of the egg of Crepidula. The maximum, minimum and mean volumes of the nuclei at different stages of the cleavage of Crepidula plana are given in tables 3 to 5 and the coefficients of growth of all the nuclei are given in table 8. It remains only to summarize the facts there presented and to give the nuclear volumes and the rate of growth in certain later stages of the cleavage. This has been done in table 9, where the maximum, miminum and mean nuclear volumes of every nucleus from the 2-cell to the 32-cell stage is given, together with the coefficient of growth for each stage. Since this table starts with the 2-cell stage the coefficients of growth are different from those given in table 8, where subsequent stages are compared with the germinal vesicle or with the germ nuclei. For the purpose of determining the usual rate of growth for each cycle of cell division during the cleavage it is desirable to start with the 2-cell stage. The germinal vesicle is an extraordinarily large nucleus, and since two nuclei are present in the egg before the first cleavage the nuclear condition at this stage is unusual; on this account the rate of nuclear growth during cleavage is

TABLE 9

Rate of nuclear growth during the cleavage of Crepidula plana

BLASTOMERES	MAXIMUM NUCLEAR VOLUMES	COEFFICIENT OF GROWTH	MEAN NUCLEAR VOLUMES	COEFFICIENT OF GROWTH	MINIMUM NUCLEAR VOLUMES	COEFFICIENT OF GROWTH
	cubic μ		*cubic μ*		*cubic μ*	
AB, CD	14,476	1.0	6,110	1.0	381	1.0
A, B, C, D	23,100	1.6	8,580	1.4	602.4	1.58
1A–1D	19,396		7,068		338.4	
1a–1d	5,748		3 620		338.4	
	25,144	1.73	10.688	1.74	676.8	1.77
2A–2D	12,220		7,068		338.4	
2a–2d	7,068		3,620		338.4	
$1a^1$–$1d^1$	3,620		1,528		235.2	
$1a^2$–$1d^2$	720		452		235.2	
	23,628	1.63	12,668	2.07	1147.2	3.01
3A–3D	8,580		5,748		150.0	
3a–3d	5,748		3,620		150.0	
$2a^1$–$2d^1$	5,748		3,620		150.0	
$2a^2$–$2d^2$	5,748		3,620		150.0	
$1a^1$–$1d^1$	3,620		1,528		235.2	
$1a^2$–$1d^2$	720		452		235.2	
	30,164	2.08	18,588	3.04	1070.4	2.80
4A–4D	12,220*		7,068		171.3	
4d	697		382		58.8	
4a–4c	2,715		1,146		112.5	
3a–3d	5,748		3,620		150.0	
$2a^1$–$2d^1$	5,748		3,620		150.0	
$2a^2$–$2d^2$	5,748		3,620		150.0	
$1a^{1.1}$–$1d^{1.1}$	3,620		452		150.0	
$1a^{1.2}$–$1d^{1.2}$	2,095		3,620		150.0	
$1a^2$–$1d^2$	720		452		235.2	
	39,311	2.71	23,980	3.92	1327.8	3.48
growth in thirty divi- s	24,835	2.715	17,870	3.92	946.8	3.48
ge growth for each divi-	827.8	1.05(=5%)	595.6	1.09(=9%)	31.5	1.08(=8%)

This volume is reached only at a much later stage, shortly before the closure
ie blastopore (fig. 6).

uring this same period from the 2-cell to the 32-cell stage the coefficient of
rth of maximum nuclear surfaces is 4.28, or an average increase of about 11
cent for each division.

best determined by comparing subsequent stages with the 2-cell stage. Furthermore the nuclear volume in the 2-cell stage is less than at any other stage, and it consequently forms a good starting point for the study of nuclear growth.

Finally, the volume of all the nuclei in the 70-cell stage, without attempting to determine the maximum volume of each nucleus, is shown in table 10.

At the 70-cell stage the ectomeres are already closing over the yolk on the oral hemisphere, and it may be assumed that the cleavage will show no new tendencies as to the growth of nuclear substance until the embryo as a whole begins to grow.

Whether nuclei are measured at either their maximum size, their minimum size or at a size intermediate between these two extremes, the rate of growth during cleavage is found to fall far short of a doubling or increase of 100 per cent at each division. The average nuclear growth during early cleavage is not more than 5 to 9 per cent for each division, and in the later cleavage it falls as low as 1 per cent for each division. A growth of nuclear substance at this rate scarcely deserves to be designated as 'phenomenal' or 'colossal.' On the other hand, the protoplasm which is generally supposed to remain fixed in quantity during cleavage, increases at a more rapid rate than the nuclei, from the 1-cell to the 24-cell stages, as shown in table 8. In view of the facts here presented, even though it be for only a single species, the generally accepted conclusion as to the great increase of nuclear substance during cleavage, as contrasted with the lack of growth of the protoplasm, evidently needs revision, as do also the theories which have been founded upon this supposed fact.

2. Nuclear growth during the cleavage of the egg of Fulgur. While my results are based largely upon the study of Crepidula plana they are not limited entirely to this species. The following measurements of the nuclei of Fulgur carica are probably not very accurate since they had to be made under a relatively low power objective (8 mm. apochromat) and since the material at my command did not permit the study of a large number of eggs, and the selection of nuclei at maximum size. Never-

TABLE 10

Actual nuclear diameters and volumes in the 70-cell stage of Crepidula plana

BLASTOMERE		NUCLEAR DIAMETER	TOTAL NUCLEAR VOLUME	COEFFICIENT OF GROWTH	
				Nuclear volume	Nuclear surfaces
		μ	μ		
2 cell stage		24	14,476	1	1
11 Entomeres	4A–4D	16	8,579		
	4a–4c	10	1,571		
	E^1, E^2	7	359		
	e^1, e^2	6	226		
4 Mesomeres	M^1, M^2	10	1,047		
	m^1, m^2	9	763		
55 Ectomeres:	*First quartet*				
	4 Apicals, $1a^{1\cdot1}$–$1d^{1\cdot1}$	10	2,094		
	3 Basals, $1a^{1\cdot2\cdot1}$–$1c^{1\cdot2\cdot1}$	9	1,145		
	1 Basal, $1d^{1\cdot2}$	12	905		
	3 Middles, $1a^{1\cdot2\cdot2}$–$1c^{1\cdot2\cdot2}$	12	2,714		
	4 Turrets, $1a^2$–$1d^2$	7	718		
	Second quartet				
	3 Tip cells, $2a^{1\cdot1}$–$2c^{1\cdot1}$	6	339		
	1 Tip cell, $2d^{1\cdot1}$	10	524		
	4 Girdle cells, $2a^{1\cdot2\cdot1}$–$2d^{1\cdot2\cdot1}$	9	1,527		
	4 Girdle cells, $2a^{1\cdot2\cdot2}$–$2d^{1\cdot2\cdot2}$	10	2,094		
	4 Girdle cells, $2a^{2\cdot1\cdot1}$–$2d^{2\cdot1\cdot1}$	9	1,527		
	4 Girdle cells, $2a^{2\cdot1\cdot2}$–$2d^{2\cdot1\cdot2}$	9	1,527		
	4 Girdle cells, $2a^{2\cdot2}$–$2d^{2\cdot2}$	5	262		
	Third quartet				
	4 Girdle cells, $3a^{1\cdot1}$–$3d^{1\cdot1}$	10	2,094		
	4 Girdle cells, $3a^{1\cdot2}$–$3d^{1\cdot2}$	9	1,527		
	4 Girdle cells, $3a^{2\cdot1}$–$3d^{2\cdot1}$	6	452		
	4 Girdle cells, $3a^{2\cdot2}$–$3d^{2\cdot2}$	6	452		
70 cells. Total nuclear volume			32,446	2.24	5.30

The total volume of these 70 nuclei is almost exactly the same as the volume of the germinal vesicle, about 50 per cent more than the volume of the germ nuclei, and 35 per cent more than the mean nuclear volume of the 32-cell stage, with which mean volume, rather than with the maximum, this actual volume of the nuclei of the 70-cell stage should be compared. In the 38 nuclear divisions leading from the 32-cell stage to the 70-cell stage the nuclear material has increased at an average rate of less than 1 per cent for each division.

TABLE 11

Diameters and volumes of the nuclei, 2-cell to 16-cell stages of Fulgur carica

STAGE	BLASTOMERES	DIAMETER OF NUCLEUS	TOTAL VOLUME OF NUCLEI	COEFFICIENT OF NUCLEAR GROWTH
		μ	*cubic* μ	
2 cells	AB, CD	40	67,020	1.0
4 cells	A, B, C, D	32	68,628	1.02
8 cells	1A–1D	32	68,628	
	1a–1d	16	8,576	
			77,204	1.15
12 cells	2A–2D	32	68,628	
	2a–2d	16	8,576	
	1a–1d	16	8,576	
			85,780	1.28
16 cells	3A–3D	32	68,628	
	3a–3d	16	8,576	
	2a–2d	16	8,576	
	1a–1d	16	8,576	
			94,356	1.40

theless they indicate the general rate of nuclear growth in this prosobranch.

In fourteen nuclear divisions there has been an increase in neuclear substance of 40 per cent, or an average increase for each division of 2.8 per cent. The rate of nuclear growth is practically the same in the other species of Crepidula as in C. plana; and in all prosobranchs the nuclear material increases but slightly during the cleavage period.

3. Nuclear growth during the cleavage of other animals. From a casual examination of the segmenting eggs of nematodes, echinoderms, amphioxus and ascidians, as well as from a study of the figures of various authors, it is evident that the nuclear growth in these forms is greater during the early cleavages than in the gastropods. In all of these forms the germinal vesicle is relatively much larger and the egg and sperm nuclei much smaller than in the gastropods, while the decrease in nuclear size in the early

cleavages is not so marked as in the gastropods, though of necessity the nuclei must grow smaller in all animals as cleavage progresses.

In the ascidian, Styela (Cynthia) partita, the maximum nuclear diameters and volumes in the different cell generations are shown in table 12:

TABLE 12

Maximum nuclear diameters and volumes in Styela (Cynthia) partita

STAGE	AVERAGE DIAMETER NUCLEUS	TOTAL VOLUME OF NUCLEI	COEFFICIENTS OF GROWTH			
			Nuclear volume			Nuclear surfaces
	μ	*cubic μ*				
Before first maturation.....	54	82,448	1.0			
Before first cleavage.....	♀12+♂12	1,809	0.02	1.0		
2 cells.....	16*μ*	4,289	0.05	2.37	1.0	1.0
4 cells.....	14	5,748	0.06	3.17	1.34	
8 cells.....	13	9,203	0.11	5.08	2.14	
16 cells.....	11	11,173	0.13	6.17	2.60	
32 cells.....	10	16,755	0.20	9.26	3.90	
64 cells.....	8	17,152	0.20	9.48	4.00	
128 cells.....	6.5	18,406	0.22	10.17	4.29	
256 cells.....	5.25	19,395	0.23	10.72	4.52	13.75

The nuclei of different blastomeres of the same generation vary considerably in size, and I have not attempted to measure each individually, as in the case of Crepidula, nevertheless the measurements given represent approximately the average nuclear diameters for each generation of blastomeres. When the cells become very numerous a very slight error in the measurement makes a big difference in the results, and the total nuclear volume in the later stages may not be very accurate. Nevertheless the table does give a true idea of the order of magnitude of the nuclei in the different generations.

In comparing this table with those for Crepidula it will be seen at once that the germinal vesicle is relatively larger, the germ nuclei smaller and the growth of the nuclear material in the early stages greater in Styela than in Crepidula. The volume of the egg and sperm nuclei represents a loss of 98 per cent as com-

pared with that of the germinal vesicle; and even in the 256-cell stage the volume of all the nuclei is 77 per cent less than that of the germinal vesicle. Comparing the nuclear volumes of subsequent stages with that of the germ nuclei, we find that up to the 32-cell stage there is an increase of 826 per cent, or an average for the first 31 nuclear divisions of 26 per cent for each division; from the 32-cell stage to the 256-cell stage there is an increase of 146 per cent, or an average increase of 0.6 per cent for each division. Since the germinal vesicle is unusually large and the germ nuclei unusually small, a better idea of the rate of nuclear growth in the egg will be obtained by comparing the nuclear volumes of later stages with that of the two cell stage, as was done in the case of Crepidula. Such a comparison is given in the last column of Coefficients in table 13. From this it appears that the nuclear growth from the 2-cell stage to the 32-cell stage is 290 per cent or an average increase for each of 30 divisions of 9.6 per cent; from the 32-cell stage to the 256-cell stage the nuclear volume increases 62 per cent, or an average increase for 224 divisions of 0.27 per cent for each division.

In the cleavage of the eggs of amphioxus and of echinoderms the rate of nuclear growth is essentially similar to that of the ascidians. Here also the germinal vesicle is very large and the total volume of the nuclei at the close of cleavage is much less than the volume of the germinal vesicle, though decidedly greater than the volume of the germ nuclei at the beginning of cleavage. In all of these cases the nuclei in the early cleavages contain little chromatin and much achromatin; while they are more densely chromatic in the later stages, showing that the chromatin has increased in quantity relatively more than the achromatin. This is probably due to the fact that the chromosomes take up less cytoplasmic substance in the smaller cells than in the larger ones, the amount of achromatin in the nucleus depending in large part upon the quantity of cytoplasm in the cell.

4. Growth of different nuclear constituents. a. Nuclear sap. All of the substances within a nucleus do not increase at the same rate. The most abundant constituent of a fully formed nucleus is nuclear sap, and this is scarcely present at all in the earliest stages of the nuclear cycle. During each resting period the nu-

clear sap increases in amount from zero until it forms the principal bulk of the nucleus, and when mitosis comes on it passes into the cell body, and as a constituent of the nucleus sinks again to zero. The substance which forms the nuclear sap is absorbed by the nucleus from the cell body throughout the whole of the resting period, only to be thrown out into the cell body again at the end of that period. Consequently the nuclear sap is no more a nuclear constituent than a protoplasmic one, belonging to both nucleus and protoplasm.[2] Studies on the growth of nuclear material should therefore be confined to the growth of the chromatin, but the difficulty of measuring the amount of chromatin at different stages will be appreciated without further comment. Also the fact that so large a part of the nuclear material belongs also to the protoplasm should be taken into account in experiments dealing with the isolation of nuclei from protoplasm; evidently the only satisfactory way in which such isolation can be accomplished is by isolating chromosomes, rather than resting nuclei.

There is good reason for believing that the nuclear sap contributes to the nourishment and growth of the chromatin and linin, and that it in turn receives substances from these, so that the materials which pass into the cell body when the nuclear membrane dissolves, are not wholly the same as those which were taken up by the nucleus from the cell body. I have elsewhere ('02) called attention to the fact that the escaping nuclear sap stains more deeply than the cell protoplasm and may therefore be called 'chromatic sap.'

As to the mechanism of this intake of protoplasmic substance into the nucleus there is every visible evidence that it is of the nature of osmosis. The nucleus becomes spherical in shape unless subjected to outside pressure, or to the action of substances which cause plasmolysis. The nuclear membrane remains entire and distinct until the last phase of nuclear growth, immediately preceding mitosis, when the nucleus swells very rapidly and the nuclear membrane becomes thin and then disappears.

The measurements given in the preceding section show that the total quantity of the more fluid part of the nucleus, the nuclear

[2] Watase (1893) says, – "The structure known as the nucleus contains a great deal of cytoplasmic substance."

sap, does not increase in quantity during the cleavage of the egg; we have seen that the total volume of all the nuclei of Crepidula at the 70-cell stage is about equal to that of the germinal vesicle, while in Styela the volume of all the nuclei at the 256-cell stage is 77 per cent less than the volume of the germinal vesicle. The conclusion is justified, therefore, that the more fluid constituent of the nucleus decreases greatly in volume during the early cleavage stages, and that the nuclei therefore become denser during this period.

b. Linin. Just as the nuclear sap is proportional in volume to the volume of the nucleus as a whole, so also it is evident that the linin is more abundant in large nuclei than in small ones. Evidently it is not possible to determine the volume of linin in a resting nucleus, but since the spindle fibers are composed largely of linin it is possible by measuring the size of spindles to determine, at least in a general way, the relative quantities of linin in different nuclei. In the following table the length of the spindle from

TABLE 13

Length of spindle in the maturation and cleavage of Crepidula plana

STAGE	LENGTH OF SPINDLE	DIAMETER OF PRECEDING NUCLEUS
	μ	μ
First maturation	42	42
Second maturation	18	—
First cleavage	30	34.5
Second cleavage, AB, CD	30	24
Third cleavage, A, B, C, D	27	22
Fourth cleavage, 1A–1D	25	21
Fourth cleavage 1a–1d	21	12
Fifth cleavage 2A–2D	24	18
Fifth cleavage 2a–2d	21	15

centrosome to centrosome is given for successive cleavages of C. plana, the measurements being made in each case in the stage of the metaphase. The diameter of the nucleus is also given for comparison with the spindle length (table 13).

In general the diameter of the spindle at its equator is, in the prophase and metaphase, about the same as the diameter of the nucleus from which it came. Spindles in the protoplasmic ectomeres are relatively larger than the size of the nucleus would lead

one to expect and this probably is due to the fact, which I ('05) have established in the ascidians, that the polar parts of the spindle are not derived from the nucleus but from the protoplasm. With this proviso, it is true that, within the same species, large nuclei give rise to larger spindles than do small ones and this may be held to indicate that the linin is more abundant in the former than in the latter.

The fact that the spindle fibers of ascidians are composed of equatorial and polar parts, the former derived from the nucleus and the latter from the protoplasm, and the fact that these two portions of the spindle, and also the polar fibers, are fundamentally alike, indicates that the linin, like the nuclear sap, is a constituent which belongs both to the nucleus and to the protoplasm.

c. Chromatin. The amount of chromatin undoubtedly increases during the cleavage; the resting nuclei in the later stages being more densely chromatic than those of the earlier stages. In each cell the chromatin is smallest in quantity when the daughter chromosomes are first separated, and it grows in quantity during the resting period. Not all of the chromatin of the resting stage goes into the formation of the chromosomes of the next mitosis, but some of it in the form of granules (oxychromatin) or chromatic sap escapes into the cell body on the dissolution of the nuclear membrane. The larger the nucleus is and the longer the resting period through which it has come, the greater the quantity of chromatin which thus escapes at mitosis. Gardiner ('98) estimated that the amount of chromatin which thus escaped into the cell body at the first maturation division of Polychaerus was five hundred times as great as that which went to form chromosomes, and conditions are similar in Styela, Crepidula, and many other forms. Consequently the volume of the chromosomes in successive stages cannot be used as a measure of the growth of the chromatin. Nevertheless the growth of the chromosomal mass, as well as the growth of the entire nuclear volume, will give some idea as to the growth of the chromatin during cleavage. Table 9, giving as it does the volumes of the nuclei and chromosomal plates at various stages, furnishes data upon which an opinion as to the growth of the chromatin of the resting stages

may be based. From the 2-cell to the 32-cell stages the growth in volume of the resting nuclei lies between 171 per cent for maximum nuclear size, and 292 per cent for mean nuclear size while the growth of the chromosomal plates is 248 per cent. It seems very probable therefore that the growth of the chromatin during these stages lies somewhere between 171 per cent and 292 per cent, or an average increase for each of the 30 divisions represented of from 5.7 per cent to 9.7 per cent. In all cases the growth of the chromatin falls far short of 100 per cent, or a doubling, in each division cycle. In Strongylocentrotus, Erdmann ('08) finds that the ratio of chromatin to plasma is seven times greater in the pluteus than at the beginning of development, and she points out that this means that plasma contributes to the growth of the chromatin.

While the chromatin as such is peculiar to the nucleus, there can be no doubt that large quantities of chromatin escape into the protoplasm. Such chromatin usually loses its distinctive staining reaction and presumably suffers chemical change. On the other hand we know that chromatin grows at the expense of substances received from the protoplasm. The work of Masing ('10) on the nucleinic acid content of the egg indicates that this important constituent of chromatin is about as abundant in early stages as in later ones; he supposes that it exists in the protoplasm.

d. Chromosomes. What is true of the quantitative relations of the chromatin as a whole is true also of the individual chromosomes; those formed from large nuclei are larger than those from small ones; the chromosomes do not double in volume in each successive cleavage, but they become individually smaller as cleavage progresses. These facts are not difficult to demonstrate, but they are difficult to express in any numerical proportion, owing to the irregular shape and small size of the chromosomes, which make it very difficult to determine their volume.

In Crepidula the chromosomes are very small and numerous, the full number being probably 60, and they are usually crowded together so that it is difficult to photograph them, or even to draw their outlines accurately, and since they are so small it is

not practicable to measure them directly with the 1/1 micrometer eyepiece. Nevertheless by selecting sections in which only a part of the chromosomes are shown I have been able to sketch the outlines of many of them with what I believe to be substantial accuracy. For the purpose of comparing the sizes of chromosomes from different cleavages I have chosen two generations of blastomeres in which the difference in the size of the nuclei is at a maximum, the nucleus in one cell being about twice the diameter of that in the other; these blastomeres are the macromeres *AB* and *CD*, and the micromeres *1a–1d* (figs. 7 and 8). In the former the diameter of the nucleus just before division is about 24μ, in the latter about 14μ. When the nuclei of the cells in question had begun to divide and the mitotic figures were in the equatorial plate stage, the chromosomes from a number of these spindles were drawn as accurately as possible with a camera lucida. In order to be certain that the stage of division was the same in each case only longitudinal sections through the spindle were chosen; and in order to avoid as far as possible individual differences in the sizes of chromosomes, only the largest and most isolated chromosomes were drawn. Fig. 9 shows chromosomes from four different spindles of the second cleavage; fig 10 shows chromosomes from the first division of the first quartet cells (*1a–1d*), also from four different spindles. In all cases the chromosomes are magnified 2000 diameters.

It is plain from these figures that the chromosomes from the larger nuclei are larger than those from the smaller ones, though the difference in the diameters and volumes of the chromosomes are not as great as the difference in the volumes of the nuclei from which they came. The average volume of the chromosomes from the large nuclei is about 5.2 cubic μ and of those from the small nuclei about 2.6 cubic μ. While the volumes of the nuclei as a whole are to each other about as 5 : 1, the volumes of their individual chromosomes are to each other as 2 : 1. In the case of nuclei which differ but slightly in volume it is not possible to be certain that the chromosomes differ in size, but in all cases in which the differences in the size of nuclei is considerable it can

be seen that the larger nuclei give rise to larger chromosomes than do the smaller ones.

Since the probable error is much greater in the measurement of individual chromosomes than of whole chromosomal plates, I have not attempted to measure individual chromosomes in each stage of the cleavage; on the other hand the dimensions of the chromosomal plates are given in table 4 for each cell up to the 32-cell stage. These measurements show that from the 2-cell to the 32-cell stage the chromosomal mass increases in volume 248 per cent or an average of 8 per cent for each of 30 divisions. The chromosomal plates, and consequently the individual chromosomes, grow smaller as cleavage advances, but in the same generation of cells small nuclei have smaller chromosomes than large ones. In short, the size of the chromosome is dependent upon the size of the nucleus from which it comes, rather than upon the cell generation to which it belongs.

In the main these observations are in harmony with those of Erdmann, and Baltzer, to which reference has already been made. In Crepidula, as in the echinids studied by the authors named, the individual chromosomes grow smaller as the cleavage advances, but this is causally related to the decrease in the size of the nuclei and of the cells, and where, in later cleavage stages, the nuclei and cells remain large, there the chromosomes also are larger than in smaller sister cells. Just as the size of the nucleus is connected with the volume of the cytoplasm in which it lies, so the size of the chromosomes is connected with the volume of the nucleus from which they come.

Montgomery ('10) has found that the sperm cells of Euschistus are of two sizes and he concludes (p. 127), that "it is probable that the large sperm possess no more chromatin than the small, though the heads in the former are much larger. The dimegaly expresses itself accordingly in differences of amount of karyolymph and of the substance (linin) that composes the mantle fibers, but much more markedly in the amount of cytoplasm." He finds also that the mitochondria (idiozome) increase directly with the amount of cytoplasm. According to my observations chromosomes from large nuclei are larger than those from small ones of

the same generation, though naturally it is more difficult to detect size differences in objects as small as chromosomes than in entire nuclei. Where the differences in nuclear volumes are great one can always detect corresponding differences in chromosome volumes.

The chromosomes of the spermatid are usually smaller than those of the oötid, but when the chromosomes of the first cleavage spindle appear, those from the sperm nucleus are usually as large as those from the egg. The reason for this is to be found in the fact that both grow, after fertilization, in the same medium, the egg plasma, and for approximately the same length of time.

e. Plasmasomes. The conclusion that large nuclei have large chromosomes, and *vice versa*, also applies to the sizes of nucleoli (plasmasomes); they are larger in large nuclei than in small ones. However in this case another factor is involved for the size of nucleoli is not only dependent upon the size of the nucleus, but also upon the length of the resting period; indeed the latter seems to be the more important factor of the two. The largest of all nucleoli is the one found in the germinal vesicle, at the close of the longest resting period in the entire life cycle. In these gasteropod eggs the next largest nucleoli are found in the cells *4A–4D* and *4a–4c* (fig. 6) in which the resting stage is particularly long. The nuclei of the cells *4A–4D* are of the same size as those of *2A–2D* viz. 18μ in diameter, but the nucleoli of the former have about three times the diameter of those of the latter.

In earlier stages of cleavage where the blastomeres are dividing rapidly it is difficult to compare the sizes of nucleoli, not only because their number varies considerably, but also because each plasmasome is usually surrounded by a layer of chromatin granules which renders exact measurements difficult. The number of plasmasomes appears to depend to a large extent upon the degree of fusion of an originally large number of separate plasmasomes. When chromosomes are isolated so that each gives rise to a distinct vesicle, each may contain a minute plasmasome, and there may be as many of these as there are chromosomal vesicles. In Crepidula the number is always greatest during the earlier stages of the resting period; during the later stages they appear to fuse

together becoming fewer and larger as the individual chromosomal vesicles fuse. For a considerable period two nucleoli are commonly found in each nucleus, one in each gonomere, or nuclear half. However, when the resting stage is long, these two fuse into a single large plasmasome.

While the nucleus continues to grow in size up to the time of the dissolution of the nuclear membrane, the plasmasome usually disappears before the formation of the spireme. In comparing the relative sizes of nucleoli it is important to compare corresponding stages; accordingly in my measurements they were measured when they had reached approximately their maximum size, and before the nucleus had reached its maximum. Nucleoli differ more or less in size even in different cells of the same generation, owing perhaps to the more or less complete fusion of the many original nucleoli; it is significant in this connection that after a long resting period they are much more uniform in size and constant in number than when the resting period is short. The following table gives the diameters of nuclei and nucleoli (plasmasomes) in various blastomeres of Crepidula:

TABLE 14

Maximum nucleolar size and nuclear size in the blastomeres of Crepidula plana

STAGE BLASTOMERES	DIAMETER OF NUCLEUS	VOLUME OF NUCLEUS	NUMBER OF NUCLEOLI	DIAMETER OF NUCLEOLI	VOLUME OF NUCLEOLI	NUCLEAR-NUCLEOLAR RATIO
	μ	*cubic* μ		μ	*cubic* μ	
1 cell, before maturation	42	32,409	1	12	905.0	35 : 1
2 cells, AB, CD	20	4,189	2	3	28.0	149 : 1
4 cells, A, B, C, D	15	1,767	2	$2\frac{1}{4}$, $1\frac{1}{2}$	7.7	220 : 1
8 cells 1A–1D	19	3,591	2	3	28.0	128 : 1
8 cells 1a–1d	13	1,150	2	2, $1\frac{1}{2}$	7.0	164 : 1
12 cells 2A–2D	14	1,437	2	2	8.3	180 : 1
12 cells 2a–2d	15	1,767	2	2	8.3	220 : 1
16 cells $1a^1$–$1d^1$	12	905	2	3, 2	18.3	50 : 1
16 cells $1a^2$–$1d^2$	7	180	2	1	1.0	180 : 1
20 cells 3A–3D	15	1,767	1	$7\frac{1}{2}$	221.0	8 : 1
20 cells 3a–3d	15	1,767	2	3	28.0	63 : 1
25 cells 4d	9	382	1	3	14.0	27 : 1
32 cells, 4a–4c	13	905	1	6	113.0	8 : 1
Ca. 100 cells, 4A–4D	15	1,767	1	9	382.0	4.6 : 1
Fulgur carica:						
Ca. 1000 cells, 4A–4D	96	462,192	1	27	10306.0	44 : 1

In eggs in which the nuclear division has been greatly delayed, if not entirely stopped, by the use of hypertonic salt solutions the nucleoli become much larger than in normal eggs. Thus in the eggs of Crepidula plana treated with 4 per cent NaCl solution for two hours, and then put into normal sea water for six hours, the sizes of nuclei and nucleoli are as follows:

TABLE 15

Nucleolar size and nuclear size in eggs of Crepidula plana in hypertonic sea water

STAGE BLASTOMERES	DIAMETER OF NUCLEUS	VOLUME OF NUCLEUS	NUMBER OF NUCLEOLI	DIAMETER OF NUCLEOLI	VOLUME OF NUCLEOLI	NUCLEAR-NUCLEOLAR RATIO
	μ	*cubic* μ		μ	*cubic* μ	
1 cell, pronuclei..... ♀	24	7,238	1	12	905	8 : 1
1 cell, pronuclei..... ♂	21	4,849	1	10	524	9 : 1
2 cells, AB, CD.....	24	7,238	1	9	382	19 : 1
4 cells, A, B, C, D.....	15	1,767	1	9	382	4.6 : 1
8 cells, 1A–1D.....	18	3,055	1	9	382	8 : 1

The great size of the single nucleolus in each of these nuclei is probably due to the fact that division has been delayed and the resting period prolonged.

f. Centrosomes and spheres. Finally we may consider in this connection the sizes of centrosomes, and spheres though they are not parts of the nucleus. In general in Crepidula, large cells contain large centrosomes and spheres, while small cells contain small ones. The maximum diameters of centrosomes in the cleavage of C. plana, vary from 2μ to 7μ, the measurements being made during the telophase of division. The maximum diameters of the sharply defined spheres, during the resting stages, vary from 5μ to 12μ; and in all cases, so far as I have observed, the largest centrosomes and spheres occur in the cells which have the largest amount of protoplasm, while the smallest occur in the cells with the least amount of protoplasm.

The centrosomes and spheres are the cell constituents which first become unequal in an unequal cell division. As soon as the spindle becomes eccentric, the centrosome and sphere which lies farthest from the center of the cell becomes smaller than the one

at the opposite pole. Only after the division wall forms do the daughter nuclei become unequal.

5. Conclusions as to nuclear growth during cleavage. The rate and amount of nuclear growth during cleavage is much less than is generally believed. Whether the nuclear volume is taken when the nuclei are at their maximum, mean, or minimum size, the nuclear growth is far from 100 per cent, or a doubling, in each division. In Crepidula the nuclear growth is not more than 5 per cent to 9 per cent for each division from the 2-cell to the 32-cell stage, and less than 1 per cent for each division after the 32-cell stage. At the 2-cell stage the nuclear volume is least and up to the 32-cell stage the chromatin increases at an average rate of about 8 per cent for each division. The stage when the volume of protoplasm is least, after the egg has reached its full size, is just before the first maturation division; between the first maturation and the 24-cell stage the protoplasm increases at an average rate of nearly 6 per cent for each division. At the end of cleavage the ratio of nuclear material to protoplasmic differs but little from the ratio at the beginning. In Fulgur the nuclear growth from the 2-cell stage to the 16-cell stage averages only 2.8 per cent for each division, and the general Kernplasma-Relation remains unchanged. In Styela the nuclear growth from the 2-cell to the 32-cell stage averages 9.6 per cent for each division; from the 32-cell stage to the 256-cell stage it averages only 0.27 per cent for each division. Such a rate of growth is not significant and indicates that the meaning of cleavage is to be found in something other than the increase of nuclear material as compared with the plasma.

In general the growth of each of the different nuclear constituents parallels the growth of the nuclear material as a whole, though this is not true of the nuclear sap, which belongs to both cytoplasm and nucleus. During cleavage the fluid content of the egg as a whole decreases, the oöplasm becoming more consistent in later stages than in earlier ones. The total fluid content of the nuclei in the early cleavage stages is much less than that of the germinal vesicle; even in the later cleavages the nuclear sap is not so abundant, in some animals, as in the germinal vesicle. In Crepidula the volume of all the nuclei at the 70-cell stage is

only equal to that of the germinal vesicle, though the volume of the chromosomal plates has increased 250 per cent; in Styela the volume of all the nuclei of the 256-cell stage is 77 per cent less than that of the germinal vesicle, though the total chromosomal volume has increased many fold during this period.

Linin is a nuclear constituent which is found also in the protoplasm, and during cleavage it grows in quantity at about the same rate as the nuclear and protoplasmic materials as a whole. The polar parts of the spindle and the astral rays arise in the protoplasm outside the nucleus, while the equatorial portion of the spindle comes from the nucleus, as is shown with great clearness in the cleavage mitoses of ascidians. Correspondingly the size of the spindle is a resultant of the volume of the nucleus and of the protoplasm.

Chromatin is more distinctively a nuclear substance than the nuclear sap or linin, though it undoubtedly grows at the expense of substance received from the protoplasm and in turn contributes material to the protoplasm. From the 2-cell to the 32-cell stage in Crepidula the growth, of the chromatin amounts to between 6 per cent and 10 per cent for each division, and as the fluid contents of the nuclei do not increase during cleavage the nuclei become more chromatic in later stages than in earlier ones.

Chromosomal material, as represented in the condensed chromosomal plates of the anaphase, increases in volume 248 per cent from the 2-cell to the 32-cell stages of Crepidula, or an average growth of about 8 per cent for each division. Individual chromosomes grow smaller as cleavage advances, but this is due to the smaller size of the nuclei from which they come rather than to the cell generation to which they belong; nuclei of the same generation which differ greatly in size produce chromosomes which differ in size, the larger nucleus producing larger chromosomes than the smaller one.

In the blastomeres of Crepidula the size and number of nucleoli (plasmasomes) are influenced by the size of the nucleus and the length of the resting period. In most of the nuclei there are two nucleoli, but when the resting period is long, these fuse into a single one. In experiments, anything which prolongs the resting

period leads to an increase in the size of the nucleoli. During the normal cleavage of Crepidula the ratio of the nuclear volume to the nucleolar volume varies from 220 : 1 to 4.6 : 1.

Centrosomes and spheres are proportional in size to the volume of the protoplasm in which they lie; they are always larger in large cells than in small ones and hence they grow progressively smaller as cleavage advances.

In general the volume of each of the nuclear constituents named is influenced by the volume of protoplasm of the cell, and by the length of the resting period. The protoplasm contributes substances to the growth of each of these constituents, and the more abundant it is the larger they grow, provided the period of growth is the same in all cases. Where the growth peroid (interkinesis) is very long the nuclei becomes unusually large and may ultimately absorb the greater part of the protoplasm.

6. Comparison of growth of chromatin with increase of chemical substances and processes during cleavage. Loeb in several important papers has shown that the nucleus is the oxidizing center of the cell, and that the chromatin is chiefly concerned in bringing about oxidations. Warburg ('08) found the oxidative power of the egg to increase at a relatively slow rate during cleavage. More recently, in view of the oft-repeated assertion that the chromatin doubles at each division, Loeb ('09) concluded that the supposed growth of chromatin in geometric ratio indicates that nuclear synthesis is of the nature of an autokatalytic reaction. Masing ('10) has shown that in the eggs of Arbacea pustulosa the nucleinic acid in the fertilized but unsegmented egg is as great as in the 'morula' with 500 to 1000 cells. He concludes that, "the colossal increase of nuclear mass in the cleavage leads to no perceptible increase of nucleinic acid in the germ. A corollary of this must be that the total quantity of nucleinic acid necessary to build up the nuclear apparatus of the germ must be preformed in the protoplasm" (quoted from Godlewski, '11). Shackell ('11) has reached a similar conclusion with regard to the nuclein content of the egg and blastula of Arbacea punctulata.

The results of my observations as to the rate of the growth of chromatin is especially significant when compared with the work

of Warburg. I find that the chromosomal mass grows at the rate of 8 per cent for each division up to the 32-cell stage. It is difficult to connect this rate of growth of the chromosomes with the lack of growth in the nucleinic acid content as shown by Masing, or with the lack of growth of the nuclein content as shown by Shackell, and it seems necessary to assume as both of these investigators have done, that these substances are already preformed in the protoplasm. If this be true, I venture the suggestion that the large amount of chromatin (oxychromatin) which escapes into the cell body when the germinal vesicle dissolves may constitute the nuclein and nucleinic acid which is distributed through the cell body.

VII. Senescence, rejuvenescence, and the ratio of nucleus to plasma.

It is well known that Minot ('90, '95, '08) maintains that the cause of senescence is the increase of plasma and its products at a rate greater than that of the nucleus. According to his view the egg at the beginning of development is in a senile condition, "in which there is an excessive amount of protoplasm in proportion to the nucleus, and in order to get anything which is young a process of rejuvenation is necessary . . . . During the segmentation of the ovum the condition of things has been reversed so far as the proportions of nucleus and protoplasm are concerned. We have nucleus produced, so to speak, to excess. The nuclear substance is increased during the first phase of development. Hence our conclusion:—Rejuvenation is accomplished chiefly by the segmentation of the ovum." He sums up his views on this subject in his four laws of age ('08, p. 250), the first two of which are: 1. "Rejuvenation depends on the increase of the nuclei. 2. Senescence depends on the increase of the protoplasm, and on the differentiation of the cells."

Richard Hertwig's views ('89, '03, '08) are apparently diametrically opposed to those of Minot, though I do not find them so definitely expressed. He finds that senescence, or rather 'depression' and 'physiological degeneration,' are accompanied by an enormous growth of the nucleus. As a result of his work on

Actinosphaerium and Infusoria, which had been overfed for a long time, he found that there was an enormous growth of the nucleus followed by physiological degeneration. The animals which saved themselves from this condition did it by the reduction of their nuclei, either by eliminating nuclear substance directly, or by the loss of the greater part of the nuclear material during conjugation, after which normal nuclear conditions were restored. He regards the immature egg cell, with its great nucleus, as in a condition of depression similar to that found in the protozoa named. By the processes of maturation and fertilization this nuclear material is greatly reduced: "Beim Beginn der Furchung und auch später ein enormes Missverhältniss von Kern und Protoplasma vorhanden ist, und dieses Missverhältniss allmählich eine Ausgleich erfährt, indem Zellsubstanz in Kernsubstanz umgewandelt wird," ('03, p. 116). Apparently then, in Hertwig's view, senescence or depression, is accompanied by too great an amount of nuclear material, which is then reduced, by maturation in the case of the egg cell, to such an extent that this enormous disproportion of nucleus to protoplasm appears; later, by means of the process of cleavage, during which the nuclear material grows at the expense of the protoplasm, the normal relations of nucleus to protoplasm are restored.

Popoff ('08) accepts Hertwig's view in all essential respects. He adds the interesting suggestion that in their period of depression preceding maturation the sex cells are so weakened that they are unable to assimilate nutriment, and they consequently store up food as yolk. The formation of yolk, glycogen and fat are, according to this author, not indications of increased activity of cells, but of incapacity to carry the organic synthesis to its end, viz., the formation of plasma.

While Minot's hypothesis differs fundamentally from Hertwig's as to the cause of senescence, the former holding that it depends upon the increase of protoplasm over nucleus, the latter that it is accompanied by an increase of nucleus over protoplasm, both agree that in the segmentation of the egg there is an enormous growth of the nuclear material as compared with the protoplasm.

Neither Minot nor Hertwig took account of the fact that a large part of the nuclear contents belongs to both nucleus and protoplasm. The 'Kernplasma-Relation' depends very largely upon the quantity of protoplasmic material temporarily in the nucleus; in the 4-cell stage of Crepidula the ratio of nuclear volume to protoplasmic volume is 1 : 6.6 when the nuclei are measured at their maximum size, but 1 : 203.8 when they are measured at their minimum size. Neither of the authors named, in describing the enormous growth of the nuclear material during cleavage, took account of the growth of the protoplasm during cleavage at the expense of the yolk.

My observations on Crepidula have yielded the following results, which bear upon the hypothesis under discussion: (1) While the germinal vesicle is absolutely the largest nucleus in the early stages of development, it is not so large with reference to the protoplasm, and hence according to Hertwig, not in so deep a depression, as the nuclei of certain blastomeres, which *ex hypothese* should be undergoing restoration to normal conditions. (2) The growth of nuclear material during cleavage is not nearly so great as has been assumed, averaging not more than 10 per cent for each division up to the 32-cell stage, and not more than 1 per cent for each division after that stage. (3) The growth of protoplasm at the expense of yolk during maturation and early cleavage is considerable, averaging about 6 per cent for each division up to the 24-cell stage. (4) The 'Kernplasma Relation,' while constant for specific blastomeres, is by no means uniform for all the blastomeres of a given stage, but may vary from 1 : 1 to 1 : 14 in different blastomeres of the same generation. (5) The 'Kernplasma-Relation' in adult epithelial cells of all three germ layers is about the same as in the majority of the blastomeres. (6) The absolute size of the nucleus depends upon the quantity of protoplasm in the cell and the length of the resting period (interkinesis). (7) The greater part of the nuclear volume consists of material which belongs to the protoplasm as much as to the nucleus; during the resting period this is taken in osmotically through the nuclear membrane, and is given out again at mitosis by the dissolution of that membrane. (8) The immature egg cell, which

according to Popoff is so weakened that it is unable to assimilate nutriment, and consequently can only store up food instead of making protoplasm, does as a matter of fact form protoplasm throughout the whole of the growth period.

So far as they go, therefore, these results do not support the view that senescence is due to either an increase or to a decrease of nuclear volume as compared with that of the protoplasm. But I think that this conflict between my results and those of Minot and Hertwig is, after all, confined to details, and that in the fundamental conception of the causes of senescence and rejuvenescence they may be brought into harmony. With the general thesis that senescence is associated with the accumulation in the cell of the products of metabolism and differentiation, and that rejuvenation consists in a return to a condition in which these products are largely eliminated, as Minot and Hertwig have urged, I am in hearty agreement; their assumption that changes in the nucleus-plasma ratio are the causes of these phenomena seems to me to be merely an error of detail.

In a very suggestive paper, Child ('11) has recently maintained that senescence and rejuvenescence are caused by a decrease or an increase in the fundamental metabolic reactions. Anything which decreases the rate of metabolism, such as "decrease in permeability, increase in density, accumulation of relatively inactive substances, etc.," will lead to senescence. "Rejuvenescence consists physiologically in an increase in the rate of metabolism and is brought about in nature by the removal in one way or another of the structural obstacles to metabolism" (p. 609).

This hypothesis finds much support in the phenomena connected with the early development of the egg. It is well known that construct ve metabolism takes place only in the presence of nuclear material, and it has long been known that the nuclei of various kinds of gland cells give off substances which play an important part in the metabolism of the cell. Loeb ('99) has shown that the nucleus is the oxidative center of the cell; Mathews identifies oxidase with chromatin; R. Lillie ('02) finds that oxidation takes place most rapidly in the immediate vicinity of the nucleus. If the rate of metabolism is associated with sen-

escence or rejuvenescence, as Child maintains, anything which facilitates the nterchange between nucleus and protoplasm should lead to rejuvenescence, anything which decreases it should lead to senescence.

During cleavage the increase in nuclear surfaces is much greater than the increase in nuclear volumes. While the increase in maximum nuclear volumes up to the 32-cell stage of Crepidula is about 5 per cent for each division, the growth in the maximum nuclear surfaces during this period is about 11 per cent for each division. From the 2-cell to the 70-cell stage the nuclear volume increases only 2.24 times, while the nuclear surfaces increase 5.30 times. In Styela the nuclear volume increases from the 2-cell stage to the 256-cell stage only 4.52 times, the nuclear surfaces increase 13.75 times. Unquestionably this greater growth of nuclear surfaces as compared with nuclear volumes, facilitates the interchange between nucleus and protoplasm. There is also a considerable increase of cell membranes during cleavage, but most of this increase is confined to surfaces of contact between cells, and free surfaces show but little growth. My observations teach that there is little, if any, interchange of materials through partition walls separating cells.

Another and much more efficient means of facilitating the interchange between nucleus and protoplasm is found in the mitotic division of the nucleus. During the cycle from one division to the next the nucleus absorbs materials from the cell body, only to throw back into the cell body these and other materials when the nuclear membrane dissolves in mitosis. The chromatin is thus brought into the most intimate relations with the protoplasm. There is thus a sort of "diastole and systole of the nucleus" (Conklin, '02), by which the interchange between nucleus and protoplasm is greatly hastened. Indeed in the paper just referred to I suggested that this function of mitosis may be quite as important as the division and separation of the chromosomes, which is usually supposed to be the one function of mitosis.

The hypothesis that the more rapid interchange between nucleus and protoplasm is associated with increased metabolism is supported by some very significant physiological work on the

maturation, fertilization and cleavage of the egg. Loeb first showed that the immature egg, with germinal vesicle intact, is metabolically inactive; it absorbs but little oxygen and gives off little carbon dioxide. On the other hand when the membrane of the germinal vesicle dissolves, metabolic activity increases, and unless the egg is started in the process of development, by fertilization or other means, it soon dies. Lyon ('04) found that during the cleavage of the sea urchin egg the evolution of carbon dioxide is more rapid during the periods of division than during those of rest.[3] Warburg ('08) found that the fertilized sea-urchin egg uses six to seven times as much oxygen as the unfertilized egg. It is well known that the condensed chromatin of the chromosomes is brought into intimate relation with the protoplasm during mitosis, and of course the same is true of the condensed chromatin of the sperm head following fertilization. We may conclude, I think, that mitosis increases metabolism by facilitating the interchange between nucleus and protoplasm, and particularly by setting free chromatin in the protoplasm, either by the dissolution of the nuclear membrane, or by the introduction of the sperm head in fetilization.

Rapid and intimate interchange between the chromatin and the protoplasm is the condition of rapid metabolism, and *ex hypothese* of rejuvenescence; slow interchange is the condition of slow metabolism, and of senescence. Such a view has many points in common with the hypotheses of Minot and Hertwig, while it avoids many of the serious difficulties which those hypotheses encounter. It is thus evident that one may hold, with Minot and Hertwig, that the germ cells before maturation are senescent, and that maturation, fertilization and cleavage represent a rejuvenescence, without necessarily connecting these processes with the nucleus-plasma ratio.

[3] R. Lillie (1910) holds that this is due to increased permeability of the plasma membrane during division.

PART II

EXPERIMENTAL STUDY OF CELL SIZE AND NUCLEAR SIZE IN THE EGGS OF CREPIDULA PLANA

I. Nuclear size and chromosome number

In Crepidula the relation of nuclear size to chromosome number is the same as in the Echinid larvae studied by Boveri ('05). By the use of various hypertonic salt solutions abnormal mitoses may be produced in Crepidula eggs; one of the most common of these abnormalities consists in the scattering of the chromosomes, so that they do not fuse together to form two daughter nuclei, one in each cell, but many small nuclei. Indeed there may be almost as many small nuclei as there are chromosomes, every isolated chromosome being capable of producing a small nuclear vesicle. In all such cases the nuclear vesicles formed from a small number of chromosomes always remain smaller than those formed from a larger number. In any given species the size of the nucleus is proportional to the number of chromosomes which go into its formation, providing the other factors which control nuclear size, viz., quantity of cytoplasm and length of resting period, are the same. On the other hand the size of the cell body is not dependent upon the size of the nucleus in the early cleavages of Crepidula, as Gerassimoff ('02) found to be the case in Spirogyra and as Boveri determined in the case of Echinid larvae, but the reverse is true.

In the eggs of Crepidula which have been treated with salt solutions the cell body frequently does not divide at all and many nuclei may be left in a single cell; where the cell itself divides there is a tendency for the blastomeres to divide in normal fashion, giving rise to macromeres or micromeres as in the normal egg, even though polyasters and abnormal mitoses are present. Consequently these eggs afford no evidence that the size of the nucleus has an influence on the size of the cell body.

II. Nuclear size and cell size in centrifuged eggs of Crepidula

While the size relations of cells and of their various constituents may be readily observed in normal eggs, it is especially in eggs which have been centrifuged at various stages of development that the factors which determine these various size relations can be most satisfactorily studied. The various constituents of a cell may be moved by centrifugal force to one pole or another, according to their specific weights, and the axis of centrifuging. In this way the yolk, the cytoplasm, the nuclei and the centrosomes, may be caused to take very abnormal positions in the cell. Even the mitotic figure may be moved out of its ordinary position in the earliest stages of its formation, but after it has reached the metaphase it can be moved only with great difficulty; from this stage on it is anchored, probably to the cell membrane by the astral radiations, while the other constituents of the cell are free to move under the influence of centrifugal pressure. In this way it happens that the cytoplasm may be centrifuged away from the spindle and the latter left in a dense mass of yolk; or the normal relations of cytoplasm and yolk to the poles of the spindle may be completely changed; or the normal size relations of the daughter cells may be quite reversed. As illustrating these changed relations, due to centrifuging, a few eggs are shown in figs. 11–37, selected from a great number which are similar to these.

These eggs were centrifuged on a centrifugal machine run by water pressure, at the rate of 2000 revolutions per minute; the radius of rotation was 6 cm., consequently the centrifugal pressure was nearly 270 times that of gravity. Eggs were centrifuged at this rate for varying lengths of time, after which they were removed from the machine and either fixed at once, or left for a longer or shorter time in sea water before fixation. All eggs were fixed in Kleinenberg picro-sulphuric mixture, were preserved in 70 per cent alcohol only long enough to wash out the fixing fluid, and were then stained in my modification of Delafield's haematoxylin and mounted entire in balsam, in the manner described in previous papers (Conklin, '02 *et seq.*)

In fig. 11 an egg is shown which was centrifuged for ten minutes after the formation of the first polar body and before the formation of the second, the axis of centrifuging being such that the lighter protoplasm was thrown to the vegetative pole and the heavier yolk to the animal pole, thus reversing the normal positions of these substances. After centrifuging, the egg was left in sea water for three hours before being fixed. The first polar body, which has partially divided, lies at the animal pole; the second maturation spindle has been greatly elongated and its axis has been turned somewhat, its lower pole having been moved to the right in the figure. The egg has begun to constrict opposite the equator of the spindle, thus leading to the formation of a giant second polar body. The nucleus of this second polar body consists only of a compact mass of chromosomes surrounded by yolk; the sphere connecting these chromosomes with the egg membrane is much elongated. The egg nucleus and sphere at the lower pole of the spindle are in contact with the field of cytoplasm and are much larger than those at the upper pole. The sperm nucleus and sphere, lying in the cytoplasmic field, are much the largest in the egg. In normal condition these relations are reversed, the sperm nucleus lying in the yolk, while the egg nucleus is in the cytoplasmic field; and in such cases the egg nucleus and sphere are larger than those of the sperm; however as the sperm nucleus approaches the egg nucleus and thus moves up into the cytoplasm it continually grows larger until, at the time the two meet, the sperm nucleus is almost as large as the egg nucleus. The fact that the normal size relations of these two nuclei may be reversed by reversing the positions of the cytoplasm and yolk, furnishes conclusive evidence of the fact that the relative sizes of the egg and sperm nuclei and asters are dependent upon the quantity of cytoplasm in which they lie.

Furthermore fig. 11 shows that the spindle itself is a structure composed of fibers more firm than the surrounding substance, and is not merely an arrangement of the granules, which happen to be present in a field of force, into lines, like iron filings in a magnetic field. The spindle remains fixed in position when all surrounding substances change position, and the spindle fibers,

though much elongated preserve their usual appearance. In this regard my work confirms the conclusions of Morgan ('10) as to the nature of the spindle in Cerebratulus, and is at variance with the work of Lillie ('09) on Chaetopterus.

In fig. 12 an egg is shown which was centrifuged for fifteen minutes during the first cleavage and was then left for three hours in sea water. The axis of centrifuging is indicated here, as elsewhere, by the lighter vacuolated substance at one pole and the heavier yolk at the opposite pole; this axis is also marked by an arrow, the head of the arrow marking the distal pole during centrifuging, the tail of the arrow the central pole. In figs. 12 to 15 the first cleavage plane does not pass through the animal pole, which is marked by the polar bodies, but is displaced to one side, and the cleavage is not meridional, as in normal eggs; furthermore the cleavage is not equal, quantitatively and qualitatively, as in normal eggs, but is markedly unequal, most of the cytoplasm having gone into the smaller one of the two daughter cells, while the larger one contains little cytoplasm and much yolk. This is evidently due to the fact that the greater mass of yolk in the larger cell has displaced the cleavage plane to one side of its normal position.

Corresponding to this difference in the quantity of cytoplasm in the first two blastomeres of these eggs, there is a decided difference in the size of the nuclei and spheres, the latter always being proportional in size to the quantity of cytoplasm in which they lie. The smaller cells with the larger quantity of cytoplasm thus have larger nuclei and spheres than the larger cells, which have a smaller quantity of cytoplasm.

The eggs represented in figs. 13, 14, and 15 were centrifuged for five hours during the first cleavage and were then fixed at once. It is evident that division took place while the eggs were on the centrifugal machine and that the daughter nuclei have grown to the size shown while the eggs were still being centrifuged. Other eggs centrifuged for the same length of time were allowed to develop further after being removed from the centrifuge, and they show that in most cases the eggs were still alive after centrifuging and not seriously injured. Fig. 13 shows a very note-

worthy fact to which attention will be devoted in a future paper, viz., that the cell axis, which is marked by the line passing through the nucleus and sphere (and centrosome), remains unchanged after centrifuging. In the stage shown in fig. 13, the spheres lie between the nuclei and the polar bodies in normal eggs, and although the positions of cytoplasm and yolk, and of the first cleavage plane have been changed in this egg, this cell polarity remains unchanged.

Fig. 16 represents an egg which was centrifuged thirty minutes and then left in sea water for twenty hours. Neither this egg nor any others of this lot developed far after being centrifuged; it is possible that the eggs were injured in some way so that none of them developed, or it is barely possible that the record of the experiment is wrong. In all the eggs of this lot the appearance is that of eggs which had been under normal conditions for about three or four hours after being removed from the centrifuge.

This egg was evidently centrifuged during the first cleavage, which was very unequal, practically all of the cytoplasm having gone into the smaller of the two daughter cells. The nucleus and sphere in this smaller cell are enormous, whereas in the larger yolk cell they are extremely small, indeed no larger than in the anaphase stage of division. The chromosomes form a compact mass which stains deeply and contains no achromatic material. The sphere is small also but the fact that it holds its normal position with respect to the nucleus shows not only that the polarity of the cell remains unchanged, but also that the material of the sphere is different from the ordinary cytoplasm. In many cases similar to fig. 16 cytoplasm slowly forms around the chromosomes in the yolk cell and ultimately such a cell may develop in a normal manner. There is no evidence that cytoplasm ever passes through the cell membrane from one cell to another, and there is positive evidence that this does not occur. The formation of cytoplasm around a mass of chromosomes in a yolk field is therefore an occurrence of more than ordinary importance. The question has been asked frequently whether the nucleus alone can form cytoplasm or the cytoplasm alone a nucleus. It is known that the latter never happens; a mass of cytoplasm without

a nucleus may live for some time and show certain vital functions, but it is unable to grow or to regenerate lost parts. It is much more difficult to test the former question, for it is usually impossible to separate the nucleus completely from the cytoplasm and yet leave it in a medium in which growth would be possible. Verworn ('91) succeeded in shelling the nucleus out of Thalassicolla, but found that the isolated nucleus was unable to grow a new cell body; but apart from the objection that the resting nucleus contains a large amount of cytoplasmic substance, this experiment is not conclusive for it is possible that the failure to grow a cell body was due to the lack of a proper nutrient medium in which the nucleus could operate.

The present experiment is free from most of these objections, though it must be confessed that one objection still remains, viz., it is not possible to be certain that every trace of cytoplasm has been removed from the yolk cell. Nevertheless the amount of cytoplasm left in the cell is very small and is quite indistinguishable, the only visible constituents of the cell being chromosomes, sphere and yolk. In the growth of cytoplasm in such a cell there first appears a very thin layer of cytoplasm around the chromosomes, then the yolk in the immediate periphery of this begins to dissolve and the cytoplasm increases in amount. Coincidently the chromosomes swell up, absorbing achromatic material from the cytoplasm, and in later stages the growth of both cytoplasm and nucleus goes forward at an increasing rate. The formation of cytoplasm takes place only in the presence of chromatin and in its immediate vicinity; on the other hand the chromosomes grow only when surrounded by cytoplasm. This indicates that some influence, probably of a chemical nature, goes out from the chromosomes and leads to the solution of yolk and the formation of cytoplasm. Whether this influence from the chromosomes may act directly upon the yolk, or only indirectly through the medium of a minimal quantity of cytoplasm, is not certain, but it seems probable that the latter is the case. After cytoplasm has been formed around the chromosomes, but not before, the chromosomes themselves begin to swell up, absorbing achromatic material from the cytoplasm, and the chromatin grows in quantity. Cyto-

plasm is essential to the growth of the nucleus and of the chromatin; on the other hand chromatin is essential to the growth of cytoplasm, or to the conversion of yolk or food substances into cytoplasm. The life of the cell consists in an interchange of materials between the nucleus and the cytoplasm; the one cannot grow in the absence of the other. This conclusion agrees with the generalization of Godlewski ('10): "Zuerst das Bildingsmaterial geliefert und von den betreffenden Regeneratskomponenten zum Protoplasm assimiliert wird, dass dagegen in der zweiten Regenerationsphase dieses Protoplasma sich wenigstens teilweise zur Kernsubstanz transformiert" (p. 88).

The question has been much discussed as to whether the nuclei, and more particularly the chromosomes of the germ cells, are the sole 'bearers of heredity,' as Weismann, and many others, have maintained. We have experimental evidence that the cytoplasm cannot form chromatin in the absence of preëxistent chromatin. On the other hand there is no certain evidence that the chromatin can form cytoplasm in the absence of preëxisting cytoplasm. The experiment described above is not entirely conclusive, for while chromosomes in a yolk field form cytoplasm, it is probable that a minimal amount of cytoplasm is left in the yolk field, and it may be said that this merely grows by assimilation of yolk. On the other hand my experiments show that where we have equal division of the chromosomes and unequal division of the protoplasm we may have regulation and normal development; whereas this never follows abnormal distribution of the chromosomes; in other words protoplasmic abnormalities are capable of regulation when the nucleus is normal, but the reverse is not the case. The nucleus is the regulating center of the cell, and it is probably also the assimilating center. And since both of these functions are involved in inheritance, to this extent at least the nucleus may be said to be the inheritance center.

Fig. 17 represents an egg which was centrifuged for four hours during the first cleavage and was then placed under normal conditions for six hours before being killed. The polar body marks the original animal pole and in the centrifuging most of the yolk was thrown to this pole, most of the cytoplasm to the opposite

pole. The first cleavage plane is nearly equatorial in position, and one of the cells contains most of the cytoplasm. The spindles for the second cleavage have formed and the spindle in the cell containing the larger amount of cytoplasm is distinctly larger than the one in the other cell; each is proportional in size to the resting nucleus from which it came and to the volume of cytoplasm in the cell. The fact that the polarity of the cells has not been changed by the abnormal position of the first cleavage plane is indicated by the fact that the spindles are parallel to each other, but not to the plane of cleavage, as in normal eggs. In short there is evidence that the spindles here attempt to take up the positions which they would have occupied in a normal egg, with meridional cleavage.

Fig. 18 represents an egg from a lot which was centrifuged fifteen minutes in gum arabic, as recommended by Lyon ('04), and which was fixed three hours after removal from the centrifuge. Fig. 19 shows an egg which was centrifuged thirty minutes, and was fixed six hours later. In both cases the centrifuging took place during the first cleavage, as is shown by the unequal distribution of cytoplasm and yolk on both sides of the first cleavage plane. In the second cleavage, which evidently occurred after the eggs were removed from the centrifuge, the cytoplasm was distributed equally to the daughter cells. In fig. 18 the second cleavage took place a little earlier in the cell rich in cytoplasm (*AB*) than in the other (*CD*), but the smaller size of the nuclei in the latter is probably due in part to the fact that these cells are poor in cytoplasm. In fig. 19 the inequality in the distribution of cytoplasm at the first cleavage is much greater than in fig. 18; nevertheless the second cleavage occurred in the cell poor in cytoplasm (*CD*) at nearly the same time as in the other cell (*AB*). Although the nuclei in the cells *C* and *D* are much smaller than those in *A* and *B*, their structure shows that they are in nearly the same stage of the cell cycle. Their smaller size is due to the smaller quantity of cytoplasm in which they lie. Figs. 17–19 indicate that the absolute size of the nucleus has little to do with the time of its division; small nuclei in yolk-rich cells divide almost as rapidly as large nuclei in cells rich in cytoplasm.

Figs. 20 to 28 show eggs which were centrifuged during the second cleavage. The first and second cleavages may always be distinguished by the fact that the polar furrow bends to the right in the first cleavage and to the left in the second (Conklin '97). In fig. 20 the distribution of cytoplasm and yolk to the daughter cells was equal in the first cleavage but unequal in the second, and the daughter nuclei are proportional in size to the volume of the cytoplasm in which they lie.

In fig. 21, which represents an egg which was centrifuged for 30 minutes and fixed at once, the second cleavage is very unequal, two of the macromeres (*B* and *C*) being small protoplasmic cells, which resemble micromeres in appearance, but which behave like macromeres as the study of later stages (figs. 24 to 28) shows.

Fig. 22 represents an egg which was centrifuged for thirty minutes during the second cleavage and then kept under normal conditions for twenty-one hours before being fixed. The second cleavage was suppressed although the nucleus divided in the upper cell, *AB*, but not completely in the lower one, *CD*. These nuclei have given rise to spindles for the third cleavage, there being two independent spindles in the cell *AB*, and two spindles which are fused at one pole in the cell *CD*, thus forming a triaster. The degree of abnormality in this case is indicated by the fact that the development has been halted at this stage, although a normal egg would have reached the 20-cell stage at least, in the time which elapsed after centrifuging.

With the exception of fig. 26, all of the figs. from 23 to 28 were drawn from the same lot of eggs which were centrifuged for thirty minutes in the 2-cell stage, and then kept for six hours under normal conditions before being fixed. In all of these eggs the second cleavage was made very unequal by the centrifuging. Two of the macromeres are not only much smaller than the other two, but are composed entirely of cytoplasm, whereas the two larger macromeres contain all of the yolk. Nevertheless the behavior of these two small, protoplasmic macromeres is almost identically like that of the large, yolk-rich macromeres; the micromeres are given off from both the protoplasmic and the yolk laden macromeres at practically the same time and in the same

direction; the micromeres formed from these abnormal macromeres are the same as in normal eggs in which all the macromeres are of the same size and contain the same quantity of cytoplasm and yolk. In short there is here a form of regulation which leads to the formation of normal micromeres from abnormal macromeres, and the exact manner in which this cellular regulation takes place is of fundamental importance, and will be discussed later.

Fig. 23 represents an egg similar in many respects to fig. 21, but of a later stage. The smaller protoplasmic macromeres preserve their original polarity as is shown by the fact that the spheres lie between the nuclei and the polar bodies. On the other hand each of the large macromeres contains a tetraster; the spindles are those of the third cleavage.

Fig. 24 represents an egg of the same type as the preceding, after the third cleavage; each macromere has given rise to a micromere which is normal in form, position, constitution and size, although the macromeres are very abnormal in these regards, two of them containing all of the yolk and very little protoplasm, and the other two being small and purely protoplasmic. Indeed the macromeres *1A* and *1D* nearly exhausted all the cytoplasm which they contained in order to form cytoplasmic micromeres of normal size; on the other hand, the size of the micromeres *1b* and *1c* is not influenced by the fact that the macromeres from which they come are small and are purely protoplasmic.

Fig. 25 is a drawing of an egg in a slightly older stage than fig. 24; the large macromeres, *1B* and *1C*, are giving off the second set of micromeres, *2b* and *2c*, while two of the first set of micromeres, *1b* and *1c*, are just beginning to divide. The four small cells which lie to the left of the polar bodies are the macromeres *1A* and *1D* and the micromeres *1a* and *1d*; these cells are purely protoplasmic and are very small, all four of them being no larger than one of the micromeres, *1b* or *1c*, in the other quadrants. Nevertheless these minute 'macromeres' have each given rise by an equal cleavage, to a micromere as large as itself. Although these micromeres are much smaller than those in the other quadrants, they are the largest that could be formed from the macro-

meres in question without making the macromeres smaller than the micromeres, thus reversing the usual inequality of this division; in short the division of these cells represents the nearest possible approach to normal conditions.

Figs. 27 and 28 show eggs of the same type as the preceding, but at a stage after the formation of the second set of micromeres (*2a–2d*) and during the division of the first set (*1a–1d*). Here also these micromeres are normal in size, although the size relations, and the cytoplasmic or yolk content of the macromeres from which they came, are very abnormal. In the cleavages which follow after the centrifuging, complete regulation has occurred, so far as this is possible. It is not possible for regulation to take place by the redistribution of cytoplasm and yolk by passage through a cell membrane.

Fig. 26 represents an egg which was centrifuged for thirty minutes during the second cleavage, and then fixed twelve hours later. At the time of centrifuging the nuclear division in the second cleavage was complete, but the division of the cell body was suppressed. Consequently each of the blastomeres, *AB* and *CD*, contained two nuclei, which by subsequent division in the manner indicated in fig. 22 have given rise to two sets of micromeres, *1a–1d*, and *2a–2d*. Both sets of micromeres have divided, as indicated by the connecting bonds, thus forming a somewhat abnormal cap of sixteen micromeres. The nuclei of the macromeres are indicated by the reference lines from the letters *2A–2D*. Other cases similar to this one will be shown and described in another paper, but this one egg shows that it is possible for both the nuclei of a binucleate cell to divide at the same time and to give rise to separate cells, each with a single nucleus, and that such cells may approximate in form and position normal blastomeres.

Fig. 29 represents an egg which was centrifuged for four hours at the close of the second cleavage, and fixed at once after centrifuging. The yolk has been forced out into lobes, which are still connected with the protoplasmic portions of the cells except in the case of one cell, where the lobe has been completely separated. It is a significant fact that the point at which the lobe forms, and consequently the point where the cell membrane is weakest lies

at the outer pole of the axis which passes through the centrosome and nucleus and these axes mark the position of the spindles of the third cleavage. Here, as in every other instance, the smallest nucleus is found in the cell which has the smallest amount of cytoplasm.

Fig. 30 is a drawing of an egg which was centrifuged ten minutes in gum arabic, during the first cleavage, and fixed four hours later during the third cleavage. Macromeres *A* and *B* are richer in cytoplasm and poorer in yolk than *C* and *D*, and correspondingly the spindles and asters are larger in the former than in the latter.

Figs. 31 and 32 represent eggs which were centrifuged four hours during the first cleavage, and were fixed six hours later. In both eggs the macromeres *A* and *B* are richer in cytoplasm and poorer in yolk than *C* and *D*. In fig. 31 the cells *A* and *B* contained more cytoplasm and divided earlier than *C* and *D;* at least one-half of the cytoplasm in the latter cells has gone into the formation of the micromeres, which are still, however, smaller than normal. The first cleavage in this egg did not pass through the animal pole, marked by the polar bodies, but was displaced to one side, and the spiral form of the cleavage is not clearly preserved in the cells *C* and *D*. While the regulation in the size of these micromeres is not complete, the tendency to approach the normal condition is evident. Fig. 32 is similar to fig. 31, though the macromeres *C* and *D* of this egg contained a larger amount of cytoplasm than in fig. 31, and the regulation in the size of the micromeres is complete.

Fig. 33 shows an egg, from the same slide as fig. 30, which was centrifuged ten minutes in gum arabic and fixed four hours later. The macromeres *1A* and *1B* contain more cytoplasm and are dividing earlier than *1C* and *1D*, but the micromeres from the former are no larger than those from the latter.

Fig. 34 represents an egg which was centrifuged for two and one-half hours during the first cleavage, and was fixed twenty-one hours later. The macromeres *2A* and *2B* contain much cytoplasm, while *2C* and *2D* contain little and yet the micromeres formed from the latter are almost as large as those from the former.

Figs. 35, 36, 37 represent eggs, from the same experiment, which were centrifuged thirty minutes during the first cleavage, and were fixed twelve hours later. The size of the micromeres of the first, second or third sets is but little influenced by the quantity of cytoplasm in the macromeres; the size regulation of the micromeres is here practically complete. In fig. 37 the cell *4c* forms at the same time as *4d*, though in normal eggs it does not form until much later; the precocious formation of this cell is probably due to the fact that the amount of cytoplasm in macromere *C* was larger than normal.

III. General results of these experiments

The results of these experiments, which have been described in the order of development from the earlier to the later stages without reference to a logical presentation of general questions, may now be classified and compared with the observations on cell size and nuclear size given in Part I of this paper. In general these experiments support in every detail the conclusions based upon the study of normal eggs and blastomeres.

1. Nuclear size in centrifuged eggs. In centrifuged eggs, as in normal ones, the size of the nucleus is always dependent upon the quantity of cytoplasm surrounding the nucleus and upon the length of the resting period. Nuclei which are normally large may be caused to remain small, and nuclei which are normally small may be rendered large by merely changing the positions of the yolk and cytoplasm in the cell.

In normal eggs of Crepidula the egg nucleus lies in a protoplasmic field near the animal pole of the egg, while the sperm nucleus enters the egg near the vegetal pole and moves up toward the animal pole through a field of yolk. As long as the sperm nucleus is in this yolk it remains very small, and only when it emerges into the protoplasmic field near the egg nucleus does it begin to grow rapidly. The egg nucleus on the other hand, grows rapidly and becomes much larger than the sperm nucleus. If now an egg is centrifuged during the formation of the second polar body so as to throw the yolk to the animal pole and the cytoplasm to

the vegetal pole, the normal size relations of the germ nuclei is reversed, the sperm nucleus becoming larger than the egg nucleus as shown in fig. 11. Godlewski ('08) holds that the size of the sperm nucleus depends upon the time which elapses before its union with the egg nucleus; it also depends, as I have shown, upon the quantity of cytoplasm in which it lies. We conclude therefore, that in all animals the relative sizes of egg and sperm nuclei are dependent upon the amount of cytoplasm in which they lie, and upon the length of the growth period (interkinesis). In this connection it may be worth while to remark that one reason why the rhythm of cleavage, in Boveri's, Driesch's, and Godlewski's experiments, follows the maternal rather than the paternal type may be found in the fact that the rate of growth of the nucleus is dependent upon the quantity and quality of the protoplasm of the egg.

In the cleavage of the egg the size of the nucleus is dependent upon the quantity of protoplasm in which it lies, as shown by figs. 12 to 20. In eggs subjected to strong centrifugal force the egg contents separate into three zones, a yellow zone of yolk at the distal (heavy) pole, a gray zone of oily and watery substance at the central (light) pole, and a clear zone of protoplasm between these two. It is the latter substance which contributes to the growth of the nucleus, as is shown by such cases as fig. 16 in which the gray substance was centrifuged out of the egg and practically all of the yolk thrown into one of the blastomeres, and most of the clear protoplasm into the other; the nucleus in the blastomere which contains yolk but little or no protoplasm has scarcely grown at all, the one in the cell containing the clear protoplasm, but without the gray substance, has grown enormously. Similar, though less striking, differences in the sizes of nuclei, depending upon the quantity of clear protoplasm in the cell, are found in all the eggs figured. In centrifuged eggs the nucleus always occupies the middle zone, and as I have just shown it grows at the expense of substance received directly from this zone. The fact that the specific gravity of the nucleus and of this middle zone are the same, is probably due to the fact that so much of the absorbed nuclear material is from this zone.

2. The sizes of spindles, centrosomes, spheres and asters. The study of centrifuged eggs shows, as was observed in the case of normal eggs, that the sizes of spindles, centrosomes, spheres and asters are dependent upon the quantity of cytoplasm in which they lie. The size of the spindle is also related to the size of the nucleus, as I have already shown, but as this, in turn, is dependent upon the quantity of cytoplasm of the middle zone, it follows that the size of the spindle as well as that of the centrosome and sphere is related to the quantity of cytoplasm in which they lie. Fig. 17 shows spindles in sister cells which are quite different in size owing to the different amounts of cytoplasm in these two cells; while figs. 11, 12, and 16 show centrosomes and sphere which vary in size depending upon the quantity of cytoplasm surrounding them.

In this connection attention should be called to the fact that the spindles from the stage of the metaphase to the end of mitosis are anchored in the cell, and can be moved only with much difficulty. The spindle fibers are tougher and more consistent than the surrounding plasm, and they are not a mere arrangement of granules in the lines of force as Lillie ('09) has maintained for Chaetopterus.

3. The rhythm of division in centrifuged eggs. The rhythm of division is not dependent solely upon nuclear size, nor cell size, nor the ratio of one to the other (Kernplasma-Relation), though it may be influenced by the absolute amount of cytoplasm present in the cell. Cleavage cells which contain a large amount of cytoplasm, and which therefore have large nuclei, usually divide a little earlier than cells poor in cytoplasm, and with small nuclei, though this is not always the case, as is shown by fig. 17, in which the large and the small nuclei divide at the same time. Nuclei which differ greatly in size may still be in the same stage of the nuclear cycle, as shown in fig. 19, and may divide at the same time. On the other hand, figs. 25, 31, 34 and 37 show cases in which nuclei of the same generation divide earlier in cells rich in cytoplasm than in cells which are poor in this substance.

4. Growth of cytoplasm at the expense of yolk. Centrifuged eggs afford an excellent opportunity of studying the way in which

cytoplasm grows at the expense of yolk. In cases in which the centrifuging occurred after the spindle was anchored in the cell, but before the division wall had formed, the cytoplasm may be thrown almost entirely to one pole of the spindle and the yolk to the other; accordingly when division occurs one of the daughter cells will contain almost all the cytoplasm, the other all the yolk, while both cells will receive the same number and mass of chromosomes, fig. 16. The chromosomes which are left in the yolk field remain small and compact since there is no cell substance which they can absorb. After some time the yolk in the vicinity of the chromosomes may begin to disappear and cytoplasm to appear in its place. It can scarcely be doubted that some substance, probably an enzyme, is given off by the chromosomes and dissolves the yolk, and that this dissolved yolk is then converted into cytoplasm through the influence of the chromosomes. Once a small field of cytoplasm is formed around the chromosomes, they begin to absorb it and to become vesicular. The process of forming cytoplasm may then go forward rapidly and in the end the yolk cell may give rise to protoplasmic micromeres in a normal manner (fig. 31). It is probable that a small amount of cytoplasm, which cannot be displaced by centrifuging, is left in the yolk cell, and it is possible that the formation of new cytoplasm would not take place in the absence of this small remnant, but it can be proved conclusively that this formation of cytoplasm takes place only in the vicinity of the chromosomes, and that in the absence of this chromatic material it never occurs at all. Under these circumstances the conclusion seems justified that the chromatin has the power of forming cytoplasm when placed in a suitable nutrient medium, such as yolk, and that the cytoplasm in turn contributes to the growth of the nucleus and of the chromatin.

5. Unequal and differential cell divisions. By centrifuging, the size and constitution of the blastomeres may be changed; divisions which are normally equal may be made unequal, and *vice versa;* cells which are normally protoplasmic may be filled with yolk and *vice versa.* In this way both the cell size and the cell content may be controlled experimentally.

Acknowledgedly the position of the spindle conditions the plane of the cleavage, the division wall passing through the equator of the spindle. When by any means the spindle is displaced from its normal position the division plane is displaced. In this way giant polar bodies may be formed, as shown in fig. 11, or macromeres may be formed which are small and free from yolk, as shown in figs. 16, 21–28, *et al.*

Are the inequalities and differentiations of normal cleavage due to similar causes, viz., external or internal pressure? Clearly external pressure cannot be involved in the unequal division of free cells, such as the maturation divisions of the egg; and the fact that isolated blastomeres of the 4-cell stage divide in the normal manner into small protoplasmic micromeres and large yolk-rich macromeres, shows that these unequal divisions during the cleavage period cannot be explained as the result of reciprocal pressure among cells. On the other hand, the formation of micromeres of normal size and constitution from purely protoplasmic macromeres, as shown in figs. 24, 27, 28, 33, 36, *et al.*, indicates that this inequality of division cannot be due to the crowding of the spindle to one side of the cell by internal pressure, such as might come from the presence of a mass of yolk—because in the cases cited, little or no yolk is present in the macromeres. If internal pressure is involved in the unequal division of these protoplasmic cells it must be pressure of a very different sort from that involved in the presence of a mass of metabolic products at one side of the cell. While the spindle may be pressed out of position by external or internal pressure this will not serve to explain the eccentric position of the spindle in such cases as I have described.

A satisfactory explanation of unequal and differential cell division must also be able to be applied to equal and non-differential cleavage, for the causes of the latter are not simple mechanical conditions, such as pressure. In the case of cleavages which are normally equal, if the spindle and yolk are moved to eccentric positions in the cell, they come back, if possible, to their normal positions when the pressure is removed; indeed they sometimes seem to come back against considerable pressure, as when

a spindle moves out of a protoplasmic field into the yolk in order to reach its normal position in the cell. When eggs like the one shown in fig. 11 are removed from the centrifuge, the egg and sperm nuclei, together with the cytoplasm surrounding them move up through the yolk until they ultimately lie in their normal position on the animal side of the egg, beneath the polar bodies. However far the germ nuclei or the first cleavage spindle may be removed from the chief axis of the egg, they invariably come back to their normal positions, with the equator of the spindle in the egg axis, and the long axis of the spindle at right angles to the egg axis, unless the spindle is held so long in its abnormal position that it is caught in that position by the divisional processes. The same is true also of the nuclei and spindles of the 2-cell stage; when moved out of the median plane of the cell they come back to that median plane, unless the cells are injured or the spindles are held in their abnormal position until the metaphase or a little later. Evidently the cause of equal cell division, such as the first and second cleavages of Crepidula, is not so simple as those have assumed who have attributed it to pressure, the line of least resistance, or the long axis of the protoplasmic mass.

Not only the eccentricity or lack of eccentricity, but also the axis of the spindle is of great importance in determining the character of the cleavage. While the former is associated with the equality or inequality of division, the latter conditions its differential or non-differential character. The polar differentiation of the egg is the first visible morphogenetic differentiation, and it is not without significance that in the first and second cleavages of the egg the spindles are at right angles to the egg axis, while in the third, fourth and fifth cleavages they are more nearly parallel with that axis.

I have hitherto spoken of the position of the spindle as if it were the one cause of equal or unequal, differential or non-differential cleavage; but for many reasons it is evident that the position of the spindle is itself the result of the structure or organization of the protoplasm, and that in this organization polarity and symmetry play an important part. Many years ago ('93) I showed that even before the spindle is formed, the shape of the

cell may indicate the position and direction of the coming cleavage, and I maintained then and in subsequent papers ('97, '99, '02) that the position of the spindle and the size, position, and histological character of the daughter cells is the result of the structure of the protoplasm, and particularly of the polarity and symmetry of the cell.

These conclusions have been confirmed by much experimental work on cell division, which I have completed but have not yet published. The position of the spindle and the plane of cleavage may be greatly changed, but the polarity and organization of the protoplasm remain unchanged, as I shall show in a future paper. Indeed it is very difficult to alter the polarity of any cell as Lillie ('06, '09) has shown, and one reason for this is to be found in the fact, as I have discovered in Crepidula, that the cell axis, i.e., the axis connecting nucleus and centrosome, can rarely be changed by artificial means.

6. Regulation in the cleavage process. Evidently connected with this persistent organization of the cell is the power of regulation which is shown in the cleavage of the egg as well as in the regeneration of adult parts. Whenever the size or constitution of blastomeres of Crepidula have been changed, or when cleavages have been suppressed, subsequent cleavages come back to the normal form so far as this is possible. The original disturbance can be righted only very gradually if at all, since neither yolk, cytoplasm nor nuclei can pass through cell membranes, and the only redistribution of substances possible is by means of new cell divisions. But in Crepidula the divisions following upon such a disturbance of the usual cleavage process are almost if not entirely normal. This is very evident in the divisions following upon disturbances of the first two cleavages. All of the yolk may be centrifuged into two of the macromeres and practically all of the cytoplasm into the other two, as in figs. 16, 19, 21, 23, *et. al;* two of the 'macromeres' may be very small and two very large, as in figs. 16, 21, 23 to 28; or one of these first two cleavages may be suppressed, as in figs. 22 and 26; but if such abnormal eggs are allowed to develop under normal conditions, the micromeres are formed in normal manner, as is shown in figs. 24 to 28 and 32

to 37. Whatever the content of the different macromeres may be, whether purely protoplasmic or entirely yolk the micromeres are always protoplasmic, even though division must be delayed until the cytoplasm which goes into the micromeres can be formed from yolk (figs. 24, 31, 35); whatever the size of the macromeres, the micromeres formed from them are approximately normal in size, even though yolk-rich cells must give up most of their cytoplasm (figs. 24, 31, 35), or protoplasmic micromeres must divide equally (figs. 25, 28), in order to give rise to micromeres of the usual size.

Such regulations of cleavage are probably caused, in the case of Crepidula, by the persistent polarity of each cell, which in turn leads to the localization of the spindle in a definite axis, with its pole at a definite distance from the surface of the cell. In what manner the polarity of the cell may cause the localization of the spindle is clearly shown in the cleavage of Crepidula. In former publications ('99, '02) I have called attention to the fact that definite movements of cell substance take place in dividing cells, and that these movements serve to orient the spindles; these movements are always related to the polarity of the cell and to that of the entire egg. Furthermore, I have elsewhere ('02) called attention to the fact that the cell membrane is weakest opposite the poles of the spindle. I was formerly of the opinion that this was due to some influence of the spindle on the cell membrane, but a further study shows that these weak places in the cell membrane are present before the spindle forms and can not therefore be caused by the spindle. In the egg shown in fig. 29 the places of reduced tension on the cell membrane are indicated by the lobes of yolk attached to the cells, and a line drawn through the centrosome, nucleus and lobe indicates the precise position which the spindle will take at the next cleavage. The axes of the third cleavage spindles are here marked out long before the spindles are formed; the weak spot in the cell membrane is not caused by the position of the spindle, but the latter is the result of the former. Experiments on eggs in the 2-cell, 4-cell and 8-cell stages of cleavage show that the positions of the points where the membrane is weakest, change in each cell generation and that they

always mark out the position of the spindle. These lobes are formed only when the egg is subjected to pressure and then only at those points on the cell surface which mark the position which will be taken by the poles of the spindle. Since the spindle axes change in successive cleavages it follows that this point of reduced tension also changes in successive cell generations.

I conclude therefore that the position of the spindle, and all the morphogenetic results which follow from this, is dependent upon the polarity of the cell; which polarity manifests itself not only in the localization of cytoplasmic substances, but also, and more fundamentally, in definite movements of the oöplasm and in reduced tension of the cell membrane at the poles of the cell.

GENERAL SUMMARY AND INDEX

Part I. Observations

1. The equality or inequality of cell division in normal cleavage is due to internal causes, rather than to the presence of metabolic substances, such as yolk, within the cell or to pressure from without. These internal causes are to be found in the polarity of the cell, in movements of the cytoplasm, and in the structure of the cell membrane. Since the position and axes of the spindles change regularly in successive divisions this protoplasmic organization must also change regularly (pp. 6–9).

2. The yolk-lobe is a temporary extrusion of yolk or oöplasm during mitotic pressure, at the former point of attachment to the ovarian wall and a little to one side of the vegetative pole. If this lobe is large, the resulting cleavage is unequal, although the furrow cuts through the chief axis and the center of the egg. The degree of inequality of the first and second cleavages is measured by the size of the yolk-lobe. The yolk-lobe is the result of an unsymmetrical distribution of yolk or egg substance with reference to the egg axis (pp. 9–11).

3. In Crepidula plana the Kernplasma-Relation varies greatly in different blastomeres and at different stages, depending chiefly upon the length of the resting period (interkinesis). In cases

where nuclei and cells are measured at their maximum size it varies from 14.5 to 0.37; at mean size from 35.7 to 1.1; at minimum nuclear and cell size it varies from 285 to 29. In protoplasmic blastomeres, which contain no yolk, the Kernplasma-Relation varies from 14.5 to 8.7, when the nuclei are at their maximum size; and from 35.7 to 7, when the nuclei are at mean size. In Fulgur, at mean size, it varies from 127.7 to 3.6 (pp. 16–24).

4. In different eggs, corresponding blastomeres have approximately the same Kernplasma-Relation; but in different blastomeres of the same egg or of different eggs the Kernplasma-Relation is neither a constant nor a self regulating ratio. It appears to be a result rather than a cause of the rate of cell division, and consequently a variable rather than a constant factor (pp. 24–25).

5. In the tissue cells of adult Crepidulas there is no marked increase of cytoplasm over nucleus, as compared with the blastomeres. The Kernplasma-Relation of various adult epithelial cells, not filled with metabolic products, varies from 28 to 7; in oöcytes and ganglion cells it varies from 6 to 3 (pp. 25–28).

6. The size of the nucleus is dependent upon at least three factors: (a) The initial quantity of chromatin (Boveri); (b) The volume of the cytoplasm; (c) The length of the resting period (p. 25).

7. The inciting cause of cell division in Crepidula is not found solely in the limitations of the working sphere of the nucleus (Strasburger), nor in the doubling of the volume of the chromosomes (Boveri), nor in a Kernplasma-Spannung (Hertwig), but rather in the coincidence of centrosomal, chromosomal and cytoplasmic rhythms, which are probably connected with the rate and nature of metabolism in the cell (pp. 29–32).

8. During the cleavage of the egg of Crepidula plana the volume of the cytoplasm more than doubles between the 1-cell and the 24-cell stage the average growth for each division being about 6 per cent; the yolk decreases in volume by nearly one-half and the entire egg is smaller at the 24-cell stage than at the 1-cell stage. This can only mean that the yolk contributes to the growth of cytoplasm during the cleavage period (pp. 32–36).

9. The average nuclear growth during cleavage is not more than 5 per cent to 9 per cent for each division up to the 32-cell stage and it may fall as low as 0.3 per cent to 1 per cent for each division after that stage; and in every case it falls far short of a doubling, or increase of 100 per cent, for each division (pp. 36–44, 54, 55).

10. Both nuclear sap and linin belong to the cytoplasm as well as to the nucleus. The chromatin is the most distinctive nuclear substance. All of these constituents are more abundant in large cells than in small ones. The mitotic spindle is of both nuclear and cytoplasmic origin and its size depends upon the volume of both nucleus and cytoplasm (pp. 44–47, 55).

11. The average growth in volume of chromatin from the 2-cell to the 32-cell stage is about 8 per cent for each division period, being about the same as the growth of the nucleus as a whole (pp. 47–48, 55).

12. The chromosomes become individually smaller as cleavage progresses, and in general small nuclei give rise to smaller chromosomes than do large nuclei (pp. 48–51, 55).

13. The size of the nucleoli (plasmasomes) depends upon the size of the nucleus and the length of the resting period; the larger the nucleus and the longer the resting period, the larger the plasmasomes become (pp. 51–53, 55–56).

14. Centrosomes and spheres of large cells are larger than those of smaller ones (pp. 53, 56).

15. The rate of growth of chromatin during the early cleavages of Crepidula (8 per cent for each division) harmonizing with the slight rate of increase of the oxidative power of the egg as determined by Warburg (p. 56).

16. My observations do not support the view that senescence is due to a decrease (Minot), or an increase (Hertwig) of nuclear, as compared with protoplasmic material; nor that rejuvenescence is accomplished during cleavage by the great increase of nuclear material relative to the protoplasm. On the other hand senescence seems to be associated with a decrease, rejuvenescence with an increase of metabolism (Child). Anything which decreases the interchange between nucleus and cytoplasm, such as products

of differentiation and metabolism within the cell, or a dense nuclear membrane, decreases metabolism and leads to senescence; anything which facilitates this interchange increases metabolism and leads to rejuvenescence. It is suggestive that in early development increased oxidation is associated with fertilization and mitosis (Loeb, Lyon, Warburg) (pp. 57-62).

Part II. Experiments

17. By centrifugal force the substance of the eggs and blastomeres of Crepidula may be stratified into a zone of heavy yolk at one pole, a zone of lighter oil and water at the other pole, and a zone of clear cytoplasm between these two; and since these eggs orient but slightly if at all while being centrifuged, the axis of centrifuging and of stratification may form any angle with the egg axis. In the early development of Crepidula the volume of yolk is much greater than the volume of cytoplasm and consequently the latter may be displaced to any side of the center of the egg or blastomere (p. 64).

18. On the other hand the mitotic figure, after the prophase, can be moved only with great difficulty, and owing to this fact the substances of a cell can be distributed in very atypical manner with respect to the poles of the spindle and the resulting daughter cells. In this way all the yolk present in a dividing cell may be thrown into one of the daughter cells, and almost all of the cytoplasm into the other (p. 64).

19. These experiments show that the spindle is a specific structure and not merely a dynamic expression of lines of force. It remains in position and functions normally when the substance in which it usually lies is completely replaced by other substance. The spindle fibers are denser than the general cytoplasm and may be stretched, shortened or bent by pressure (p. 65).

20. If centrifuging occurs during the second maturation division, when the poles of the egg are clearly marked, the yolk may be driven to the animal pole and the cytoplasm to the vegetal pole, the spindle may be much elongated and a giant polar body may be formed (fig. 11). In such cases the sperm nucleus, which

enters the egg near the vegetal pole, lies in a cytoplasmic field, the egg nucleus in a yolk field, and the former grows more rapidly than the latter, thus reversing the usual size relations of the germ nuclei. The relative size of the germ nuclei is dependent upon the volume of the cytoplasm in which they lie as well as upon the length of time that the sperm nucleus has been in the egg (pp. 67, 75).

21. If centrifuging occurs during the cleavage almost all the yolk present may go into one daughter cell, almost all the cytoplasm into the other (figs. 16, 19). Under these circumstances the subsequent growth of the daughter nuclei is proportional to the volume of the cytoplasm of the middle zone in which they lie. Neither the yolk nor the substances of the lighter zone contribute directly to the growth of the nucleus (pp. 75–76).

22. The size of spindle, centrosome, and sphere in any cell is not definitely fixed, but may be modified by altering the quantity of cytoplasm; the larger the quantity of cytoplasm in a cell, the larger are all the structures named (p. 77).

23. The rhythm of division may be modified, but only to a slight extent, by altering the quantity of cytoplasm in a cell. In general, cells rich in cytoplasm divide a little earlier than those poor in this substance; but though the quantity of cytoplasm in a cell and the size of its nucleus may be greatly changed by centrifuging, the rhythm of cleavage is but slightly changed (p. 77).

24. When the daughter chromosomes at one pole of a spindle are left in a cell composed almost entirely of yolk, they do not form a vesicular nucleus until yolk has been dissolved and a certain amount of cytoplasm has been formed around the chromosomes. It is evident that something, perhaps an enzyme, is given off from the chromosomes or chromatin, which leads to the transformation of yolk into cytoplasm; this cytoplasm is in turn taken up by the chromosomes and ultimately contributes to the growth of the chromatin, (pp. 77–78).

25. The typical size, position and constitution of blastomeres, and consequently the type of cleavage, do not depend upon external or internal pressure, but upon a definite polarity, symmetry and movement of the cell contents, and upon reduced surface

tension at the poles of the cell. Therefore, the causes of equal or unequal, differential or non-differential divisions are intrinsic rather than extrinsic (pp. 78–81).

26. Whenever the size, constitution or number of blastomeres is changed from the typical condition, subsequent cleavages come back to the normal form so far as this is possible. This regulation in cleavage is connected with a persistent polarity of the cell, which is not changed by centrifuging, and which manifests itself in a definite cell axis passing through nucleus and centrosome, in typical movements and localizations of cell contents, and in reduced tension of cell membrane at the poles of the cell (pp. 81–83).

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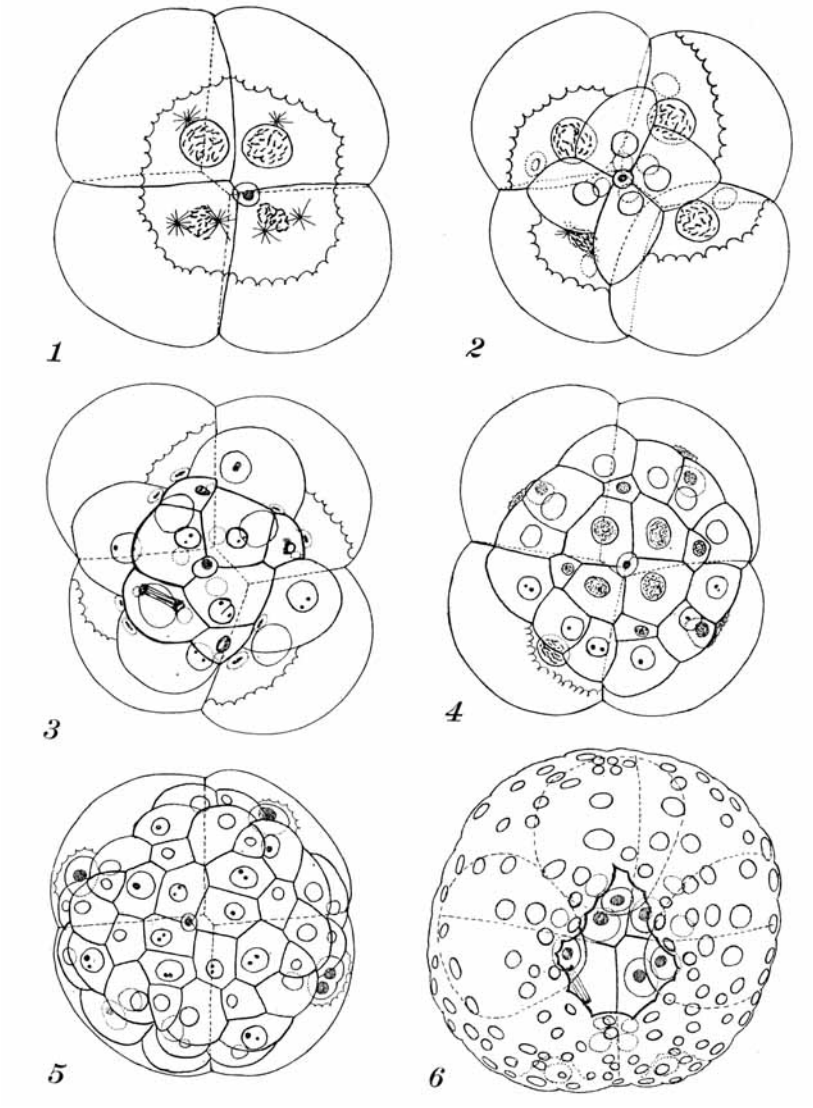
DESCRIPTION OF FIGURES

All figures (with the exception of figs. 9 and 10) represent entire eggs of Crepidula plana, fixed, stained, and mounted on slides. They were drawn with the aid of a camera lucida under Zeiss Apochromat 3 mm., Ocular 4, and represent a magnification of 333 diameters. In the centrifuged eggs, the axis of centrifuging is, in many cases, indicated by an arrow, the head of the arrow marking the distal (heavy) pole and the tail of the arrow the central (light) pole. In figs. 12 to 19, and 29 to 37 the first cleavage is in the long axis of the page, the second cleavage (figs. 18 and 19) is at right angles to this. In figs. 21 to 28 the first cleavage runs across the page, the second, lengthwise of it.



Figs. 1–6 Successive stages in the development of the egg of C. plana, showing the maximum sizes of the nuclei of the macromeres. Fig. 1, 4-cell, just before third cleavage; fig. 2, 8-cell, just before fourth cleavage; fig. 3, 16-cell, just before fifth cleavage; fig. 4, 24-cell, just before sixth cleavage in macromere 3D; fig. 5, 42-cell, just before sixth cleavage in macromeres 3A-3C; fig. 6, Gastrula, just before seventh cleavage of the macromeres.

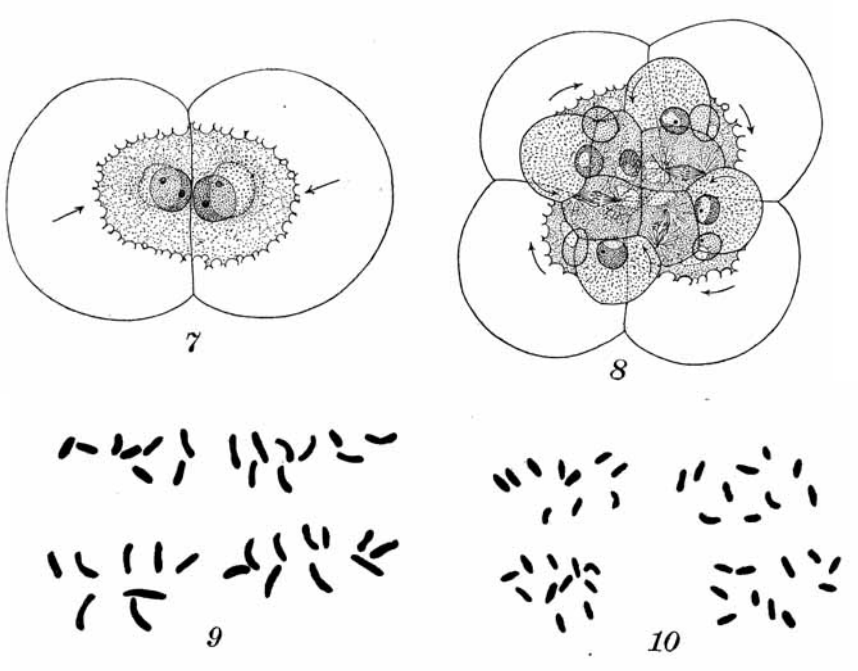


Fig. 7 2-cell stage of C. plana. The nuclei just before the second cleavage are 24μ in diameter.

Fig. 8 12-cell stage of C. plana. The nuclei in the first quartet of micromeres, 1a–1d, three of which are dividing, are 14μ in diameter at their maximum size.

Fig. 9 Chromosomes from four different spindles of the second cleavage, all in the metaphase and all magnified 2000 diameters.

Fig. 10 Chromosomes from four different spindles of the cells 1a–1d, all in the metaphase and all magnified 2000 diameters.

Fig. 11 Egg centrifuged ten minutes after formation of first polar body and during formation of second; fixed three hours after centrifuging. Telophase of second maturation division; indication of formation of enormous second polar body. The size of nuclei is dependent upon quantity of cytoplasm in which they lie.

Fig. 12 Centrifuged fifteen minutes in gum arabic, fixed three hours later. Evidently centrifuged during first cleavage; almost all of the cytoplasm is in the smaller cell. The size of the nuclei is proportional to the quantity of cytoplasm.

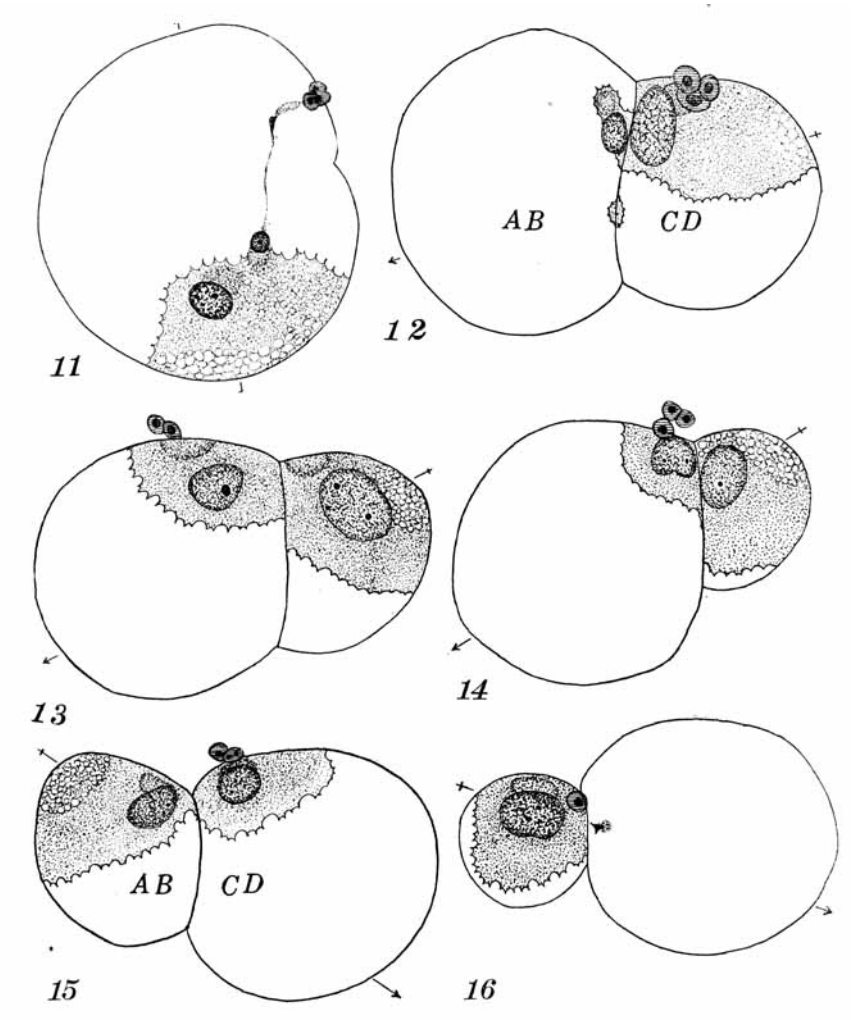


Fig. 13 Centrifuged five hours (2000 revolutions per minute) during the first cleavage; fixed at once; structure similar to preceding.

Fig. 14 From the same experiment as the preceding. Size of nuclei is proportional to the quantity of clear (granular) cytoplasm; yolk and oily or watery constituents of the cytoplasm do not influence nuclear size.

Fig. 15 From the same experiment as the preceding, and showing similar results.

Fig. 16 Centrifuged thirty minutes; fixed twenty hours after centrifuging. Egg has not developed. Enormous difference in the size of sister nuclei.

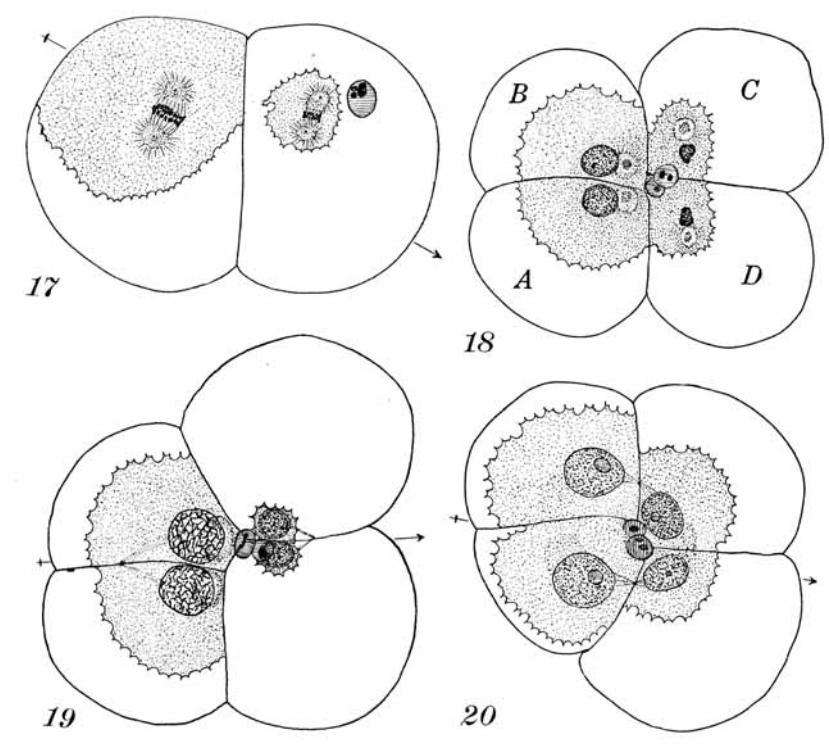


Fig. 17 Centrifuged four hours (2000 revolutions per minute); fixed six hours after. Evidently centrifuged during first cleavage. The cleavage plane does not pass through the polar axis. The spindles are proportional to the size of nuclei from which they were formed, and to the volume of cytoplasm in which they lie. They are not parallel to the plane of the first cleavage, which is here out of its normal position.

Fig. 18 Centrifuged fifteen minutes in gum arabic; fixed three hours after. Evidently centrifuged during first cleavage. The second cleavage appeared earlier in the more protoplasmic cells (*A* and *B*), than in the others.

Fig. 19 Centrifuged thirty minutes; fixed six hours later. Evidently centrifuged during the first cleavage. The size of the nuclei is plainly dependent upon the volume of the cytoplasm in which they lie.

Fig. 20 Centrifuged four hours, (2000 revolutions per minute); fixed at once. Evidently centrifuged during the second cleavage; the daughter nuclei are proportional in size to the volume of cytoplasm in which they lie.

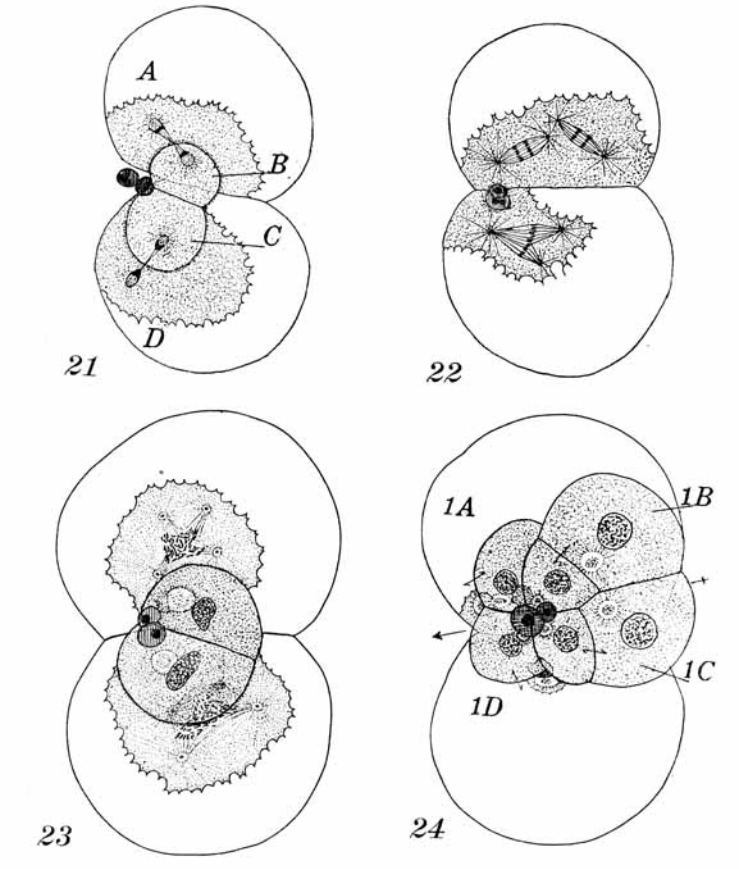


Fig. 21 Centrifuged thirty minutes; fixed at once. Centrifuged during the second cleavage, which was thus made very unequal, two of the macromeres (*A* and *D*) containing all the yolk and the other two (*B* and *C*) being small and purely protoplasmic.

Fig. 22 Centrifuged thirty minutes; fixed twenty-one hours later. The second cleavage was suppressed. Two spindles for the third cleavage are present in each cell, but the cell body shows no signs of division.

Fig. 23 Centrifuged thirty minutes, during the second cleavage; fixed six hours later; two of the macromeres are small and protoplasmic; tetrasters are present in the other two.

Fig. 24 Same slide as preceding. All of the macromeres have given rise to normal micromeres of similar size, although two of the macromeres are small and purely protoplasmic while the other two are large and contain much yolk and little cytoplasm.

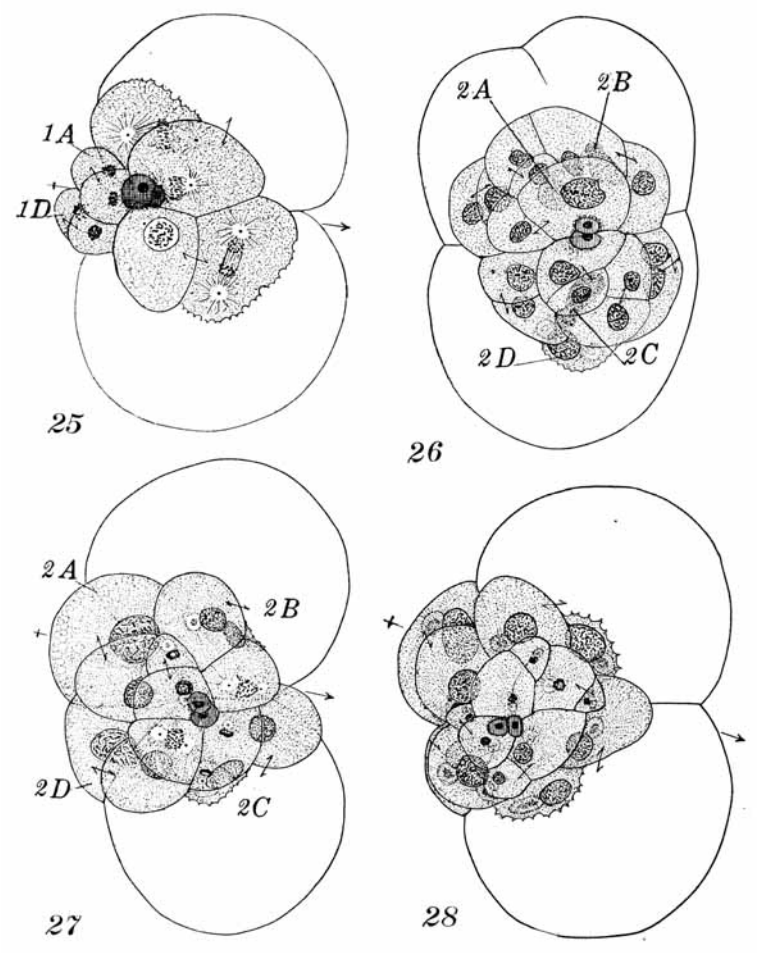


Fig. 25 Same slide as preceding. The minute protoplasmic 'macromeres' (*A* and *D*) have divided equally into the macromeres *1A* and *1D* and the micromeres *1a* and *1d*. The other macromeres (*B* and *C*) have given rise to micromeres somewhat larger than usual.

Fig. 26 Centrifuged for thirty minutes during the second cleavage; fixed twelve hours later; the nuclear divisions of the second cleavage were completed, but the cell divisions were suppressed. Each of these two binucleate macromeres has given rise to two first, and two second quartet cells, just as if four macromeres were present, and each of these micromeres has subdivided in approximately normal manner and is uni-nuclear.

Fig. 27 Centrifuged for thirty minutes in 2-cell stage; fixed six hours later. Micromeres formed from protoplasmic macromeres are of the same size as those formed from large yolk macromeres.

Fig. 28 Same as preceding. The regulation in the formation of micromeres is complete.

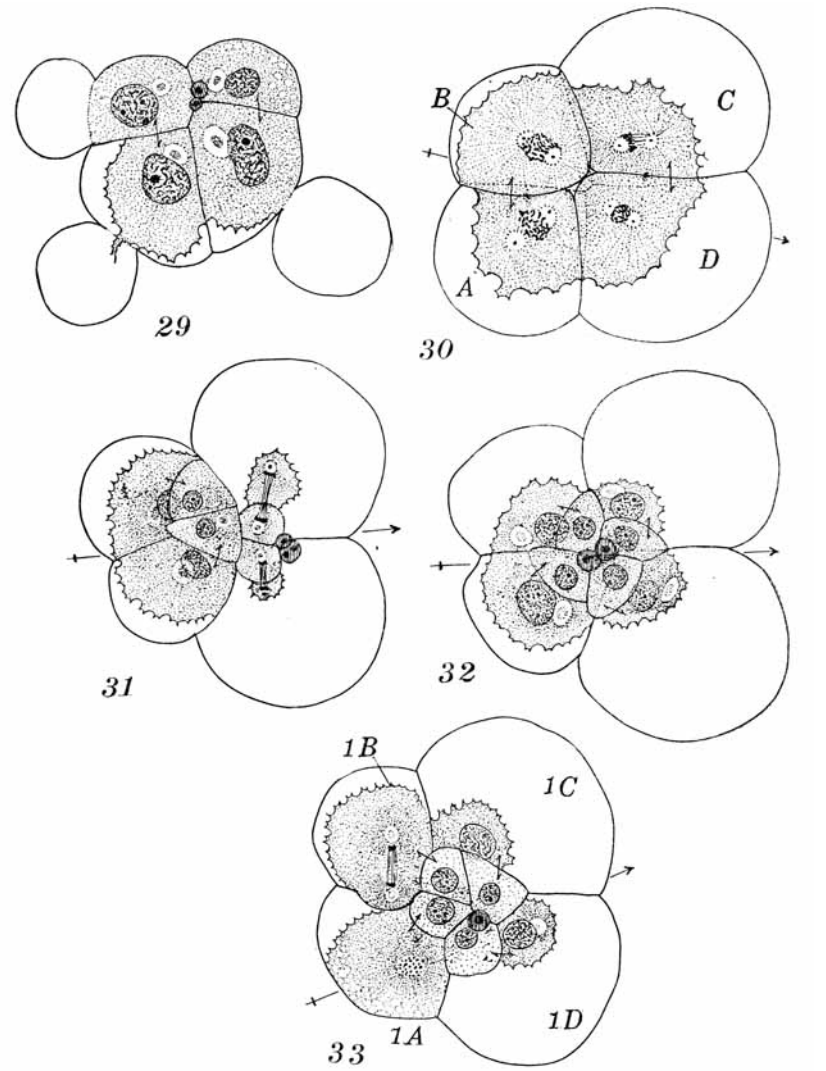


Fig. 29 Centrifuged four hours (2000 revolutions per minute); fixed at once. The yolk was thrown out into lobes, one of which has been detached; the smaller nuclei are in the smaller cells.

Fig. 30 Centrifuged ten minutes in gum arabic during first cleavage; fixed four hours later. Asters and spindles are proportional to the volume of the cytoplasm.

Fig. 31 Centrifuged four hours during the first cleavage; fixed six hours later. Most of the cytoplasm is in the smaller macromeres and these have divided earlier than the larger ones. At least one-half of the cytoplasm in the larger macromeres goes into the micromeres. The first cleavage is not strictly meridional and the spiral form of division is lost. (For explanation of figs. 32 and 33, see p. 98).

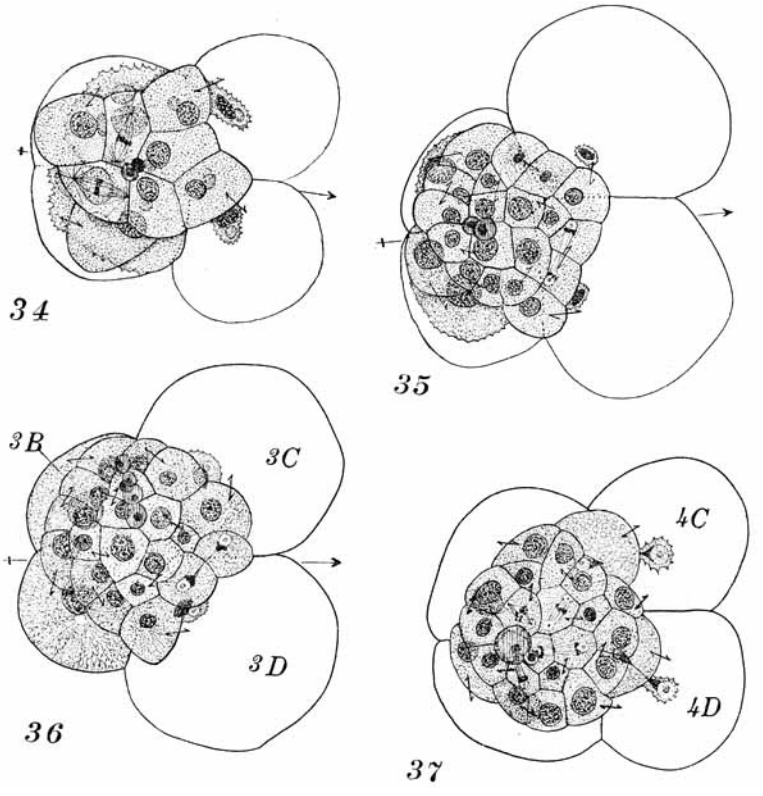


Fig. 32 From the same slide as the preceding. Though the macromeres differ in size and protoplasmic content, the micromeres are all of the same size.

Fig. 33 Centrifuged ten minutes in gum arabic; fixed four hours later. The protoplasmic macromeres are dividing earlier than the others. The size of the micromeres does not depend upon the quantity of cytoplasm in the macromeres from which they came.

Fig. 34 Centrifuged two and one-half hours; fixed twenty-one hours later. The size of the micromeres is almost irrespective of the size of the macromeres; also it is nearly independent of the amount of cytoplasm in the macromeres.

Fig. 35 Centrifuged thirty minutes during the first cleavage; fixed twelve hours later. The micromeres from the protoplasmic macromeres are but little larger than those from the yolk cells.

Fig. 36 From the same slide as the preceding, showing essentially the same conditions.

Fig. 37 From the same slide as the preceding. The cell *4c* forms at the same time as *4d*, though in normal eggs it is formed much later.