THE GENESIS OF THE PLASMA-STRUCTURE IN THE EGG OF HYDRACTINIA ECHINATA

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SIXTY-SIX FIGURES (EIGHT PLATES)

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I. INTRODUCTION

This paper is offered as a contribution to the discussion concerning the origin and relationship of the protoplasmic[1] granules, especially the chromidia and mitochondria. Although this complex and difficult problem has received much attention, it is still far from a satisfactory solution. A considerable group of observers, including Goldschmidt and his followers, have endeavored to extend Richard Hertwig's conception of chromidia in Protozoa, to certain of the protoplasmic granules in the metazoan cell, attributing a nuclear origin to the latter (chromidia) and regarding them as only temporary elements of the protoplasm. A second group of observers, led especially by Meves and Duesberg, are highly skeptical in regard to this hypothesis and have reached the conclusion that the most constant and characteristic of the granules (mitochondria, plastochondria) are purely protoplasmic in origin. According to them, the granules in question form an essential constituent of the protoplasm and are permanent cell-elements which exhibit a genetic continuity, and are the bearers of protoplasmic heredity ('plastochondrial germ-plasm'). The present paper is concerned especially with the conclusions of Schaxel, which are more or less intermediate between the two foregoing conceptions. He finds both types of protoplasmic granules present, 'extra-nuclear chromatin-granules' (corresponding to chromidia) which control differentiation, and mitochondria of purely protoplasmic origin.

[1] The term 'protoplasm' is used throughout the paper instead of 'cytoplasm', according to the usage of the earlier writers on the subject.

II. REVIEW OF SCHAXEL'S WORK

Since Schaxel's results are rather complicated, it may be well to review them briefly here. His interesting conception has been derived from observations on the eggs of animals from widely varying groups (Hydrozoa, '11b; Scyphozoa, '10; echinoderms, '11a; annelids, '12; ascidians, '09) in all of which he describes essentially the following conditions. After the last oogonial division the nucleus is reconstructed in the typical fashion, that is, the chromosomes elongate as smooth threads to form a bouquet-stage. These threads then lose their smooth contour, become granular, and, by further diffusion, soon form a typical granular reticulum, the threads of which as a rule center in the nucleolus. Only a small layer of protoplasm (which at this time takes an acid or 'plasma' stain) surrounds the nucleus. This is the 'pre-emission stage' and the protoplasm is said to be in a state of 'primary achromasie.'

This stage merges gradually into the next or 'emission' stage, characterised by the accumulation of chromatin-granules on the nuclear net, especially at those points where the threads of the net touch the nuclear membrane. At the same time, by filtration through the membrane, groups of granules collect on the outside of the nucleus directly against the wall, at the ends of linin-threads which are poor in granules. The stage of actual emission of material is of rather brief duration and at its close the protoplasm is in a state of 'chromasie.' This 'extra-nuclear chromatin' stains with basic dyes.

The 'post emission' stage follows rapidly and is characterised by further reconstruction of the nucleus, spreading of the extra-nuclear chromatin throughout the protoplasm ('complete chromasie') and rapid growth and differentiation of the yolk. The latter is formed indirectly from the extra-nuclear chromatin, as a small island of yolk in a 'nest' of granules, at the expense of which it increases. In his earliest paper on ascidians, Schaxel ('09) describes a 'secondary achromasie' of the protoplasm of the mature egg, any extra granules not used in the formation of the yolk being absorbed by the phagocytic action of the test-

cells. In the other forms described, Schaxel emphasizes the fact that that part of the extra-nuclear chromatin, not used in the formation of the yolk, remains between the yolk-spheres as 'intravitelline chromatin.' The latter has been traced in the echinoderms and annelids to the end of cleavage, when it has completely disappeared, having, presumably, been used in the process of differentiation. The cells of the blastula are again in a state of 'achromasie.' In Aricia, in which later stages of development were studied, a 'secondary chromatin-emission' occurs in the cells of the gastrula. The granules of this emission are used in the differentation of cells into body tissues. In later papers[2] Schaxel describes mitochondria in the cell, *in addition to* the extra-nuclear granules. He considers the two elements distinct, since the mitochondria are present in the egg before the chromatin-emission occurs and also remain as constant cell-constituents from cell generation to generation, while the protoplasmic chromatin disappears from the cell in the process of differentation.

Schaxel's conclusions regarding the chromatic nature of the extra-nuclear granules are based on both staining reactions and morphological evidence. He is well aware that not too great emphasis can be placed on the fact that the granules stain as chromatin, yet this fact combined with the morphological evidence, has some weight. The morphological argument seems stronger, since granules outside the nucleus at the end of threads having few or no granules, strongly suggests the passage of those granules through the membrane. His figures are most convincing.

My immediate purpose has been to obtain more accurate evidence for or against these interesting conclusions by testing the staining reaction of the extra-nuclear granules in order to determine if possible to what extent such reactions can be relied upon as an indication of their nature; but I have also endeavored to study the origin and ultimate fate of the granules, their relation to the yolk and mitochondria, and the origin and fate of the

[2] Mitochondria as such are not described in Schaxel's early papers. They are described carefully in his later ones on echinoderms and annelids to correct the mistaken conception that the nuclear granules represent the chromatic origin of the mitochondria.

latter. Since Hydractinia echinata shows both mitochondria and protoplasmic granules which take 'nuclear' stains, it forms a favorable object for study of the origin and relation of these elements, the results of which form the first portion of the paper. In a second part a few observations are briefly presented which are intended to supplement previous descriptions of maturation and fertilization in Hydractinia echinata and Eudendrium ramosum.

This investigation was undertaken at the suggestion of Prof. E. B. Wilson, to whom I wish to express sincere thanks for his kindly direction of the work. For suggestions concerning certain experimental aspects of the problem I am indebted to Prof. T. H. Morgan. I wish also to express my thanks to Prof. F. R. Lillie for his generosity in putting a room at my disposal during several summers at the Marine Biological Laboratory at Woods Hole.

III. PROTOPLASMIC GRANULES AND MITOCHONDRIA IN HYDRACTINIA

A. MATERIAL AND METHOD

The material for this work was obtained at Woods Hole during the summers of 1910, 1911 and 1912. The egg of Hydractinia is very favorable for staining tests, since all stages of development, from the early egg in the entoderm to the mature egg in the gonophore, are present on the same stalk, making it certain in staining tests that all stages receive the same treatment. Since a change in staining reaction occurs during development of the egg, this is of much importance. One possible difficulty with this material is the well-known fact that the nucleus early loses its affinity for basic stains and may take acid stains but slightly. If care be used in determining the staining reaction of the chromatin in the very early stages, no confusion need result from this condition.

Various killing fluids were used, the results of which for the sake of brevity and clearness have been tabulated (table 1). Material fixed in fluids containing chromic and osmic acids is best for the study of both nucleus and protoplasm, since the integrity of the elements is much better preserved than in other

TABLE 1

Young egg

STAIN	KILLING FLUID	NUCLEUS	NUCLEOLUS	PS. CHR. GRAN.	LARGE GLOBULES
No stain	Meves[2]				brown
Benda[3]	Meves	brownish yellow	violet or brown[4]	brownish yellow	brown or violet[4]
Bensley[5b]	Bensley[5]	purplish red	clear red	greenish gray	clear red
No stain[5c]	Bensley				light brown
Bleached (no stain)	Bensley				bleached
Bleached (Iron-hem)	Bensley	gray or black	black	gray	black
Iron-hematoxylin and Licht Grün	Flemming's	gray	brownish	dark[1] gray	black
	Meves'	gray	brownish	gray	black
	sub-acetic	blue-black		black	black
	pic-acetic	blue-black	black	black	black
	formalin[6]	black	black	black	black
	100 per cent alc.	blue-black	black	black	black
	alc.-acetic	blue-back	black	black	black
	hot water[7]	black	black	black	black
Thionin and eosin	Flemming's or Meves'	red, blue, young	blue	blue	blue
	sub-acetic	red, blue young	blue	blue	purplish blue
	pic-acetic	red, blue, young	blue	blue	purplish blue
	formalin	blue	blue	blue	blue
Lithium carmine and Lyons blue	Subacetic separate	red	red	blue	blue
	Subacetic together	red	red	red	red
	pic-acetic	red	red	red	red
Auerbach[12]	sub-acetic	red, green, young	blue	greenish blue	greenish blue
	pic-acetic	red, green	blue	greenish blue	greenish blue
	alc. 100 per cent	red, green	blue	green	greenish blue
Saffranin and Licht Grün	Flemming's	purplish or red	red	purplish or red	red
	Meves'	purplish or red	red	purplsh or red	red
Saffranin and Methyl-violet	Flemming's	violet	red	violet	red

[1] The color of the globules is given if they take a different stain from the ground-substance of the sphere. In that case the ground-substance always takes the 'plasma' stain.

[2] Meves' killing fluid for mitochondria is a modification of Flemming's fluid, i.e., the acetic acid content is much reduced (Erg. d. Anat. u. Entw., Bd. 12; Lee, last ed.).

[3] Benda's stain for mitochondria is a double stain of sulphalizarinate of soda and crystal-violet. (Erg. d. Anat. u. Entw., Bd. 12, or Lee, last ed.)

[4] The color of the oil depends on the degree of extraction. With long extraction the violet is removed, leaving the blackening caused by the osmic acid in the fixative.

[5] Bensley's mitochondrial methods (modification of Altman's methods):

a. Killing fluid:

Osmic acid 4%	2 cc.	24 hours
Potassium bichromate 2.5%	8 cc.	
Acetic acid	2 drops	

b. Stains:

Altman's anilin fuchsin	6 min.
1% methyl green 00	dip
Wash in 95% alcohol	

c. Bleach to be used after the above killing fluid:

30 sec	1% potassium permanganate
30 sec	5% oxalic acid
Wash in water	
Dip in	2.5 % potassium bichromate

TABLE 1—Continued

Old egg

NUCLEUS	NUCLEOLUS[11]	PROTOPLASM	MITOCHONDRIA	COMPOUND YOLK	FINE YOLK	OIL
			ight brown			rown
brownish yellow	violet or brown[4]	rownish yellow	leep violet	violet	rown and violet	violet or brown[4]
reddish	red (clear)	eddish purple	ed (purplish)	ed (clear)	urplish red	greenish or brown dark brown ight brown
gray	black	gray	black	black	lack	black
green	black	green	black	black	lack	brownish black
green	black	green	black	brownish black	rownish black	brownish black
blue-black	black	green	[9]	green and black	green and black	black
blue-black	black	green	[9]	green	green	black
green	black	green	black	black	green	black
blue-black	black	green	[10]	black	lack	black
blue-black	black	green	[10]	black	green	black
green	black	green	black	black	green	black
red	blue	reddish	reddish	bright blue	pale blue	brownish blue
red	blue	red	[9]	red	red	red
red	blue	red	[9]	red	red	red
red	blue	red	[11]	blue	pale blue	blue
red	red	blue	[11]	blue	blue	blue
red	red	red	[11]	bluish	bluish	bluish
red	red	red	[11]	bluish	bluish	bluish
bluish red	blue	red	[9]	red	red	red
bluish red	blue	red	[9]	red	red	red
bluish red	blue	red	[10]	red	red	red
green	red	green	bright red	bright red	light red	brownish red
green	red	green	bright red	bright red	light red	brownish red
light violet	red	violet	violet	red	reddish violet	deep purple

[6] Five per cent formalin was made in normal salt solution and the formic acid neutralized with sodium carbonate (Mann,'02).

[7] Hot water proves an excellent fixation both for nuclear and protoplasmic structures.

[8] The pseudochromatin-granules lie in a protoplasmic net taking the 'plasma' stain.

[9] The mitochondria are absent, probably dissolved by the acetic acid of the fixative.

[10] The mitochondria cannot be identified because of the poor fixation given by alcohol.

[11] The mitochondria cannot be differentiated because they stain exactly like the other protoplasmic elements.

[12] Auerbach's stain, used as a chemical test, is a mixture of acidulated methyl-green and acid fuchsin. Jenaische Zeitschrift, Bd. 30, 1896.

fluids. Chemical tests were made on material fixed in indifferent killing fluids, such as alcohol, hot water and formalin in which the formic acid is neutralized. A large number of stains were also used, the results of which are again shown in table 1. Some experiments with 'intra-vitam' stains gave most useful results, which are recorded in table 2.

Other methods to determine the chromatic nature of the protoplasmic granules have been tried, such as artificial digestion of fresh or alcoholic tissue, and tests for nucleo-histone and phosphorus (Mann '02). The possible proteid nature of the granules was also investigated by the use of Millon's reagent. Experiments on the staining reaction of egg-albumen which had been fixed with various ones of the same killing fluids were also made.

B. PROTOPLASMIC STRUCTURES OF THE MATURE EGG

It is essential to differentiate the protoplasmic structures of the mature egg in order to make clear their relation to the extranuclear granules which I prefer to call 'pseudochromatin-granules.' The nucleus will therefore not be considered at present. The egg of Hydractinia is large and filled with various granules and spheres which fall into four groups:

1. *Small simple yolk-spheres* which vary greatly in size and occur throughout the egg.

2. *Compound yolk-spheres,* the largest elements in the egg. They form in general a crescentic layer around the egg, a little below the surface and are found to some extent throughout the center of the egg.

3. *Oily bodies,* about the size of the small yolk-spheres, which darken in osmic acid and are often irregular in shape. These pervade the whole egg.

4. *Mitochondria,* small bacillus-like rods, slightly more abundant at the surface of the egg, but rather evenly distributed throughout the protoplasm.

These elements of the mature egg lie in a finely granular protoplasm, which stains entirely with plasma stain, that is, there are

at this stage no basic-staining granules that correspond to Schaxel's intra-vitelline chromatin[3] (fig. 14).

No one staining method differentiates these various types of structure, since, as a rule, all stain the same way with certain dyes used after some killing fluids. For example, as shown in table 1, if iron-hematoxylin is used on material in Flemming's or Meves' killing fluids, all the elements take the hematoxylin stain in slightly varying degrees of intensity (fig. 16). By the use of various killing fluids and stains, however, they can be shown to be distinct elements in the following way (table 1).

The *oily bodies* are most easily distinguished from the yolk since the former darken somewhat in osmic acid. After staining in saffranin and methyl-violet they take a deep violet color, while the yolk is red. Also, the oil can be completely separated from the other cell-elements by centrifuging the egg.

The *two kinds of yolk*, simple and compound (often indistinguishable from each other when iron-hematoxylin is used after Meves' or Flemming's fixation) show their individuality when stained either with Benda's stain or with saffranin and methyl-violet. These stains reveal, in certain of the spheres, drops or globules which stain differently from the ground-substance of the sphere. The geometrical regularity of these is shown in figure 15, in which young and old compound spheres are represented. After the use of Benda's stain the mature simple yolk is uniformly violet in color, while the compound spheres show brilliant violet globules in a yellow ground-substance. When stained in saffranin and methyl-violet, the globules of the compound sphere are brilliant red and the ground-substance light lavender, while the simple yolk is reddish violet. The most striking differentiation appears in material fixed in picro-acetic killing fluid and stained in iron-hematoxylin and light green, for the simple

[3] These elements are diagrammatically represented in figure 14 in the following manner: Young yolk is represented by solid gray circles; mature simple yolk is represented by hollow circles; mature compound yolk is represented by hollow circles containing small circles; oil is represented by hollow circles containing dots; mitochondria are represented as black rods; the protoplasm is uniformly gray.

yolk takes the plasma stain, while the globules of the compound spheres are the only bodies in the egg which take the hematoxylin.

Since the globules of the compound yolk-spheres are of similar size and may take the same stain as the mitochondria (the violet of Benda's stain) they at first suggest nests of dividing mitochondria. This is an impossibility however, since the compound yolk-spheres are the most conspicuous structures in eggs fixed in killing fluids which dissolve the mitochondria (picro-acetic).

The *mitochondria* are so identified because of their rod-like shape and their typical mitochondrial behavior; that is, they dissolve in fixatives containing acetic acid, do not dissolve in alcohol, are darkened by osmic acid, and give the typical response to the so-called mitochondrial stains (Benda, Bensley or Altman, and iron-hematoxylin). In living material they are highly refractive bodies which take Janus green as a vital stain.

C. HISTORY OF THE YOUNG EGG, BOUQUET-STAGE

The youngest eggs studied were found in the proliferating area of the stalk of the gonophore. Here the entoderm cells are smaller than those of other regions, their protoplasm is not vacuolated, and their nuclei are more deeply staining. The eggs, when differentiated from the surrounding entoderm cells, are somewhat larger and have nuclei in the bouquet-state; a nucleolus is present in which the chromatin-loops center (figs. 1 b, 2,). A slightly later stage shows the nucleolus in which the chromosomes center, pressed against one end of the nucleus (fig. 3). The origin of these cells was not determined. Since they are evidently the result of a recent division (they are usually found in pairs) it seems probable that they are the product of an oogonial division (fig. 1). The egg cells are conspicuous in this stage, since the chromatin and nucleolus take the basic stains intensely. The protoplasm, which is a very thin homogeneous layer around the nucleus, stains but lightly with plasma stains and thus contrasts strongly with the nucleus. This stage corresponds to Schaxel's 'pre-emission stage' The structure is the same, whatever fixative is used. No attempt to study synapsis was made, although many cells showed some evidence of double threads (fig. 1 a).

D. EARLY GROWTH PERIOD

1. Nuclear reconstruction

The growth-period begins directly after the preceding stage, both nucleus and protoplasm increasing in size. Nuclear changes appear, which result in the reconstruction of the nucleus; that is, the densely staining smooth chromatin-loops break up into a coarse, open, granular net, the threads of which center in the nucleolus and radiate to the periphery of the nucleus (figs. 4, 5, 6). The granules of chromatin are conspicuous in this period at the nodes of the net, while a few fine granules lie against the inner wall of the membrane. By the time this stage is reached the chromatin takes most basic stains somewhat less intensely than previously, and may even fail to stain with most basic dyes or take the plasma stain instead. The latter condition is striking when eggs fixed in either sublimate-acetic or picro-acetic killing fluids are stained with thionin and eosine or Auerbach's fluid. As the egg grows, the radial arrangement of the nuclear net is lost (figs. 7, 8, 18) until by the time the egg has reached the gonophore the chromatin is coarsely granular, with little evidence of the net-like arrangement left. At the same time, its affinity for basic dyes has continued to diminish (figs. 19, 20, 22, 25, 26, 27, 28). A more complete account of the disappearance of the net is given later.

2. Pseudochromatin-granules

When but a slight increase in the size of the egg has occurred and when the above nuclear changes are occurring, there appears throughout the protoplasm a fine granular precipitate ('Emission stage' of Schaxel) which, as a rule, takes the basic stains (figs. 4, 5, 6). The granules of which this substance consists I will call 'pseudochromatin-granules.' Striking differences appear in the form and staining reactions of these granules as a result of different modes of fixation. After Meves' method of fixation, they are fine and evenly distributed throughout the egg. If stained with iron-hematoxylin they appear gray in color while the chromatin is deep gray or black. If stained according to

Benda's method, the protoplasmic granules and the chromatin both take the yellow color of the alizarine. Flemming's killing fluid produces a slightly coarser but still evenly distributed precipitate which stains somewhat more intensely with iron-hematoxylin than that killed in Meves' fluid. Several other fixatives (sublimate-acetic, picro-acetic) give very striking pictures, since they produce a strong coarse precipitate, which is not evenly distributed, but more or less massed in the region of the nucleus, and which has a great affinity for basic stains. If stained with iron-hematoxylin, the pseudochromatin-granules are intensely black. If stained with Auerbach's fluid or double stained with thionin and eosin, a striking contrast is produced since the nucleus here takes the 'plasma' stain (fuchsin or eosin), thus emphasizing the fact that the protoplasmic granules stain with the basic dyes (methyl-green or thionin).

In my material I can discover no such striking picture as Schaxel finds of groups of granules on the outside of the nuclear wall at the ends of nuclear threads, regarded by him as centers of distribution and diffusion into the protoplasm. Poor fixation, resulting from the use of sublimate-acetic and picro-acetic fluids, may give rise to some such appearance, but in good fixations I find a uniform distribution of the granules from the beginning (figs. 4, 5, 6, 7, 17). Furthermore, the egg is constantly increasing in size and yet the granular mass which increases with it, retains at all times its uniform distribution. By the time the egg has reached the gonophore, it is of considerable size and is completely filled with these densely staining granules (figs. 8, 9). In the figures they are always represented by gray granules.

3. Basic staining globules

In the young egg, in addition to the pseudochromatin-granules, a second element, consisting of basic-staining globules, appears against the nuclear membrane (fig. 17). Since these globules ordinarily stain as the granules do (iron-hematoxylin), and since they appear very much like similar bodies figured by Schaxel in Pelagia, which he interprets as centers of dispersal of the 'extra-

nuclear chromatin,' it is important to determine their nature. They are differentiated from other cell elements diagrammatically in figures 4, 5, 7, 8 and 10 by the use of circles containing parallel lines. Although they are so closely applied to the nuclear membrane, again I find no evidence of a direct nuclear origin. Their distinction from both pseudochromatin-granules and chromatin is apparent for several reasons. (1) These globules darken in osmic acid while the chromatin and granules as seen in unstained preparations, do not. (2) After Benda's stain, they take a deep violet color, while the chromatin and granules stain yellow. (3) Also in material stained with saffranin and methyl-violet the pseudochromatin-granules are violet, while the globules stain a brilliant red. It is evident therefore that the globules are identical, neither with the nuclear chromatin, nor with the pseudochromatin-granules. Since I find, as shown in the figures, no grouping of the granules around the globules, they can hardly be dispersion-centers. The time when globules first appear in the egg is variable, for they may be present as soon as the pseudochromatin-granules appear, or not until later. They are found up to the time the yolk begins to appear, sometimes lying a short distance from the nucleus, but never far away (figs. 7–10).

To sum up: There are in the early growth-period two protoplasmic elements, one a fine granular precipitate (pseudochromatin-granules) which is scattered throughout the protoplasm and takes basic stains, the other, large drop-like masses which appear near the nuclear wall and which are also probably not chromatin. Neither of these elements appear as such in the mature egg, both being completely used during development.

During this period the egg is migrating from its original position in the entoderm of the stalk toward the gonophore, where it becomes established in the ectoderm. The increase in the size of the egg is considerable during this process, but still further growth takes place after the egg has reached the gonophore before any change in the condition described occurs, or any evidence of the structures characteristic of the adult egg appear.

E. DIFFERENTIATION OF THE EGG

1. Nucleus

The nuclear structure will be described in detail in a later section, but may be considered briefly here. After certain killing fluids (Flemming, Meves, neutral formalin, hot water) the nucleus early loses its affinity for all basic stains and shows a very fine, nearly homogeneous structure, in which little or no evidence of a fine net or chromosomes appears. It now takes even the plasma stains very slightly (figs. 21, 28). After some killing fluids (sublimate-acetic, picro-acetic, alcohol), a heavy net-like precipitate, which takes basic stains strongly, is formed (fig. 22). The nucleus, which reaches a relatively enormous size, lies at first in the center of the egg (fig. 19) and moves to the periphery only a short time before growth of the egg is completed (fig. 23). A basic staining nucleolus, which contains one or several vacuoles staining with acid dyes, is constantly present.

2. Protoplasmic changes

a. Development of the simple yolk. The first elements to appear in the protoplasm are small yolk-spheres which develop into the the simple yolk and possibly the compound yolk-spheres of the mature egg. It seems evident that these spheres develop directly from the pseudochromatin-granules as described by Smallwood ('09). Material fixed in Flemmings or Meves' fluid or formalin is best for these observations, since the individual yolk-spheres are kept distinct. In eggs about one-fourth grown, which have been stained according to Benda's method, the extra-nuclear granules can be seen to have enlarged slightly and uniformly (fig. 9). They still take the yellow-brown color which Benda's stain gives the granules. In a slightly older egg (about one-third grown) some of the spheres are seen to have grown more rapidly than the others, so that the uniformity in size is lost (fig. 10). A few now increase in size very rapidly, giving yolk-spheres of very unequal sizes (fig. 11). It is important to notice that the spheres still stain like the pseudochromatin-granules (yellow-

brown). In my material, as shown in the figures, there are no nests of 'extra-nuclear' granules within which yolk-material with a different staining reaction is secreted, as described by Schaxel. There is, rather, a graded series from the smallest spherical granules to the largest spheres; and these latter do not as yet differ in staining reaction from the pseudochromatin-granules, a condition subsequently seen.

A little before the egg is half grown, however, a change of staining reaction begins. A few of the largest yolk-spheres change their reaction in the Benda stain, the brown of the alizarin being replaced by violet. Figure 19 shows such an egg under low magnification, while figure 12 represents this change diagrammatically. As the egg grows, more and more of the yolk-spheres take the violet color until the whole egg is dominated by violet, a few small yellow ones remaining among the large violet spheres (figs. 13, 14, diagrammatic). This change in staining reaction is very striking on a slide in which all stages in the growth of the egg are present, when obviously they have all received the same treatment in the staining process.

These points are less strikingly but well shown when some of the same material is stained in iron-hematoxylin, since the pseudochromatin-granules are grayish, the young yolk gray, the nature yolk black (figs. 16). Although picro-actic and sublimate-acetic fixations are poor for determining the development of the yolk, the change in staining reaction is even more strikingly shown when iron-hematoxylin counterstained with light green is used, since the granules of the young egg take an intense black, while the yolk-spheres of the mature egg are green. This fixation also makes it evident that the pseudochromatin-granules as such are not present in the mature egg between the yolk-spheres ('intravitelline chromatin' of Schaxel) for there are no deeply staining granules present in such eggs, while young eggs after the same treatment are dominated by black granules.

b. Development of the compound yolk. Since the young stages of the simple yolk-spheres and the compound spheres stain alike, they cannot be distinguished from one another until they are nearly grown, when internal globules appear in the compound

spheres. These are differentatied rather suddenly about the time that the staining reaction of the simple yolk begins to change (fig. 13). The Benda stain shows one or several small globules staining with crystal-violet, within a large compound yolk-sphere, the ground-substance of which is yellow brown (figs. 14, 15). The size of these spheres varies considerably, some of them forming the largest elements in the egg. The globules are arranged symmetrically, and increase in size until, in the mature egg, they may merge and practically fill the sphere. Usually a notched edge indicates such an origin (fig. 15). Certain stains always differentiate the compound from the simple yolk. Thus, saffranin followed by methyl-violet gives the inner globules a bright red color and the external ground-substance a pale violet color. Some of the spheres never reach this state of development but appear in the mature egg with small distinct globules within. It seems probable from staining reactions that certain of the simple yolk-spheres are utilized for the storing of a different material in the form of globules.

c. The appearance of the mitochondria. The mitochondria are not seen in the egg until after the yolk is well formed, but before the staining reaction of the latter has changed. As is usual for mitochondria, special fixatives (Meves' fluid, Flemming's fluid, Bensley's fluid, formalin, hot water) are essential. When Benda's staining method is used after fixation with Meves' killing fluid, a few small rounded bodies, violet in color, appear here and there among the yellow spheres, uniformly distributed throughout the egg (fig. 12). These are larger than the pseudochromatin-granules, about the size of some of the smaller yolk-spheres, and of uniform size. They are comparatively few at first, but gradually increase in number and size until they are so numerous in the mature egg that they fill all the spaces between the yolk-spheres. From their first appearance they take the typical mitochondrial stains, that is, violet in Benda's stain; intense red in Bensley's stain; and deep black in iron-hematoxylin. Since the question of the origin of the mitochondria has been variously answered, it is of interest to determine that point in Hydractinia. As they are scattered throughout the

egg from the beginning, they are certainly not of direct nuclear origin. Again I find no evidence for their origin from the pseudochromatin-granules; for, when the mitochondria first come into view, they are larger than the granules and also stain entirely differently if the Benda method be used. Also they can hardly originate from certain of the yolk-spheres which are of similar size, since again the staining reaction is different and yolk-spheres much larger than the mitochondria are yellow in this stain. These facts all point to their formation de novo in the protoplasm.

d. Oil globules. These appear very early while the egg is still in the entoderm of the stalk; that is, before the yolk formation has begun and even before all the pseudochromatin-granules are formed (figs. 7, 8, 9, 10). They lie scattered amoung the granules, the number varying much in different individuals. They are in some cases few in number, but in others are frequently very numerous even in this early stage (fig. 8). At this time they are easily confused with the globules which appear against the nuclear wall, since they usually take the same stain. That they are distinct from the nuclear globules is apparent by their greater blackening in osmic acid. Also, in double staining with saffranin and methyl-violet the oil is violet while the globules are red. Again, after Benda's stain, the oil is brownish and the globules violet. Auerbach's stain distinguishes the oil from the pseudochromatin-granules, for the latter are green while the former are red. In later stages the oil globules increase greatly in number until the egg is profusely dotted with them (figs. 13, 14). The oil is also formed de novo throughout the growth-period in the protoplasm of the egg.

Since none of the elements of the mature egg correspond to the large globular masses which appear against the nuclear wall in the early growth-period, it is possible that they form a source of elaborated food which is used during the process of growth.

F. THE NATURE OF THE PROTOPLASMIC GRANULES

Because of the theoretical interest connected with this question, it is essential to determine, if possible, whether the extra-nuclear granules are chromatin, extruded from the nucleus as such, or whether they are of protoplasmic origin. Certainly, the first impression given by these granules, staining intensely in basic dyes, favors the conclusion urged by Schaxel, that they are chromatin sent into the protoplasm. The picture is so striking that after many staining tests I was still convinced that such was the case; for all ordinary dyes show identical staining reactions for the granules and the chromatin. It was only after an extended study of the effect of many dyes, both on fixed and living material, that I finally reached a different conclusion, based on the following facts:

1. The uniform distribution of the basic-staining granules, as described above, makes their direct nuclear origin doubtful. In my material no accumulation of the granules against the nuclear wall, no corresponding accumulation of chromatin-granules within the nucleus, is to be seen at any time. This is especially noticeable in the nucleus of late stages when the basic granules are rapidly increasing, for the nucleus is now uniformly homogeneous with an affinity for plasma-stains only.

2. The amount of granular material in Hydractinia can hardly be accounted for if it is all given off in the early growth-period as Schaxel finds in his forms, since this period is of too short duration. An enormous increase in amount takes place after the stage corresponding to Schaxel's 'emission' stage which cannot be explained by simple separation and distribution of pre-existing granules. And since the granules are of the same size in all stages, they cannot be formed by repeated separation without the addition of further material.

3. If further material is added, there is no evidence that it arises from the nucleus, since at the time when the pseudochromatin-granules are increasing rapidly, the nucleus is changing into the so-called resting state, during which time it takes the plasmatic stains while the granules are colored by the nuclear stains. It

seems no more probable that an emission should occur with the chromatin in a diffuse state than when it is in the condensed condition of the early growth-period, at which time no emission could be proved.

4. It is true that globular masses appear against the nuclear wall which after certain stains may be colored like both the pseudochromatin-granules and the chromatin. That they are not the same was shown by Benda's stain and double staining with saffranin and methyl-violet (table 1). This similarity of staining reactions in some cases makes confusion of the three elements easy. The globules also, as stated above, do not form dispersion centers.

5. Although in the majority of cases the pseudochromatin-granules take the basic stains, there is still strong evidence from staining reactions against their chromatin character. Paradoxical as it may seem, the most convicing evidence against their being extruded chromatin is, in fact, from staining tests.

As can be seen from table 1, which presents the results of staining tests on sections after many fixations, the granules usually stain with the basic stains like the chromatin. For example, the result is the same when iron-hematoxylin is used after any killing fluid, whether it contains osmic acid, a heavy metal, or is an indifferent fluid such as neutral formalin, alcohol, or hot water. In fact, in these indifferent fluids, the granules stain as intensely as in material fixed in acid fluids, such as sublimate-acetic and picro-acetic fixatives, while they stain much more strongly than in eggs killed in the usual osmic acid containing fixatives. Again, in material fixed in Meves' fluid and stained according to Benda's method, the granules and the chromatin stain alike (i.e., yellow). Also, staining with thionin or Auerbach's fluid gives the same results, the granules take the basic stain. This is the result with the large majority of stains.

There are, however, some exceptions to this condition. The first fact to come to my notice was the variable results obtained with saffranin and light green when used after Meves' and Flemming's killing fluids. In the early stages the nucleus stains with

the saffranin very intensely while the protoplasm is colored green. The color of the granules differs, however, according to the length of time the slide is left in the solution of light green, and consequently to the degree of extraction of the saffranin, while the chromatin stains in the same way under all circumstances. For example, if the saffranin is but slightly extracted, the granules are bright red like the chromatin, the protoplasm green. If the saffranin is somewhat further extracted, the granules appear purplish (i.e., a combination of the two colors), while the chromatin remains bright red. If the extraction is more complete, the granules become entirely green while the chromatin still appears bright red.

Since the above experiments suggested that the granules may not respond to all chromatin-stains, further tests were made. Several investigators (Crampton '96, Foot '96) have used solutions of lithium carmine and Lyons blue to distinguish the yolk-nucleus from the chromatin; the yolk-nucleus is stained blue, the chromatin stains with the carmine. Obviously, if the protoplasmic granules in Hydractinia are chromatin, they should stain with carmine. I find, however, that here again the granules stain differently from the chromatin, since they take the blue stain and the nucleus the red, this last even in late stages when the nucleus has usually lost its affinity for basic dyes. Further differences between the granules and the chromatin are shown by slight variations in their staining reactions when iron-hematoxylin is used after different killing fluids. As previously indicated, the granules are gray after Meves' killing fluid, slightly darker after Flemming's fluid, and intensely black after fixation in sublimate-acetic, formalin, alcohol, and hot water. The staining tests on preserved material indicate then, that the granules are not necessarily the same in their reaction as chromatin.

More striking and decisive results are given by experiments with stains on fresh material (table 2). I first tried a dilute solution of methyl-green slightly acidulated with acetic acid, as recommended by Lee ('03) for a chromatin stain. Here the results are as in the majority of experiments on fixed material, i.e., the granules take the basic stain (methyl-green) strongly

while the nucleus is unstained. Auerbach's stain, which combines acidulated methyl-green and acid fuchsin, also gives similar results since the granules take the green basic stain and the nucleus stains with the red plasma stain.

Since the above stains contain an acid, several of the more usual basic intra vitam stains were now tried (neutral red, methylene-blue and dahlia). Different results appeared immediately, since both the granules and the chromatin are stained intensely with the neutral red or the methylene-blue. A still different result was obtained with dahlia, for while the nucleus is stained intensely purple, the granules were but slightly tinged.

Since Lee states that these intra vitam stains are harmful to the cell and the results not trustworthy, some further tests were made with some comparatively new stains (footnote 4, table 2), which have been found by other workers to be perfectly harmless to the cell and to stain chromatin (Kite '13). These give the most striking results. Three stains were used; new methylene-blue G.G., new methylene-blue R., and diamond-fuchsin. A very dilute solution was made by adding a small amount of the stain to sea-water containing Hydractinia eggs. After a few hours the eggs were mounted in glycerine. Methylene-blue G.G. and R. gave the most striking results, although diamond-fuchsin gives convincing preparations. The *nucleus in every case takes the basic stain strongly while the pseudochromatin-granules are left colorless.* A noticeable difference in staining reaction occurs between fixed and living material, for in living material the nucleus stains in all stages with these basic stains while in fixed material the nucleus in late stages stains only in acid stains. Since in certain cases the granules in living material do not take the chromatin-stains, while the nucleus does, they cannot be the same as the chromatin in the nucleus and are therefore not formed chromatin extruded from the nucleus. And since with a stain that is acid, the staining reaction is reversed, i.e., the granules stain with the basic dye and the nucleus is either non-staining or stains lightly with acid stains, it seems probable that the presence of the acid is the determining factor in this reaction. The same explanation would hold for the behavior after many killing fluids.

In indifferent killing fluids (alcohol, neutral formalin, hot water) the reversed staining reaction of the granules must be due to some other cause than the presence of the acid. It is possible that mere precipitation changes the chemical composition of the granules, giving it an affinity for basic dyes.

Similarity in staining reaction for the identifying of materials has long be questioned. The physical and chemical nature of staining processes has been ably discussed by many writers (Fisher '09, Hardy '99, Lilienfeld '93, Heidenhain '11, Němec '10, Mathews

TABLE 2

Vital stains and digestion tests

Young eggs

KILLING FLUID		STAIN	NUCLEUS	NUCLEOLUS	PS. CHR.-GR.	LARGE GLOBULES
Fresh Material: 95 per cent or 100 per cent alcohol	Control[1]	iron-hem. and light green	blue black	black	black	black
	Digested		black	black	black	black
	Control[1]	Auerbach	green(young)	blue	greenish blue	greenish blue
	Digested		green	green	green	green
Hot water	Control[1]	iron-hem. and light green	black	black	black	black
	Digested		black	black	black	black
	Control[1]	acidulated meth-green	no stain	[2]	intense green	[2]
	Digested		no stain	[2]	green	[2]
	Control[1]	Auerbach's fluid	no stain	[2]	intense green	[2]
	Digested		no stain	[2]	green	[2]
	Control[1]	New methylene blue	intense blue	[2]	no stain	[2]
	Digested	G. G. (4)	no stain	[2]	green	[2]
	Control[1]	New methylene blue R. (4)	intense blue	[2]	no stain	[2]
	Digested		no stain	[2]	green	[2]
	Control[1]	Diamond fuchsin	intense pink	[2]	no stain	[2]
	Digested		no stain	[2]	pink	[2]
	Control[1]	Millon's reagent	no stain	[2]	brick red	[2]
	Digested		no stain	[2]	no stain	[2]
		Neutral red	deep pink	deep pink	deep pink	[2]
		Methylene blue	deep green	deep green	deep green	[2]
		Dahlia	deep purple	deep purple	deep lavender	[2]

[1] Sections or fresh material were digested in artificial gastric fluid (1 per cent pepsin in 0.2 per cent HCl) at body temperature from two to three hours. In the adult egg the yolk and mitochondria are digested, leaving a framework of protoplasm. Millon's reagent shows that some proteid from the protoplasm of the young egg is removed while the pseudochromatin-granules are not affected.

[2] The fixation is so poor that the mitochondria cannot be identified.

'98, Mann '02, Prenant '10 a, Lundegård '12, et al.). The consensus of opinion on the subject is that even though staining may be chemical it cannot be relied upon as indicative of chemical similarity of cell materials. This is clearly shown by Mathews ('98) and Zacharias ('97) who have demonstrated that previous acid or alkaline treatment determines what stain is selected by any cell element. The above experiments confirm the conclusion that similarly staining elements can hardly be considered identical. Again, experiments with egg albumen fixed in the various killing fluids according to Mann's method, confirm his results in that an

TABLE 2—Continued

Mature eggs

NUCLEUS	NUCLEOLUS	PROTOPLOSM	MITOCHONDRIA	COMPOUND YOLK	SIMPLE YOLK	OIL
blue black	black	green	[2]	black	black	black
greenish gray	black	greenish gray				[3]
bluish red	blue	red	purplish	red	red	red
greenish red	green	red				
green	black	green	black	black	green	black
green	black	green				
blue green	[5]	light gray green	no stain	no stain	no stain	[5]
blue green	[5]	gray				
purplish pink	[5]	grayish pink	no stain	no stain	no stain	[5]
purplish pink	[5]	grayish pink	no stain	no stain	no stain	[5]
intense blue	[5]	pale gray	no stain	no stain	no stain	[5]
no stain	[5]	pale gray	no stain	no stain	no stain	[5]
intense blue	[5]	pale gray	no stain	no stain	no stain	[5]
no stain	[5]	pale gray	no stain	no stain	no stain	[5]
intense pink	[5]	pinkish gray	no stain	no stain	no stain	[5]
no stain	[5]	pinkish gray	no stain	no stain	no stain	[5]
no stain	[5]	no stain	no stain	no stain	no stain	[5]
no stain	[5]	no stain	no stain	no stain	no stain	[5]
pink	pink	pale pink	no stain	no stain	no stain	[5]
pale green	pale green	very pale green	no stain	no stain	no stain	[5]
deep purple	deep purple	pale purple	no stain	pale purple	pale purple	[5]

[3] The oil globules were not identified in any of these tests.

[4] These stains furnished by the Casella Color Company, New York, were found to be perfectly harmless used in dilute solution (Kite '13).

[5] The nucleolus, large globules and oil have not been identified in fresh material.

apparently differential stain may be obtained on coagulated egg-albumen which may depend on purely physical differences or differences in density. Experiments were also made to test Heidenhain's contention that selective staining is most successful when the stains are applied simultaneously and progressively. Since different results are obtained when stains are used successively, sumultaneously, progressively, or regressively, a true selective value can hardly be maintained. The evidence from all sides indicates that staining reactions are unreliable as chemical tests.

6. Artificial peptic digestion tests were tried to determine if possible, whether the granules are chromatin (table 2). If one accept the non-digestion of any material in the cell as proof of its chromatic character, then this test supports the nuclear origin of the granules. Material killed in alcohol and in boiling water as well as fresh material, was used in these tests. The fixed material was sectioned and digested on the slide in a 1 per cent solution of pepsin in a $\frac{1}{5}$ per cent solution of hydrochloric acid. Such digested sections together with an undigested control were stained with iron-hematoxylin and light green or with Auerbach's fluid. The fresh material was stained both before and after digestion with acidulated methyl-green, Auerbach's fluid, new methylene-blue G.G., new methylene-blue R., and diamond fuchsin, and mounted in glycerine. In all cases, after two to three hours of digestion at body temperature, the yolk, mitochondria and the bulk of the protoplasm were digested in the adult egg, leaving a slight framework or net which stained only with plasma stains. In the young eggs the protoplasmic granules remain undigested and take the basic stains, while the nucleus is non-staining. This reversal in staining of digested material as compared with fresh material when stained with the same neutral vital stains may well be due to the acid in the digestive medium, a point which supports the view that staining depends on previous treatment.

Even though the granules in fixed material, whether digested or undigested, stain with basic dyes, a slight difference between the two occurs. If two slides, one containing digested, the other undi-

gested sections, are run back to back through staining jars to ensure similar treatment, the granules in the digested sections after staining with iron-hematoxylin, take the stain much less intensely than those of the control slide. Also after Auerbach's stain the granules are grayish in the digested material, rather than green as in the undigested sections.

Still another point suggests that there may be other materials than chromatin in the cell which may not be digested by peptic digestion. In sections placed in a digestive medium in which the acid content is strong 0.5 HCl and digested either a long time (18 to 20 hours) at room temperature, or a short time (2 to 3 hours) at body temperature, all the material in the nucleus is digested while the granules remain undigested. This confirms the impression that the granules are of a different nature from chromatin. Again, the failure of a certain material to be digested by peptic digestion is not necessarily a proof of its chromatic character, since peptic digestion depends on the degree to which a substance is penetrated and therefore on the density of the material. In the above case it is possible that the acid aids in the penetration of the nuclear material but is unsuccessful in penetrating the granules. On this basis peptic digestion is no test for chromatin. Since pancreatic digestion digests the whole cell, it is evident that an alkaline medium is essential for the digestion of the pseudochromatin-granules.

7. Millon's proteid test also is in harmony with the possible chromatin nature of the granules. Before digestion, young eggs containing granules (as well as mature eggs) give the typical brick red reaction very strongly, while no proteid test is obtained after digestion, although the granules are still present as stated above. The proteid reactions in the undigested egg must then be given either by the protoplasm or some proteid associated with the granules, rather than by the granules themselves.

8. Attempts to locate chromatin by testing for histone as described by Mann ('02) were only partially successful. The results, however, support the view that the granules are not the same as the chromatin. The strongest color reaction indicating the presence of histone occurred in the nucleus, while the proto-

plasm of the young eggs containing the granules, although responding to the test slightly, did so no more strongly than the adult egg in which no granules are present.

9. Attempts to locate chromatin derivatives in the protoplasm by tests for phosphorus as described by Mann were also tried. These again are useless since Bensley ('06) has recently shown that the standard tests for phosphorus are unreliable.

To sum up: The balance of the evidence in Hydractinia decidedly indicates the nonchromatic nature of the granules in question. In all cases which seem to indicate the contrary conclusion (some staining and digestive tests and tests for proteid) the result can be interpreted in some other way. When to this are added the definite results from staining reactions in both fixed and living material and the morphological evidence that has been given above, we are, I think, forced to the conclusion that the granules are not chromatin extruded as such from the nucleus.

The above conclusions differ decidedly from Schaxel's, which appear to be based on very careful and detailed observations. Whether these conflicting views are due to the different forms studied, or to the fact that the large number of methods of fixation and staining used on Hydractinia has made the nature of the granules more evident, it is impossible to say. Since Hydractinia behaves, when Schaxel's methods are employed, in the same way that his material does, it seems probable that if the above methods were used on the forms studied by Schaxel they would yield similar results.

G. OTHER ACCOUNTS OF CHROMATIN-EMISSION IN HYDROIDS

Chromatin in the protoplasm has recently been described by several observers in a number of Hydroids. C. T. Hargitt ('13) finds a chromatin-emission in Campanularia brought about by the fragmentation of the nucleolus. The yolk develops from this extruded chromatin. Stschelkanowzew ('06) has described in Cunina a similar chromatin-emission through chromatin-nucleoli (secondary nucleoli). This condition is not found in Hydractinia, as the description of the nucleolus in a later section indicates. A

still different method of chromatin-emission is described by Smallwood ('09) in Hydractinia. Soon before maturation small particles of chromatin leave the nucleus and wander into the protoplasm. Smallwood says, "The reason for regarding them as chromatin is because they give the same color reactions as similar shaped bodies in the nucleus, and for the further reason which is obvious in figure 1, namely, the actual migration of the chromatin from the nucleus." An exactly similar chromatin-emission is described by Smallwood ('07) for Pennaria, taking place here, however, after maturation rather than before. Also in this form the chromidia are stated to arise from both male and female germ-nuclei. In these two Hydroids the protoplasmic chromatin is connected with neither yolk formation nor differentiation. As will be shown in the next section, I have traced the nucleus in Hydractinia through the growth-period, the reappearance of the chromosomes and maturation stages, and in no case are bodies of the type described to be found. From Smallwood's figures, it is evident that the fixation is defective, since a very strong coagulation net is present which does not appear with good fixation. It seems to me the bodies in question may well be artifacts.

Trinci ('07) has described basophilic granules in the oocytes of a number of Hydroids, which he considers as a differentation of the protoplasm brought about by the influence of the nucleus and as belonging in the same category as chromidia, mitochondria or plastosomes. The granules disappear during the development of the egg.

Van Herwerden ('13) has recently tested Schaxel's hypothesis of the nuclear origin of the basophylic granules by the use of nuclease. After treating the mature echinoderm egg (in which mitochondria are visible in life) with a preparation of nuclease, he finds that the mitochondria (basophilic granules) have disappeared. He concludes that the mitochondria are a nucleinic acid compound and therefore properly chromidia. Since in the young, living egg he can see none of the basophilic granules which appear in fixed material, he believes the latter are artifacts. Unlike Schaxel, however, he holds the mitochondria to be developed from this basophilic substance, since it is also a nucleinic acid

compound as shown by its digestion with nuclease. Direct observation on young, living eggs failed to show a direct migration of material from the nucleus into the protoplasm. Diffusion currents present in the egg, coincident with a slight nuclear shrinkage, he feels, favors the diffusion of a soluble substance through the nuclear wall. That he is dealing with a substance that differs from the granules of Hydractinia is apparent, since vital stains give different results in the two cases. His granules are stained with dahlia, those of Hydractinia are not. With methylene-blue and neutral red his granules do not stain, while those of Hydractinia do. Also the fate of the granules differs in the two cases. They can hardly then be homologized. Although he has been unable to furnish any more definite proof of a direct migration of nuclear material into the cytoplasm, the nuclease digestion indicates nucleinic acid present in the granules and mitochondria.

H. MITOCHONDRIA

1. Experimental

The presence and behavior of the mitochondria as seen in sections have been sufficiently described above. Further investigation of the function and behavior of these bodies was carried on by means of some centrifuging experiments. In previous experiments of this sort, no attempt has been made to locate the mitochondria after centrifuging the egg and to determine their further behavior in development. Hydractinia eggs within 5 minutes after fertilization were placed in a water-centrifuge and revolved at a moderate speed for $1\frac{1}{4}$ to $1\frac{1}{2}$ hours, or until the control eggs had divided once. The cell-materials are separated into three layers (fig. 61, a, b,). Sections of eggs killed as soon as removed show the oil at the small end of the pear-shaped egg, forming an oil-cap. A clear protoplasmic layer lies below this, while the broad end of the egg is filled with a mingled mass of yolk and mitochondria (fig. 62). If such eggs were removed to sea water and allowed to develop it was found that the first cleavage-plane cuts the egg without reference to the stratification

(fig. 63). The majority of the eggs cleave so that the different materials are equally distributed in the two blastomeres; often, however, the distribution is unequal. *Complete separation of the kinds of material may occur, as when the cleavage plane comes in through the protoplasmic layer between the ends* (figs. 63, *b;* 65, *g;* 66, *a, b, e, f*). Sections show this to be due to the position of the nucleus in regard to the stratification. Since the eggs are not centrifuged sufficiently to move the nucleus, it may lie in any relation whatever to the egg-materials. It is, however, usually found in the protoplasmic layer (fig. 64, *a, d*) in which case the egg divides so as to distribute the materials equally (fig. 63, *f*). The nucleus may also lie in the yolk-end of the egg (fig. 64, *c*) in which case the cleavage-plane comes in through the yolk (fig. 63, *a*). The nucleus not infrequently appears at one side of the protoplasmic layer (fig. 64, *d*), in which case the first cleavage-plane separates the two kinds of material, one blastomere receiving in addition to part of the protoplasm, yolk and mitochondria and the other the oil mass.

Individuals showing various distributions of the materials were isolated and their development was followed, apparently normal swimming larvae resulting (fig. 65). The yolk and mitochondria are confined to one region of the resulting planula, the oil to another. Also eggs which cleaved so that the different materials segregated in the two blastomeres were cut apart, separating the blastomere containing yolk and mitochondria from the one containing oil (fig. 66). These were isolated and the development followed, and although many died, I succeeded in getting a considerable number of these half larvae. If the protoplasmic area were nearly evenly divided between the two blastomeres, both were apt to live. So far as I could tell those that lived were perfectly normal, except in size, the one being small and white from its oil content, the other large and greenish from the yolk content. Both were ciliated planulae. Sections of these planulae which were killed in Meves' killing fluid and stained with Benda's method, show the mitochondria apparently unchanged in one, while the other contains none. It is apparent, then, that up to this point of development the mitochondria are not essential for

development. Hydroid planulae are hard to carry beyond this point of development in the laboratory, since any disturbance prevents the planula from attaching, which is essential for further development. Even so slight a disturbance as changing the water in the dish is sufficient to prevent attachment, so that further development after so great a disturbance as separating the blastomeres is impossible.

The above experiments are of value only in indicating that the mitochondria are not essential for differentation as far as the planula-stage and that they can hardly be vital constituents of the protoplasm since they may be centrifuged out of the protoplasm like any metaplasmic body, such as yolk.

2. Discussion

Mitochondria in Hydractinia do not agree in a number of points with descriptions of these bodies in other forms. An extensive review of the subject will not be attempted, however, since it has been so well discussed and reviewed by many recent investigators (Fauré-Fremiet '10, Prenant '10 b, Montgomery '11, Duesberg '11). Duesberg presents a monumental review of the literature on the subject in which he brings together in classified form and in the most exhaustive way, all papers concerned with mitochondria and chromidia. The opposing views as to the nuclear or protoplasmic nature of these bodies have already been stated. The above writers assume the identity of mitochondria and chromidia. Schaxel, on the other hand, considers them quite distinct, as has been sufficiently indicated above. It is also apparent from the aforegoing description that Hydractinia corresponds with Schaxel's observations in this respect, that is, that two distinct elements are present, mitochondria of undoubted protoplasmic origin and basophilic granules in the protoplasm. The latter, however, are not chromatic in Hydractinia and therefore not chromidia (extra-nuclear chromatin of Schaxel).

I also agree with Schaxel in finding that the pseudochromatin-granules first appear in the early growth-period of the egg, thus giving no evidence of continuity from cell generation to generation.

Again, Schaxel finds mitichondria already present in the early growth-period of the egg before his 'chromatin-emission' occurs. In this respect results with Hydractinia are different, since the mitochondria appear only after the egg is a third grown. Hydractinia is an exception to the rule in this, since in most forms the mitochondria are either already present or appear in the early growth-period. The possibility of direct nuclear origin of the mitochondria in Hydractinia is also excluded, since they arise de novo throughout the egg and are not collected in a definite body against the nucleus (yolk-nucleus) as described in many forms.[4] The mitochondria in Hydractinia do no contribute to the formation of the yolk, as described for other forms by a number of workers.[5] I also find no grouping of the mitochondria around an idiozome, indicating their origin through the retrogressive development of the 'centroplasm' as suggested by Vedjovsky. Again, Hydractinia gives no evidence of the mitochondria forming a part of the architecture of the protoplasm (Fauré-Fremiet and others), since in centrifuged eggs they are carried to one pole of the egg together with the yolk, leaving a free layer of protoplasm which has the usual protoplasmic structure. Since the blastomere of a centrifuged egg containing no mitochondria develops into a swimming larva, they can hardly be vital units of the protoplasm. I find no indication of their multiplication by division

[4] A yolk nucleus of mitochondria is described in the eggs of the stint (Lams '04, Arch. Anat. micr., T. 6); Rana (Lams '07, Arch. Anat. micr., T. 9); Proteus (Schmidt '04, Anat. Hefte, Bd. 27, and M. Jörgensen '10, Festschr., R. Hertwig); Testudo (Loyez '05, '06, Arch. Anat. micr., Bd. 8); chick (D'Hollander '04, Arch. Anat. Micr., T. 7); some birds (Loyez '05, '06, Arch. Anat. micr., T. 8); human egg (Van der Stricht, '05, Bull. Acad. Belgique); cat, (Russo, '09, '10, Arch. f. Zellfor., Bd. 4–5); bat and guinea-pig (Van der Stricht '05, Compt. Rend. Assoc. Anat., Geneve); Ascaris (Schoonjans '09., Bull. Soc. Roy. Sci. Med., Brussels); Ciona intestinalis (Loyez '09, Assoc. Anat., Nancy).

[5] Yolk is described as being formed directly from the mitochondria by Russo ('09, '10) in cat; Loyez ('09), ascidians and human egg, Compt. rend. Assoc. Anat., Paris; Fauré Fremiet ('10, Arch. Anat. micr., T. 11); in Lithobius; Zoja ('91, Mem. del R. Inst. Lomb. di Sci., vol. 16) etc. Yolk is described as formed indirectly under the influence of mitochondria by Van der Stricht ('05) in the bat; Lams et Devorene ('08, Arch. de Biol., T. 23) in some mammals; Van Durne ('07, Ann. Soc. Med. de Gand, T. 88); Schooonjans ('09) Ascaris; Bluntschli ('04, Morph. Jahrb., Bd. 32) etc.

at any time during the growth-period or cleavage, as suggested by Duesberg ('10) and Fauré-Fremiet ('10 a). Their origin and behavior in Hydractinia indicate that they may be either precociously differentiated portions of the protoplasm (Vedjovsky' 07) or metaplasmic bodies.

Scepticism as to the identity of the bodies described as plasmosomes, chondriosomes, chromidia, ergastroplasm, etc., has been expressed by a number of observers (Veratti '09, Penas '11, Lundegård '10, Gurwitsch '10 and others) who believe that they have nothing in common but their name. Such an impression is certainly gained in reviewing the literature. The experiments with staining tests lead the writer to join these investigators in the belief that structures which have the same staining reactions may have been confused. If the standard tests for mitochondria are to be relied on for identifying them, then the mitochondria of Hydractinia, which respond to these tests, do not conform in many respects to the conditions found in other forms.

IV. MATURATION PHENOMENA AND AMITOSIS IN HYDRACTINIA AND EUDENDRIUM

When this work was begun it was my purpose to re-examine the eggs of several Hydroids which had been described as showing no mitotic figures during maturation, a nuclear disintegration or fragmentation occuring at the time of the disappearance of the germinal vesicle. The suggestion was made "that reduction phenomena of maturation may well be accomplished without any of the complex and spectacular processes of mitosis" (Hargitt '06). Nuclear reconstruction was described as occurring later through the collection of these fragments in several 'nuclear nests' throughout the egg (C. W. Hargitt, Pennaria, '04, Eudendrium '04, Clava '06; Allen, Tubularia crocea, '00); and the cleavage of the egg as frequently amitotic. Smallwood ('09) and G. T. Hargitt ('09), have since established the occurrence of typical maturation phenomena and mitotic cleavage in Pennaria and Tubularia, while independent studies by the writer ('09) gave the same result both in Pennaria and in Clava. Since

Eudendrium, among the above forms, has not been re-examined, a brief account of the maturation stages occurring in this form will be given here. This section is concerned chiefly with a brief account of the maturation stages in Hydractinia to supplement Smallwood's account which as he states is incomplete because of lack of material. I have been fortunate in finding the stages lacking in his description.

A. HYDRACTINIA

1. Nucleus of the growth-period

As described in the previous section, in the early growth-period the nuclear net is centered in the nucleolus from which it radiates (figs. 2, 3, 4). As the egg grows this radial arrangement is lost, the chromatin assuming a reticular form (figs. 7, 8, 25). While these early stages show the same nuclear structure after preservation with any killing fluid, later stages are profoundly modified. When preserved in sublimate-acetic or picro-acetic killing fluids, a coarse, deeply staining, granular reticulum appears in a colorless ground-substance (fig. 12). That this reticulum is a coagulation phenomenon is suggested by comparing this nucleus with those shown in figures 19, 20, 21 and 23, which are sections of eggs killed in Meves' killing fluid. Here the nuclear net, which has changed into a fine net, stains more lightly in basic dyes than after the former fixatives, and lies in a finely granular, homogeneous ground substance which stains lightly with plasma stains (figs. 18, 19, 25). Figures 20 and 28 show a slightly older stage in which a process of diffusion of the chromatin net, previously begun, has proceeded until there is just a suggestion of the net in the homogeneous ground-substance. This leads directly to the condition shown in figure 21, in which all trace of the net has disappeared and only the ground-substance is left. The question of the method of disappearance of the chromatin will be taken up in detail a little later. The net completely disappears before the egg is one-third grown. The nucleus lies at the center of the egg until near the end of the growth-period when it moves to the free surface of the egg.

2. Maturation stages

The maturation phenomena take place while the egg is still in the gonophore as Smallwood ('09) states, and not after leaving it (Bunting '94). The lightly staining, homogeneous condition of the nucleus, which has long been recognized as a characteristic of the hydroid egg, and which has led to much confusion concerning the maturation stages, persists until the nucleus breaks down to form the chromosomes. The first indication of reappearing chromosomes occurs in gonophores killed from 20 to 30 minutes before eggs from the same colony are deposited. Since the reformation of the chromosomes is best seen in material preserved in fluids which do not cause a heavy precipitate, the following description is based on material fixed either in Meves' or Flemming's fluid, neutral formalin, or hot water. The deeply staining nucleolus is usually still present at the inner border of the nucleus; its history will be described more fully later.

Out of the apparently homogeneous ground-substance of the resting nucleus, there appear very lightly staining threads on which are groups of granules staining a little more intensely than the threads. These threads appear in pairs, either parallel or X like in form (figs. 30, 31). In either case many fine branches merge from the main threads into the general homogeneous ground-substance. The number of these groups of threads in a single nucleus corresponds to the haploid number of chromosomes (12 or 14). The exact haploid number has not been determined but since 14 such pairs of threads is the number most frequently found in a nucleus and since 14 tetrads is the usual number found in the later stages (figs. 4, 46), it seems certain that this represents the haploid number and that they are bivalent chromosomes. The chromosomes now condense rapidly into tetrads which are very much smaller than the crosses. The initial stage in this process consists in a shorting of the arms of the *X* and the collection of the granules in a mass at the center, the latter taking a slightly deeper stain than before (fig. 32). In a later stage (fig. 33) the ends of the arms of the *X* are still visible, although the bulk of the granules appear at the center. The condensation

consists apparently in the migration of the granules along the linin-threads toward the central point, leaving the lightly staining net merging into the ground-substance. The chromosomes now condense rapidly into small compact tetrads which stain intensely. A number of stages in the formation of a tetrad are shown in figure 24. The nuclear membrane breaks down about this time and the chromosomes which have been scattered through the nucleus collect at the center (fig. 24).

Up to this point there has been no evidence of a spindle. When it does appear, the chromosomes are already collected in the center of the nuclear area (fig. 35) and the spindle apparently arises in connection with them from the achromatic portion of the nucleus. The spindle, which is many times smaller than the nucleus, differs entirely in structure according to the fixation. The general topography of such a spindle in the center of the nuclear area after Meves' fixation is shown in figure 37. The much enlarged spindle shown in figure 38 makes it clear that no centrosomes or astral radiations are present, the blunt spindle lying free in the nuclear area. If material is fixed in sublimate-acetic solution, in addition to the spindle, small asters appear, which are continuous with the coarse net present in the nuclei of such eggs (fig. 36). They give every appearance of being part of the coagulation phenomena caused by the killing fluid. In some cases a small centrosome-like body occurs at the center of the aster. But since it is not constant and may be asymmetrically placed in regard to the spindle, it also seems to be a result of the coagulation. In fact, I have not been able to find a true division-center, either outside or within the nucleus. The spindle fibers arise, apparently independently of an aster or centrosome, directly out of the nuclear ground-substance, for the spindle appears directly in the center of the large nuclear area, a considerable layer of the nuclear plasma surrounding it. The tetrads are now drawn on to the spindle and become arranged in an equatorial plate (figs. 37, 38). As stated above, the number of tetrads in the equatorial plate has not been definitely determined, the number being between 12 and 15. Since 14 was more commonly present and it is the number appearing in the polar body, it seems probable

that this is the haploid number (figs. 39, 40, 46). A small element near the center of the plate is characteristic.

The spindle, still without asters or centrosomes, now rotates 95° until it is perpendicular to the surface, and then moves out of the nuclear area toward the surface, where the first polar body is formed (figs. 43, 44, 45). As seen in some of the figures, a single or double granular mass—the remains of the nucleus—is left behind in the protoplasm, where it is absorbed. Since the manner in which the tetrads are formed is not determined, it is impossible to interpret this division in terms of reduction. The second polar spindle is formed immediately, as shown in figure 46. The egg is now shed from the gonophore, a small female germ nucleus being very rapidly reconstructed from the remaining chromatin (fig. 47). The very great difference in size between this nucleus and the germinal vesicle has been sufficiently emphasized by previous writers on Hydroids. The egg, which has been somewhat flattened in the gonophore, rounds up when shed.

This description of the formation of the first polar spindle differs from that given by Smallwood, who finds it appearing with asters in the protoplasm in connection with a very small nucleus which is many times smaller than the typical germinal vesicle of Hydractinia. The small size of the nucleus and the position of the spindle outside of the nucleus makes it probable that he has figured the first cleavage-nucleus and spindle. Since I have traced consecutive stages in the breaking down of the germinal vesicle and the formation of the chromosomes and spindle, (stages which Smallwood lacked) it seems conclusive that a blunt spindle minus asters and centrosomes lying in the center of the large nuclear area, is typical for Hydractinia. This conclusion is supported by the condition found in other forms, since a blunt spindle minus asters has been described in a number of Hydroids (Gonothyrea, Wulfert, '02; Clava squamata, Harm '02; Clava leptostyla, Beckwith '09; Eudendrium, present paper; Linerges, Conklin '08; Cordylophora, Morningstein '01; Cunina, Stschelkanowzew '06). Further the formation of the spindle within the nuclear area itself also find support in the condition described

for Clava by Harm ('02) who finds the first polar spindle very small and formed within the germinal vesicle from the achromatic portion, and the same condition in Cordylophora, as described by Morningstein ('01).

3. Fertilization

The egg is fertilized as soon as it is shed, the spermatozoon entering at any point on the surface. A fetilization-membrane is formed (fig. 42). The method of union of the two germ-nuclei depends on the point at which the spermatozoon enters. If, as often happens, it enters near the female germ-nucleus (fig. 47) the sperm head may enter the egg-nucleus bodily, without expanding (fig. 49). Here the chromatin of the egg-nucleus is already collected in masses to form the chromosomes of the first cleavage-spindle. An aster accompanies the sperm head in this case as in others, no spindle as yet having formed, however. If the sperm enter at some distance from the female germ-nucleus, it enlarges as usual before union and a spindle develops in connection with it. The degree of enlargement varies, as shown in figures 48 and 50. Complete fusion of the two nuclei may take place before the breaking up into chromosomes but this is not essential.

The entrance of the sperm head directly into the egg nucleus without expansion is described in some other Hydroids (Wulfert, '02, Gonothyrea; Harm, '02, Clava squamata; I have also observed it in Pennaria). Also as the figures show, I do not find the sperm head in Hydractinia forming a group of vesicles as figured by Smallwood ('09) in Hydractinia and Smallwood ('09) and Hargitt ('09) for Pennaria. It expands directly into a single vesicle.

4. Fertilization membrane and mitochondria

The hydroid egg is usually spoken of as naked (Wilson '00, Hargitt '04). Smallwood ('09), however, has described a membrane for Pennaria, formed at fertilization. I have found this membrane easy to demonstrate in fresh material if intra vitam staining methods described by Kite ('12) are used. In Hydrac-

tinia a membrane is also present. Sections of an unfertilized egg show a yolk-free area at the surface of the egg in which mitochondria are scattered irregularly (fig. 41). No distinct membrane can been seen at this time. Sections of fertilized eggs show the mitochondria arranged in a distinct layer directly at the surface of the egg, a more or less free space being left between them and the yolk-spheres. Outside the layer of mitochondria a very thin, transparent layer or membrane appears, which as a rule clings closely to the surface of the egg (fig. 42). The method of formation of the membrane was not determined.

5. Nucleolus

As stated earlier, a nucleolus is already present in the egg after the last oogonial division, when the chromatin is in the bouquet-stage (figs. 1, 2, 3). Its origin was not determined and it stains intensely in basic dyes. As the chromatin breaks up into a net, it is still pressed against the nuclear wall and may already show vacuoles (fig. 4). When the radial arrangement of the net is lost, the nucleolus is no longer flattened against the nuclear wall, but may be well toward the center of the nucleus (figs. 7, 18). In general, however, it retains its excentric position (figs. 19, 21, 22). During the growth-period the nucleolus increases in size and becomes vacuolated, the skeleton retaining its intense staining capacity for basic dyes while the vacuoles stain with acid dyes. One large vacuole may occupy the center, leaving a rim of basic staining material (fig. 29 b), or many fine vacuoles may appear, making the nucleus more or less spongy (figs. 27, 28, 29 a). Both conditions are typical for Hydroids. The increase in size continues throughout the greater part of the growth-period, a point in which Hydractinia differs from Pennaria, as described by Hargitt, since in the latter form growth stops and nucleolar disintegration begins as soon as the spireme is completely broken up. About the time of maturation—usually before the breaking down of the nuclear membrane—the nucleolus dwindles and disappears in the substance of the nucleus by a process of dissolution and not by fragmentation as described by Hargitt for Tubularia. It may have

disappeared before the reappearance of the chromosomes (fig. 23), or, as is more common, it may be disappearing as the chromosomes are reforming (fig. 29, a).

Because of its staining reaction, the nucleolus was at first thought to be chromatic. Since the nucleus, throughout the greater part of the growth-period, is non-staining with basic dyes while the nucleolus takes these stains intensely, it at first seemed evident that the chromatin which is to form the chromosomes is stored in the nucleolus during this stage, a condition described for a number of forms.[6] This conclusion proves to be unfounded after further staining tests, since the nucleolus does not always take the characteristic chromatin-stain (table 1.) For example, in Hermann's saffranin methyl-violet stain, the nucleolus is red while the chromatin of the 'resting' nucleus is violet. Also, in Bensley's acid fuchsin methyl-green stain the nucleolus stains with the fuchsin or plasma stain. Again, in Auerbach's stain, which ordinarily stains the chromatin green, the nucleolus is stained blue. The strongest evidence that the nucleolus is not chromatin is given by Benda's stain, after which the chromatin is yellow-brown and the nucleolus violet. This point is confirmed by Dublin ('05) who finds decisive proof with Auerbach's stain that the basic staining nucleoli of Pedicellina are not chromatin. The nucleolus in Hydractinia has at no time any direct connection with the chromosomes as described by Guenther ('03) and Dublin ('05). There is no evidence then that the disappearance of the nucleolus at the time of the reappearance of the chromosomes bears any relation to the same. From the above description of the nucleolus in Hydractinia, it is evident that no fragmentation of the nucleolus contributes to the formation of basic granules (pseudochromatin-granules) which lie in the protoplasm, as Hargitt finds in Campanularia.

[6] Chromatic nucleoli have been described among hydroids in Forskalia and Agalma by Schaxel ('11); Gonionemus, Bigelow ('07); Campanularia, Hargitt ('13); Cubomedusa, Conant ('98) Hydra, Downing ('09); Gonothyrea, Wulfert ('02). Günther ('03) finds the nucleolus in the echinoderm egg forming out of the nuclear net, and the chromosomes reappearing from the nucleolus.

6. Chromosomes and continuity

My results contribute little to the solution of this question, but since some evidence in its favor appears in the nucleus of Hydractinia eggs, it can hardly be passed by without comment. As previously stated, when the chromatin becomes diffuse, the net-like arrangement of the chromatin is lost. This is brought about by the threads becoming gradually arranged in groups of two either parallel or cross-like threads (figs. 25, 26). These crosses or parallel threads gradually fade out completely, leaving the characteristic lightly staining nucleus (figs. 21, 27, 28). That the chromosomes reappear in exactly the same way in which they disappear, that is, as *X*'s and parallel threads, is evident by comparing the figures just described with those of reappearing chromosomes (figs. 29, 1, 30, 31). In fact, they are so exactly alike in form, in many cases, that whether they are disappearing or reappearing can only be decided by other conditions in the egg and nucleus. It is hard to resist the impression that this identity in their method of disappearance and reappearance is significant of some sort of continuity.

B. EUDENDRIUM RAMOSUM

Maturation and fertilization

The complete history of the chromatin in the maturation of Eudendrium has not been worked out, but such stages as I have establish its regular character. Maturation, fertilization and development of the egg up to the planula stage take place in the gonophore. The position of the nucleus after it leaves the center of the egg differs from most other hydroids, since it does not lie at the free surface of the egg as it ordinarily does, but at the inner end which leads to the cavity of the gonophore (fig. 51). The maturation and fertilization stages take place at this point. I have sections only of material fixed in sublimate-acetic killing fluid, so that there is always present in the resting nucleus the heavy net-like structure caused by this fixation.

During the growth-period the nucleus consists of a fine net with a deeply staining nucleolus present (fig. 52), the whole very similar to a corresponding stage of Hydractinia after the same killing fluid (fig. 22). The nucleus grows enormously as is shown by comparing figures 52 and 53, the latter being ready for maturation. Because of the coagulation phenomena, I have been unable to trace the reappearance of the chromosomes in this form. Clumps of chromatin lying in the nuclear net give the first indication of reappearing chromosomes (fig. 53). The nucleolus here, as in Hydractinia, increases much in size throughout the growth-period, becomes vacuolated, and disappears before the polar spindle is formed. The origin of the spindle was not determined since the first spindles seen were completely formed and, in the equatorial plate state (figs. 52, 55), perpendicular to the surface. The spindle is of much smaller size than the nucleus and is also without asters and centrosomes. The chromosomes are not in the form of tetrads and, since their origin is not known, nothing can be said of their quadripartite condition (fig. 55). I have also too few sections of the equatorial plate to establish definitely the haploid number. Since 13 is the most constant number appearing, it is undoubtedly near the reduced number (fig. 56). Two stages in the formation of the second polar spindle are shown in figures 57 and 58. A female germ-nucleus (fig. 59), characteristically smaller than the germinal vesicle (fig. 53), is reconstructed after the last polar division. The only cases of fertilization which I have observed show the two germ-nuclei of equal size (fig. 60), indicating that the sperm enters the egg early and so expands before meeting the egg nucleus. I have found no spindle in connection with the fusion nucleus, but the fact that the protoplasm killed at this period is not well fixed, may account for this. Development was carried no farther, the question of cleavage and amitosis not being studied in this form. The establishment of a single cleavage-nucleus makes nuclear fragmentation and subsequent reorganization in 'nuclear nests' impossible and amitotic cleavage improbable.

V. SUMMARY

1. The bulk of the evidence from staining reactions and morphological conditions indicates that those protoplasmic granules in Hydractinia which often take the chromatic stains are not extruded from the nucleus as such, and points to their formation de novo throughout the protoplasm. The granules are therefore not comparable to the chromidia of Hertwig and the term 'pseudochromatin-granules' is justified.

2. There is no evidence of formed material passing through the nuclear membrane into the protoplasm either early (Schaxel) or late (Smallwood) in the growth-period. Globules, which may be mistaken for such material, are formed during the growth-period, flattened against the nuclear wall, but their staining reactions under certain conditions differ from those of chromatin.

3. We are still unable to differentiate with certainty by any of the above methods, the nuclear derivatives in the protoplasm, i.e., staining tests cannot be relied on as tests for chromatin.

4. The yolk is formed in Hydractinia directly from the scattered pseudochromatin-granules and not from nests of granules.

5. The pseudochromatin-granules correspond in a general way to the yolk-nucleus of many other forms. Here the granules are never gathered into a distinct body.

6. The pseudochromatin-granules are completely used up in the formation of the yolk; that is, none, as such, are left in the protoplasm between the yolk-spheres (intravitelline chromatin of Schaxel) to determine further differentiation.

7. The yolk is not formed from mitochondria in Hydractinia and there is no yolk-nucleus consisting of mitochondria.

8. The mitochondria are not of nuclear origin in Hydractinia, but arise de novo in the protoplasm after the formation of the yolk has begun. I find no evidence of multiplication of mitochondria by transverse division, or of their genetic continuity from one cell-generation to another.

9. The mitochondria and chromidia (extra-nuclear granules) are not identical in Hydractinia.

10. The mitochondria are not a vital part of the protoplasm in Hydractinia but are a highly differentiated product.

11. Maturation and fertilization are typical in Hydractinia and Eudendrium and cleavage is mitotic.

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PLATES

PLATE 1[7]

EXPLANATION OF FIGURES

Hydractinia echinata[8] × 2625

1 Two egg cells resulting from the last oogonial division; *a*, diplotene stage of spireme-thread; *b*, early bouquet-stage.

2 Early bouquet-stage, showing loops centered in the nucleolus.

3 Late bouquet-stage, showing the nucleolus pressed against one end of the nucleus.

4 to 8 Five stages in the early growth-period, showing the accumulation of 'pseudochromatin-granules' throughout the protoplasm, the appearance of globules against the nuclear wall and oil in the protoplasm; semidiagrammatic. Granules are represented by gray, oil by circles containing dots, nuclear globules by circles containing parallel lines.

5 to 11 Three later stages of the growth-period, showing the direct development of the yolk-spheres from the pseudochromatin granules; semidiagrammatic. Young yolk-spheres shown in gray.

12 Egg about one-third grown, showing the appearance of mitochondria between the simple yolk-spheres, some of which are now mature and possess a different staining reaction; semidiagrammatic. Mature simple yolk is represented by circles; mitochondria by black rods, young yolk and oil as above.

13 A nearly mature egg showing compound yolk-spheres in addition to the above elements, as well as more mature simple spheres; diagrammatic. Compound spheres are represented by circles containing small circles.

[7] All figures except those of plate 8 and figure 61 were drawn with a camera.

[8] All drawings were made from material fixed in Meves' or Flemming's killing fluid unless otherwise stated.

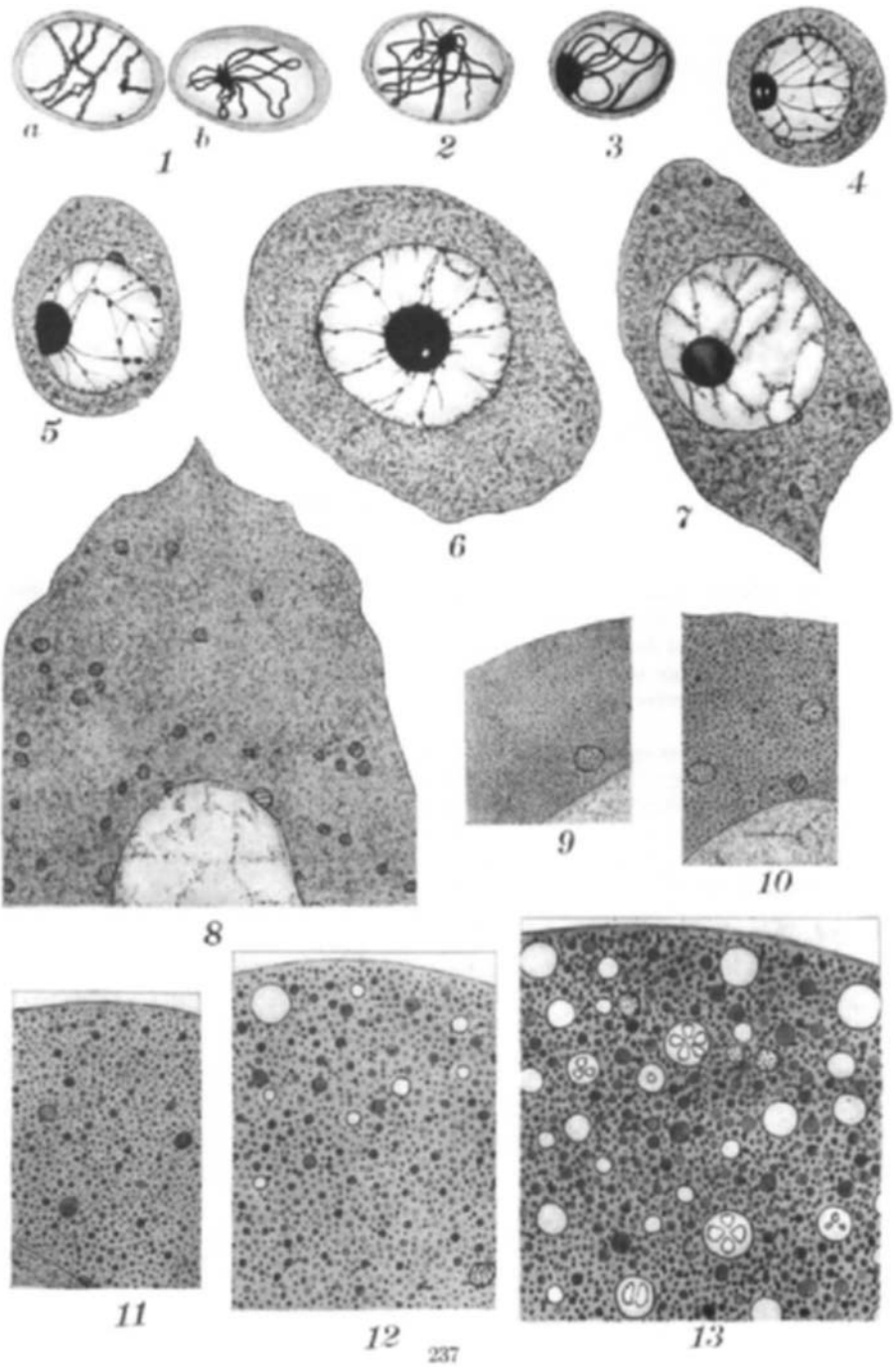
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PLATE 2

EXPLANATION OF FIGURES

Hydractinia echinata

14 Mature egg, showing the arrangement of mitochondria, simple and compound yolk, and oil; diagrammatic. Symbols as in plate 1. × 2625.

15 Types and development of compound yolk-spheres. × 2625.

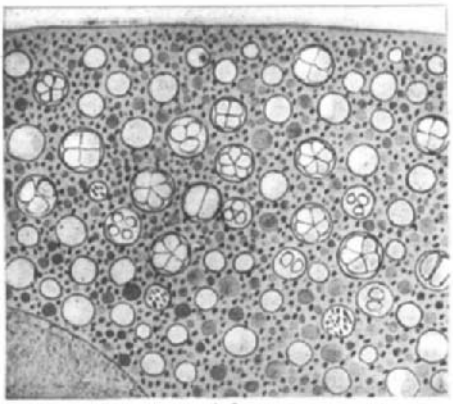
16 Mature egg as shown after staining with iron-hematoxylin. × 2625

17 Young oocyte, showing radial arrangement of nuclear net. Meves' killing fluid. × 825.

18 to 20 Older eggs showing the gradual disappearance of the nuclear net in a homogeneous ground-substance. Meves' killing fluid. × 600.

21 Nucleus showing the complete disappearance of the nuclear net, a granular ground-substance only remaining. Meves' killing fluid. × 600.

22 The same, after sublimate-acetic killing fluid, showing a strong coagulation net in a colorless ground-substance. × 600.



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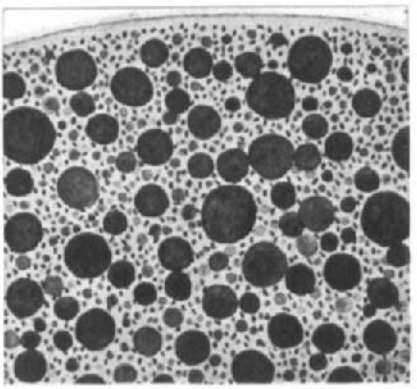

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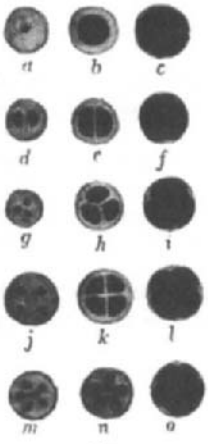

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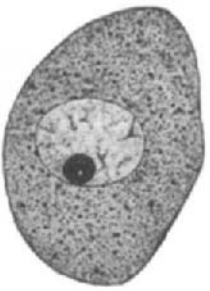

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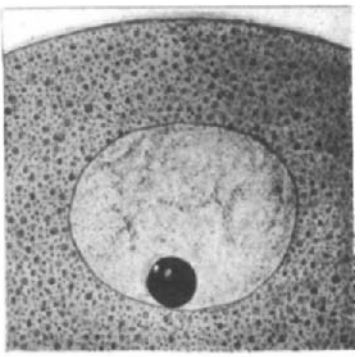

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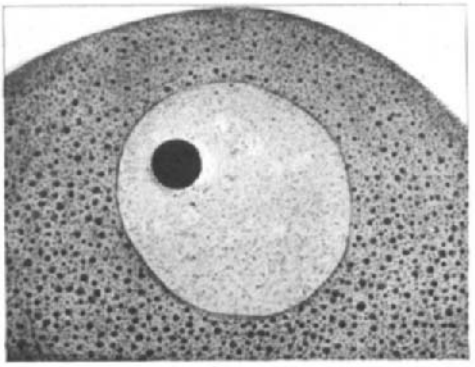

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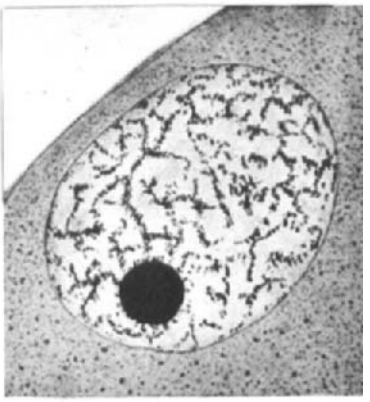

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PLATE 3

EXPLANATION OF FIGURES

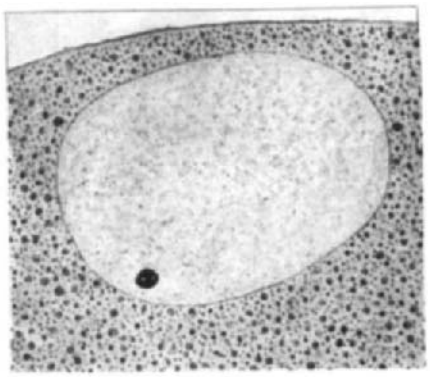
Hydractinia echinata

23 Nucleus just before the reappearance of the chromosomes, showing the homogeneous ground-substance devoid of chromatin net and the nucleolus already much reduced in size. × 600.

24 Chromosomes reappearing in the nuclear area, the nuclear wall having broken down. × 600.

25 to 28 Four stages of nuclei in the early growth-period, showing the disappearance of the nuclear net by the chromatin arranging itself in crosses and parallel threads which become gradually fainter. × 1225.

29 a The reappearance of a chromosome, just before maturation, in the form of a cross, the nucleolus with many vacuoles. *b*, nucleolus with one large vacuole. × 1225.



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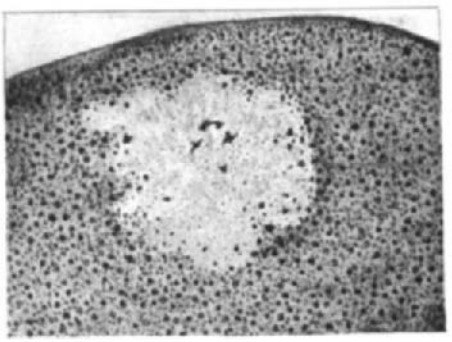

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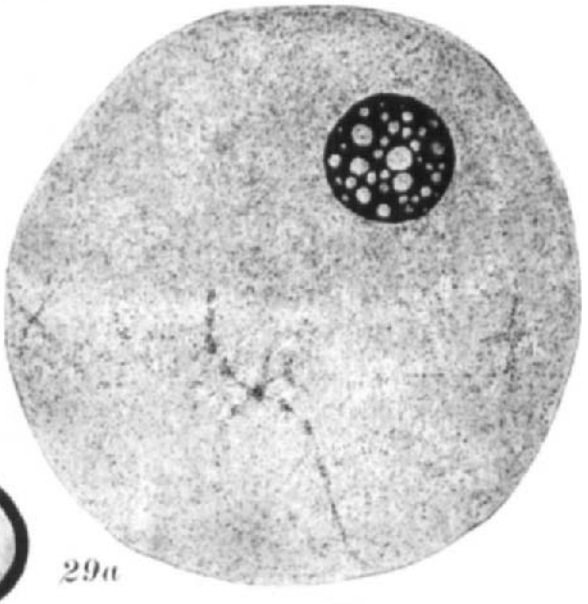
29b



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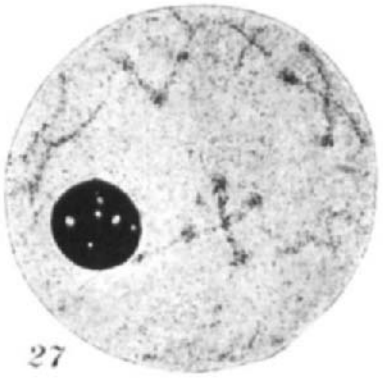

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PLATE 4

EXPLANATION OF FIGURES

Hydractinia echinata

30 and 31 Two drawings from the same nucleus, showing the reappearance of the chromosomes in the form of crosses or parallel threads. × 1225.

32 and 33 Two stages in the condensation of a cross-shaped chromosome. × 1225.

34 Various stages in the late condensation of the crosses and parallel threads into tetrads, all found in the nucleus of figure 24. × 1818.

35 Partially condensed tetrads arranged in an equatorial plate. × 1818.

36 First polar spindle in the center of the nuclear area, showing astral radiations which form a part of the heavy nuclear net caused by sublimate-acetic fixation. × 600.

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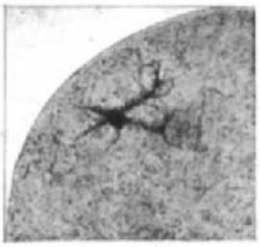

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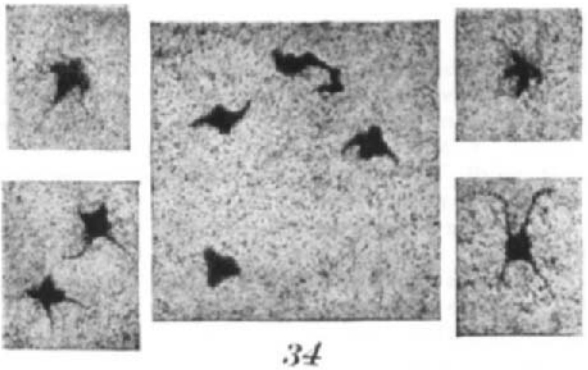

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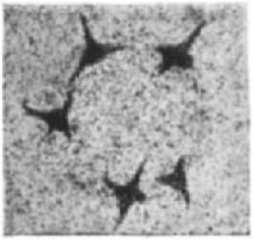

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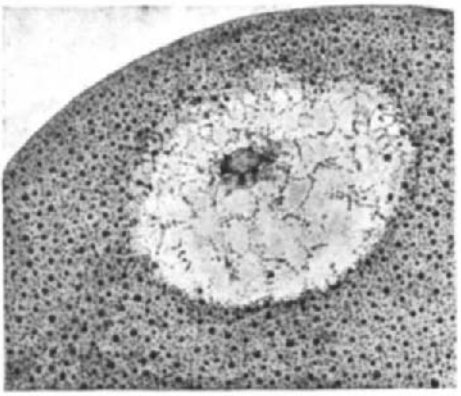

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PLATE 5

EXPLANATION OF FIGURES

Hydractinia echinata

37 The first polar spindle (having no asters) in the center of the homogeneous nuclear area, which is characteristic after Meves' fixation. × 600.

38 The first polar spindle, showing the tetrads becoming arranged at the equator. × 2625

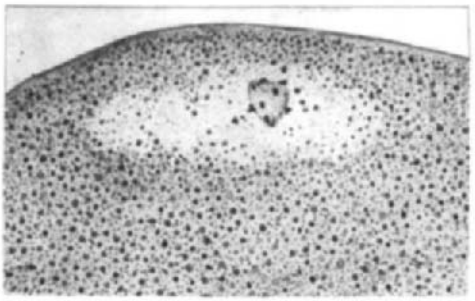
39 and 40 Two equatorial plates of the first polar spindle, showing 15 and 14 chromosomes respectively. × 2625

41 Section to show the arrangement of cell-elements before fertilization. × 2625

42 Section to show rearrangement of mitochondria and fertilization membrane after entrance of the sperm. × 2625.

43 to 45 Three stages in the formation of the first polar body, the spindle having moved out of the nuclear area which remains as two homogeneous areas in the protoplasm. × 2625

46 The formation of the second polar body. The first polar body shows 14 chromosomes. × 2625



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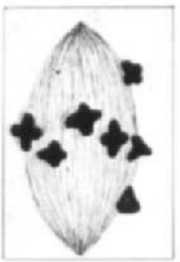

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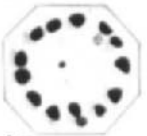

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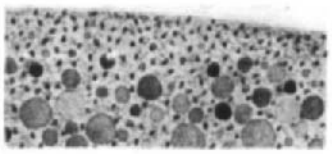

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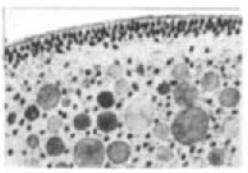

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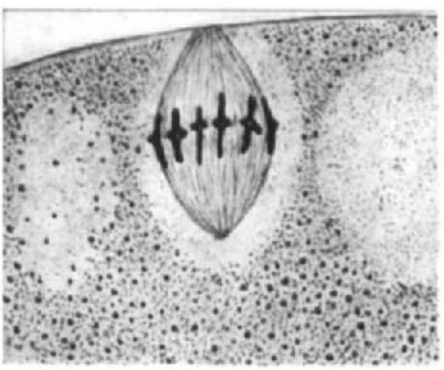

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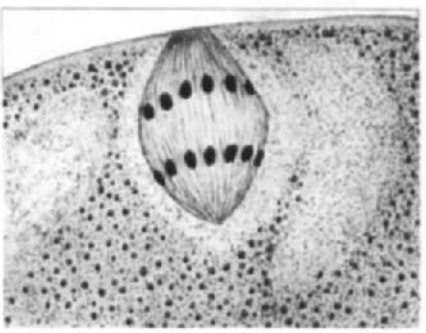

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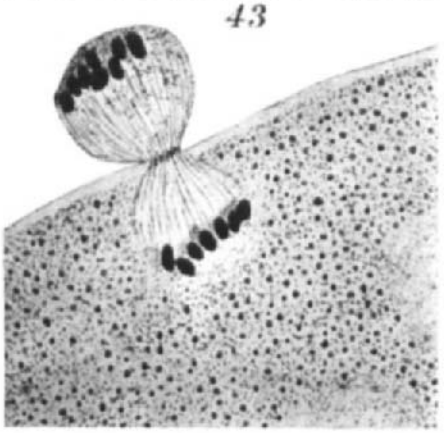

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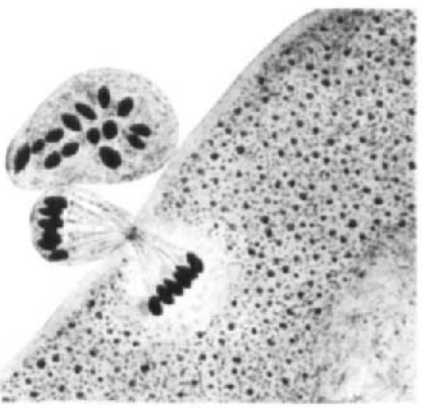

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PLATE 6

EXPLANATION OF FIGURES

Hydractinia echinata

47 Early fertilization stage in which the sperm has entered near the female germ-nucleus. × 2625.

48 Later fertilization stage showing the male germ nucleus somewhat expanded. × 2625

49 Fertilization stage in which the sperm head has entered the female germ-nucleus before expanding, the chromosomes of the egg nucleus being already formed. × 2625.

50 Fertilization stage in which the two germ nuclei are of more nearly equal size before union. × 2625.

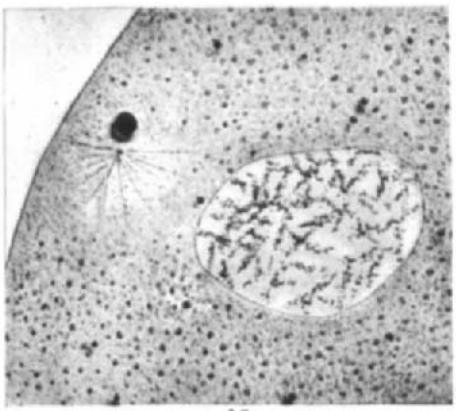
Eudendrium ramosum[9]

51 Gonophore containing an egg showing the position of the nucleus at the inner end. × 75

52 Same nucleus much enlarged, growth period. × 2625.

53 Nucleus ready for maturation, showing the chromosomes forming. × 2625.

[9] All Eudendrium sections are of material fixed with sublimate acetic.



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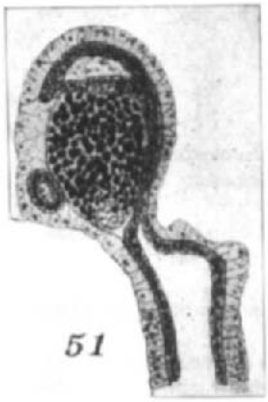

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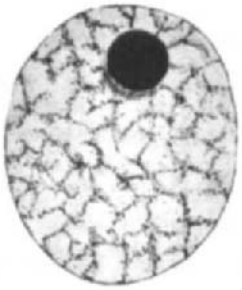

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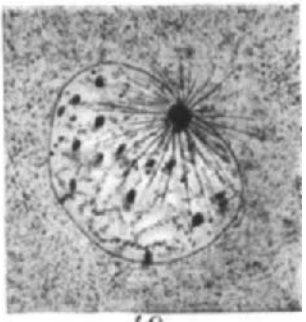

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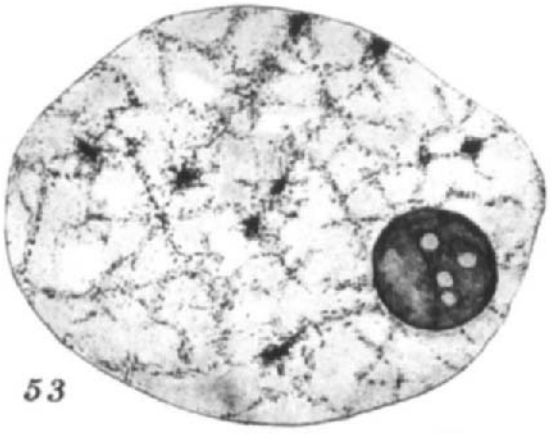

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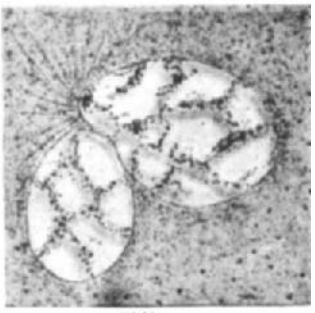

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PLATE 7

EXPLANATION OF FIGURES

Eudendrium ramosum

54 and 55 Two stages of the first polar spindle. × 2625.

56 Equatorial plate of first polar spindle, showing 3 chromosomes. × 2625.

57 and 58 Two stages in the formation of the second polar body. × 2625

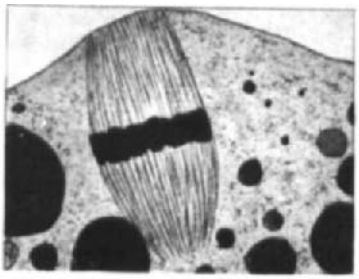
59 The female germ nucleus. × 2625.

60 Fertilization stages, showing the two germ-nuclei of equal size. × 2625.

Hydractinia echinata

61 Two centrifuged eggs showing the egg materials separated into three layers. × 85.

62 Section of a centrifuged egg showing the distribution of the cell-elements, oil at the narrow end, a protoplasmic layer below, yolk and mitochondria at the broad end. × 338.



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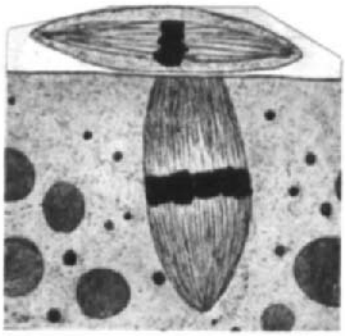

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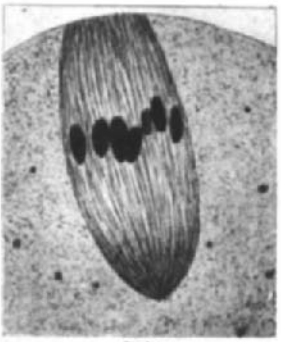

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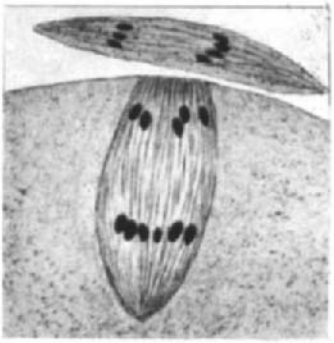

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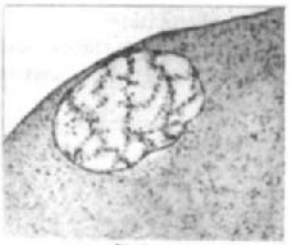

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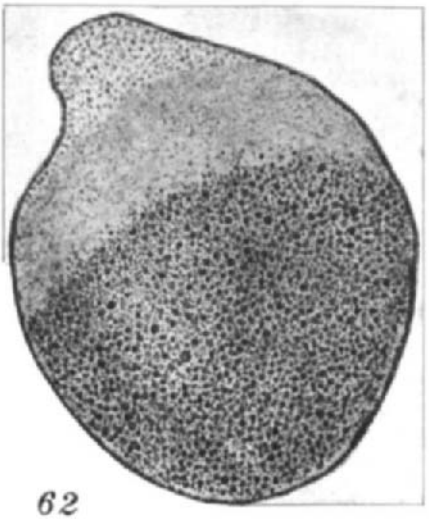

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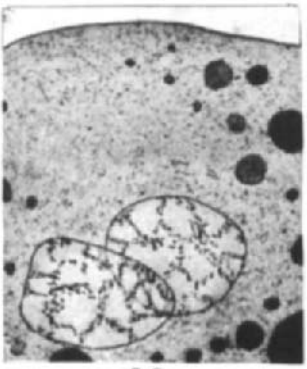

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PLATE 8

EXPLANATION OF FIGURES

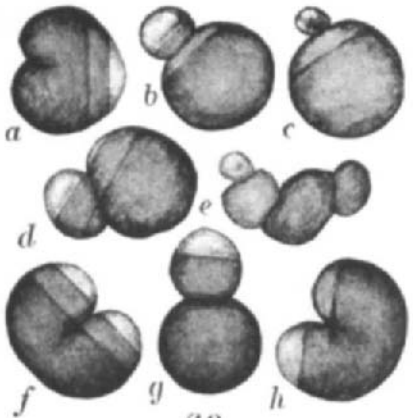
Hydractinia echinata. × 85

63 Centrifuged eggs, showing the direction of the first cleavage plane in reference to the stratification.

64 Sections of centrifuged eggs, showing the position of the nucleus in reference to stratification.

65 Centrifuged eggs which were isolated and development to planula followed: *J* and *K* show the resulting distribution of the materials in the planula.

66 Centrifuged eggs in which the blastomeres were separated in the two-cell stage, isolated, and followed to the formation of the planula. *F* and *G* show several stages in this development.



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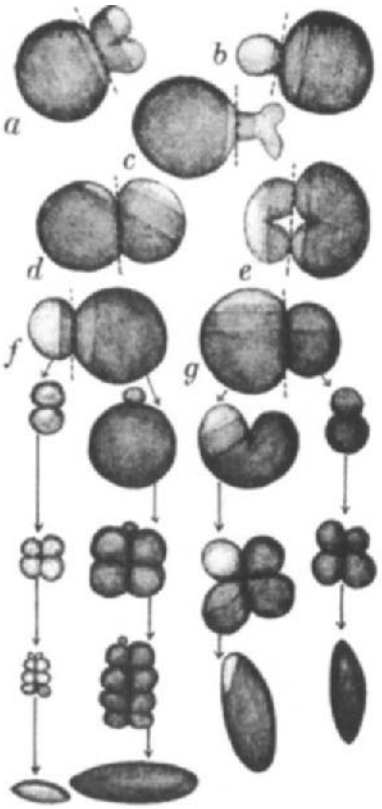

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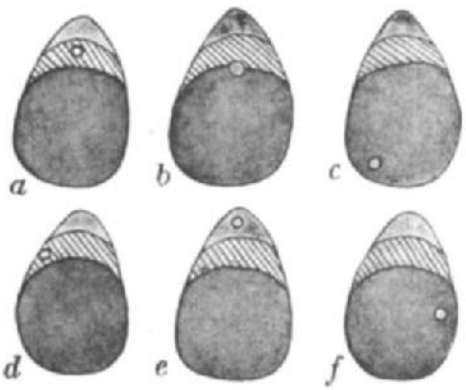

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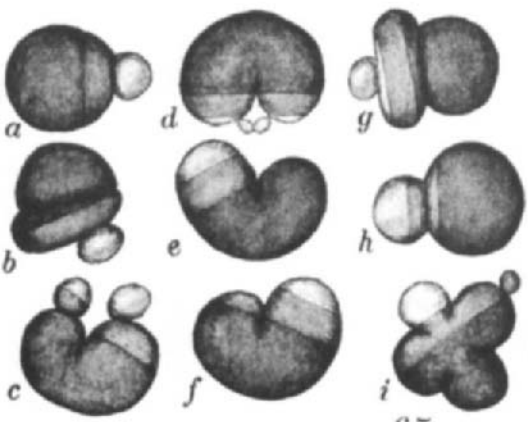

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