

GERM CELLS OF COELENTERATES

I. CAMPANULARIA FLEXUOSA

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TWENTY-ONE FIGURES

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INTRODUCTION

In an earlier paper (G. T. Hargitt '09) an account was given of the maturation, fertilization and segmentation of two of the tubularian hydroids, namely, *Tubularia crocea* and *Pennaria tiarella*. In the present consideration of one of the campanularian hydroids the complete oogenesis will be referred to, though not with equal attention to all phases. It may seem superfluous to add more to what has already appeared on the cytology of the coelenterates, and to call attention once more to the old view of Weismann concerning the origin and behavior of the germ cells of the Hydromedusae. But some features, such as continuity of the chromosomes, peculiarities and uniqueness of the germ cells, have come to have such a large place, on account to their relation

to heredity, that an examination of some of the assumptions on which the theories of heredity rest is not only of interest but more or less necessary. Weismann, who has been the great exponent of the uniqueness (one might almost say the sacredness) of the germ cells, based his hypothesis principally and primarily upon his work on the Hydromedusae. It has been found by Goette and others that many of his views on the place of origin of germ cells in hydroids, their place and manner of ripening, what he called the 'germinal track,' etc., are quite erroneous, but they are still referred to as bases for conclusions on other problems and theories.

Since it was found that *Campanularia flexuosa* offered such an unequivocal answer to some of these very questions it has seemed to be worth while to make a careful study of it and the results are here set forth.

MATERIALS AND METHODS

The material was collected, some in 1908 in Great Harbor at Woods Hole, some in 1909 in Casco Bay, Maine, at South Harpswell. I wish to thank the directors of the biological stations at these places for the courtesies extended to me. The material so collected was killed in Zenker's fluid, in Bouin's picro-aceto-formol, in Mann's picro-corrosive-formol, and in several of the other common killing mixtures. As noted in the earlier paper, it was found that material allowed to remain in alcohol for any length of time deteriorated somewhat, particularly as regards the finer details of the nucleus. When this was demonstrated the material not imbedded at once and preserved in paraffin was used sparingly or for comparison on grosser points of structure; the details here shown have all been worked out from material which was imbedded as soon as possible after being killed, in each case within two weeks after the capture of the material. Since then I have made a practice of imbedding the material within a few hours or a day after killing, with excellent results. As has been suggested by others (Smallwood '09, C. W. Hargitt '11) this is a very necessary precaution, since it does make a noticeable difference in the results.

Material killed in the mixture of Bouin seems to be especially favorable for study, showing in fine detail even very delicate structures; on the whole the fixation from this mixture seems to behave better for coelenterates than any other I have used. One can depend on the results given by it, since a close comparison of results obtained from this and from other fluids, as mercuric chloride, osmic acid, picric acid, potassium bichromate, platinic chloride, and so forth, show no essential differences, it being mainly a difference as regards staining. The very granular appearance found after the use of mercuric chloride mixtures, however, is in large part artificial, due to the vigorous precipitation of the colloid substances by the mercury. The material killed in Bouin's fluid has been used more than any other, though the results have always been checked by reference to other methods of fixation.

I should like to call especial attention to the fact that during the study of this form there has been a study of living material as well. Of course there are many features that could not be seen in the living eggs, but a great many structures of the nucleus and of the cytoplasm are almost as clear in the living material as in the sectioned and stained preparations. To check the work on *Campanularia*, living eggs of *Obelia* were also observed and the conditions found are the same as the ones figured for *Campanularia*. The sections show that all these features observed in life were faithfully preserved and clearly shown, and one may conclude, therefore, that the things not seen in life have probably been well preserved also.

In the paper on the tubularian hydroids I discussed the matter of stains; some or all of these have been used here. Heidenhain's iron-hematoxylin, as might be expected, has given the best results in the delineation of the form of the nuclear and cellular constituents. Combinations of various sorts have been used to determine, as far as possible, the similarity or dissimilarity of various constituents and their possible significance, origin and fate. The sections were usually cut 5 to 7 μ thick in order to have the nucleus in few sections, with the result that where chromosomes were present they usually appeared in a single section.

OBSERVATIONS AND DISCUSSION

1. The gonophore

The general position of the germ cells and their relation to each other is shown in the longitudinal section of a single gonophore in figure 1. In this particular instance the eggs are all in different stages of growth, though there is great variety in this. Especially characteristic is the definite arrangement of the eggs, so far as the stage of development is concerned, the older eggs at the distal end while in the proximal region the eggs are so young as to have hardly started their growth. It is this arrangement of the eggs which, in part, makes *Campanularia* so favorable for study. There need be no uncertainty of the stage of development of a certain egg, for if this egg does not itself show clear evidences of its stage of development there are those distalwards which are certainly older and the ones lying proximally are younger; both of these, therefore, serve to indicate the probable age of the egg under consideration.

Another advantage comes in the ease of comparison of stages. It often happens that the eggs at the proximal end of the gonophore are just beginning to grow, or are half-grown, and the distal eggs are in stages of cleavage or perhaps are planulae ready for liberation. In a single gonophore therefore, it is possible to get a number of different ages and to be certain of their relative stage of development, a condition that is not easily attained in eggs that develop freely and separately in the water, particularly in those cases, as the hydroids, where there are not many eggs ready for fertilization at the same time. This makes possible, not only a more certain but also a closer gradation of stages than is easily obtained under the ordinary conditions of development. These things make very clear the order in which certain things happen and therefore make a logical interpretation more precise. It seems very certain that, under the circumstances, no mistake has been made in the arrangement of the series, and no important stages have been omitted.

2. Origin of the egg

In figures 2, 3 and 4 the earliest recognizable egg cells are shown, and from this to the mature egg ready for fertilization an unbroken line has been traced, so there is not the slightest question that the

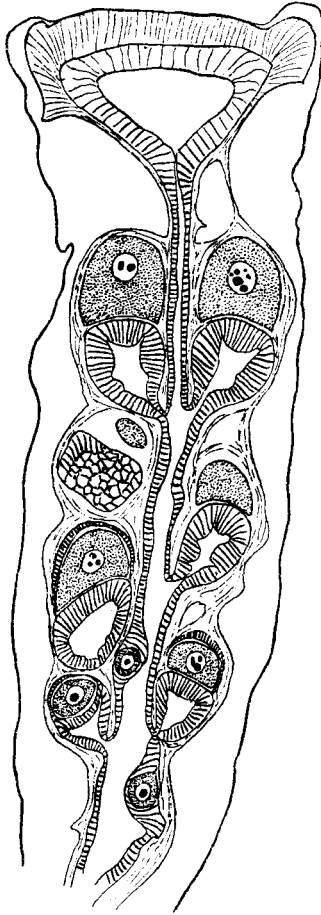


Fig. 1 Longitudinal section of an entire gonophore, showing eggs in various stages of growth. $\times 130$.

cells shown are indeed egg cells. These cells are developed in the pedicel of the gonophore only and in no case were they found in the stem adjoining; and in the pedicel they came only from the

entoderm. In the ectoderm of this region no cells were found which looked at all like those figured, or any others that had any of the characteristics of germ cells, so it is certain that we have an entodermal origin of the egg cells. Goette ('07) found also that the eggs of *Campanularia flexuosa* which he examined, as well as those from most other campanularian hydroids, came from the entoderm and from the entoderm alone. He was not able to determine whether in this case the cells came from a single transformed epithelial cell as he had found in some hydroids or from the basal half of a divided epithelial (entoderm) cell as he found in other cases. Figures 3 and 4, especially the former, leave no doubt that an entodermal cell divides, the basal half forming the egg cell. Figure 2, however, seems to suggest the transformation of a single entodermal cell into an egg cell. There is no reason why both methods may not be active; certainly after it has been found, as Goette did, that some hydroids produce germ cells from ectoderm or entoderm indifferently, it might be expected that some would produce germ cells from entire or from half-cells indifferently, and this I believe to be the case in *Campanularia flexuosa*.

The place of origin of the egg cells of the hydromedusae, once of great interest, has ceased to have very much importance. Weismann ('83), in his actual observations, found evidences of some germ cells arising from the ectoderm and others coming from the entoderm. (His statement on p. 145 concerning *Campanularia flexuosa*, that in the ectoderm there are cells which appear similar to the youngest egg cells, is not correct for the material which I have studied.) But later, especially in his volumes on *Evolution* ('04) he refers the origin always to the ectoderm, and if they are not actually to be distinguished there, says they must at any rate originate there and later migrate into the entoderm where they become demonstrable as egg cells. Entirely aside from the theoretical importance which he claims for the place of origin and the subsequent migrations, views long since shown to be without any firm foundation or real significance, it is interesting to see the many cases where an undoubted origin from the entoderm is demonstrated. C. W. Hargitt ('06) showed in *Clava*

leptostyla that the entoderm was the place where the egg cells originated; Goette ('07), in his very extensive paper on the germ cells of hydromedusae, showed conclusively that in many forms the germ cells came from the entoderm, others from the ectoderm and still others indifferently from one or the other. He says, for example, referring to *Podocoryne carnea*, that egg cells were found in the entoderm of the bud, in the ectoderm, and from all developmental stuffs of the medusa bud (p. 81). Again: "For me, therefore, no doubt exists that the germ cells of *Clava multicornis* proceed only from transformed half-entoderm cells" And later (p. 414) he says: "However, after it has once been established that the germ cells of *Hydropolyps* originate sometimes in the entoderm, sometimes in the ectoderm . . . it is naturally of little fundamental concern which mode is current for the separate species." Smallwood ('09) in *Hydractinia* finds the egg cells arising in the entoderm. C. W. Hargitt ('11) in a general paper on coelenterate ontogeny calls attention to the work of Goette and of others and of the stand which Weismann has taken in regard to these facts, and reference should be made to these papers for a fuller discussion.

It may not be amiss, however, again to call attention to the fact that the work of Goette, the present paper and others furnish exactly the evidence demanded by Weismann himself as a proof of the origin of germ cells from the entoderm. Weismann says (p. 237) "If the egg cells were of entodermal origin, they must proceed by cross division of ordinary entodermal cells, with the result that the distal half bordering the enteric cavity remain epithelial cells, the basal half becoming only germ cells." The work of Goette shows this, and the present paper in figure 3 represents that just this thing has occurred, the basal half of the cell becoming a germ cell, the remainder continuing as an ordinary epithelial cell. Figure 4 is another drawing of a similar stage; in this the nucleus of the distal half of the original epithelial cell is present in another section. As already stated, not all egg cells of *C. flexuosa* are so formed, some coming from a transformed entire entoderm cell (fig. 2) but in all cases from the entoderm.

The entodermal cells from which the egg cells have arisen appear not to differ in any way from any of the other entoderm cells of the region; they are all cells which line the coelenteric cavity of the pedicel of the gonophore. Before the multiplication of the cells which led to the formation of the gonophore took place these cells were regular entodermal cells lining the coelenteric cavity of the stem of the hydroid; there is nothing that would set one cell apart from any other in position, size or appearance. In other words, previous to and in the early stages of gonophore formation *all* the entodermal cells of this region would be considered as ordinary differentiated epithelial cells. And any entoderm cell has the power of becoming a germ cell, provided only that it is in the position where the gonophore is to form. If no question of assumed theoretical importance came up, as of the origin of the germ cells, probably no one would even think that one of these cells had any different history, or any different potentiality than every other cell. Much less would he say that even though no difference could be discovered by the most delicate technic and with the latest and most refined optical apparatus, yet one particular cell must have had a different history, must have different capabilities, must be of a sort fore-ordained for a particular purpose since, forsooth, it came to a different end from its neighbor. And yet is not this just the argument of those who insist that the germ cells must of necessity be different, and have a different history from the somatic cells?

In *Campanularia flexuosa* there seems not to be the slightest doubt that the egg cells have arisen from differentiated epithelial (entoderm) cells lining the coelenteric cavity, which are not different from any others in the same place. The first indication of any difference comes when the nucleus increases very much in size; forms a spireme of chromatin, more or less regular in its arrangement (figs. 2, 3, 4); and a little later the cytoplasm is a little more compact and stains more deeply (figs. 3, 4, 5). As already mentioned, this does not occur in the prophase of dividing cells not forming germ cells. And in the egg cells the apparent prophase is not followed by any division, but from this time on, as shown in figure 2 to 6, and so forth, there is an unbroken series

of stages through the entire history of the egg. Furthermore, there is no division of the cell to form generations of oogonia and oocytes, but from the earliest stage found, up to the mature egg, it is the growth and development of a single cell. In other words, an individual cell (or half of a single cell) of the entoderm transforms directly into a single mature egg without any divisions in the process, until the polar bodies are formed.

Smallwood ('09) found in *Hydractinia* that egg cells are transformed directly without any immediately preceding cell divisions and C. W. Hargitt ('11) refers (p. 525) to similar occurrences in *Pachycordyle* and *Eudendrium*, and others have found similar cases. The point here made is that the form under consideration shows, in a manner so clear and striking as to leave no doubt, that it is not possible to speak of a 'continuity of the germ-plasm' in the sense of cells early set aside and more or less carefully guarded from contact with, and participation in, the activities of the so-called somatic cells. For it is clearly shown that the egg cells arise from, and are a further development of, an individual (already differentiated) entoderm cell, which up to the time of the transformation was performing the same functions as its neighbors lining the coelenteric cavity. Here, somatic and germ cells, that is somato-plasm and germ-plasm, are one and the same thing. It is proper to speak of a continuity of germ-plasm in the sense that all cells in the body, germ cells and somatic cells, are descendents of the original fertilized egg cell; but no cell in the body appears to retain any initial peculiarity that does not also apply to every other cell. If it be objected that this removes from the concept 'germ plasm' the very thing that has characterized it, I can only reply that such is precisely the case; but from the evidence as presented in *C. flexuosa* no other conclusion can be drawn. The work of Goette ('07) and of others give the same results when they say germ cells may be formed from any of the developmental stuffs of the body. Jorgensen ('10) finds the same thing to be true in sponges and comes to the same conclusion. It is not necessary to insist that these conclusions be applied to all other groups of animals, for exactly the opposite conclusion has been drawn from the evidence shown in forms such as *Ascaris*,

where there appears to be little doubt that there is an early separation of the primordial germ cells. Just as the weight of evidence in this form is admitted, so it must be in *Campanularia*.

3. *Later development of the egg*

a. *The cytoplasm.* Reference has been made to the fact that in the entoderm cells which are just starting their development as egg cells, there is little or no difference in the appearance of the cytoplasm from that of other cells. Figure 2, for example, shows at *a* and *b* two developing egg cells and one other entoderm cell; also some ectoderm cells, in all of which the cytoplasm is the same in appearance. But very soon there occurs the change which has been familiar to all workers for a long time, namely, that the cytoplasm which is granular becomes more compact and the stains take hold with greater intensity (figs. 3 to 5). Whether this is due to physical or to chemical changes within the cytoplasm is not certain; chemical changes are assuredly taking place and the physical configuration is also altering. No further changes occur while the egg is in its place of origin, but when it is migrating the short distance up the pedicel of the gonophore into the latter, it is increasing in size, due to the absorption of food from the enteric cavity. In a stage like that shown in figure 6, in which the egg is in the base of the gonophore, and figure 7, which represents an egg in its final position within the gonophore, the cytoplasm shows well marked changes which appear as irregular spaces within the cytoplasm. This is not purely an artefact (though it may be in part) since the spaces show some definiteness, as though caused by currents or streams within the cytoplasm. At the same time there is certainly an exchange of substances between the nucleus and the cytoplasm, as shown in figures 6 to 8.

All this time the cytoplasm has remained finely granular, with few or no protoplasmic bodies, even at the time when the egg has grown to be a quarter of its final size. This growth occurs very rapidly up to this point, and I interpret the lack of deutoplasmic bodies as meaning that food taken into the body of the cell is used up immediately, with nothing left over for a reserve.

In the stage represented in figure 8 (the egg being a quarter of the final size) we have the first indication of these yolk bodies, present in greater numbers near the periphery of the egg; when these bodies become more abundant (figs. 9 to 13) they are present near the nucleus as well. Figures 9 and 10 show lines in the cytoplasm radiating from the nucleus, which is evidence of currents which are proceeding outward from the nucleus. I conceive, therefore, that the yolk is built up in the cytoplasm out of material which has come from the nucleus, or from the material in the cytoplasm through the aid of material which has come from the nucleus. The simplest explanation of this appears to me to be that perhaps an enzyme from the nucleus gets into the cytoplasm and there elaborates and synthesizes the food brought into the egg. The presence of radiating lines—that is, of currents going out from the nucleus—is present for a considerable time; indeed up to the time when the polar bodies are about to be formed. This activity continues, then, throughout the entire history of the egg cell, and when it ceases the egg is loaded down with yolk granules which are so closely packed as to leave little space between them. The granules of the cytoplasm, at first the only material present, are now arranged around these yolk bodies and between them in a sort of an alveolar arrangement, but apparently not much greater in amount than in the young eggs. The difference in the appearance of the yolk bodies shown in figures 9, 10, and so forth, is apparently without significance, being due to the variation in the tenacity with which the bodies hold the stain.

b. The nucleolus. It is in the peculiarity of the nucleolar structure and history that one of the most interesting phases of the development of the egg of *Campanularia flexuosa* lies. And let it be remembered that many of the conditions and appearances referred to and figured have been seen in the living egg. The living egg of *Obelia* has also shown similar things so that there is no question of the effect of killing agents and so forth, for these appearances are faithfully shown in the sections; that is, the nuclear constituents have been normally preserved in the killed eggs, even to the delicate features.

In figures 2 to 4 the youngest egg cells show a single spherical nucleolus which stains deeply with iron-hematoxylin, though, with

double stains of acid and basic reactions, this body assumes some of the protoplasmic tint. In these same figures the nucleoli of the entoderm and ectoderm cells are much larger than those in the egg cells, though with the nucleus the opposite is true. Until the eggs assume their place in the gonophore, the nucleolus remains single; sometimes it is present as a single body after the eggs have come to rest and have increased in size in their permanent place in the gonophore (fig. 1). In the short migration which the egg cells go through from the pedicel of the gonophore into the body of the latter certain changes occur, principally in the cytoplasm and nucleus. The chromatin thread of the youngest eggs disappears and the nucleus contains only a delicate reticulum in which, as figures 5 and 6 show, are several deeply staining strands. In figure 6 there is also shown a darker and denser mass of cytoplasm just outside the nucleus, and scattered throughout this, but principally close against the nuclear membrane, several very deeply staining small granules. These are of interest because of what happens in later stages and are explained as being due to some substance passing from the nucleus into the cytoplasm.

After the eggs have reached their place in the gonophore, the nucleolus soon undergoes great changes, consisting of the breaking up of the nucleolus into pieces of various sizes and shapes, and never again in the history of the egg is the nucleolar matter in one body. It is the history of the changes of these nucleolar bodies and their possible significance that this section hopes to describe and make clear. Concerning the staining reactions of these fragments the following will show in general, and may suggest something of the nature of the changes involved. Double stains such as hematoxylin and eosin, hematoxylin and picric acid, picrocarmine and Lyons blue, hemalum and eosin and so forth, show some selective action. The result is that some of the nucleolar fragments show the colors assumed by the protoplasmic portion of the cell, some those assumed by the chromatic constituents of the nucleus, and some a tint intermediate between, or rather compounded from, the two tints. That is, the nucleoli behave as chromatic material, as non-chromatic material, or as a mixture

of the two substances. In some egg cells all the nucleolar fragments appear as non-chromatic, in others all appear to be mixtures, and in still other eggs some of the fragments seem to be chromatic and some non-chromatic or mixtures; in no case were the fragments in one egg all chromatic. In some instances these differences have been indicated in the drawings by differences in shading. The term nucleolus is simply a general term, as used here, for it has some of the characteristics of a plasmosome and behaves in part as a karyosome.

In the stage represented by figure 7 (about one-quarter the size of the mature egg) the fragmentation of the nucleolus begins and thereafter is characteristic, the variety in size and shape of these bodies being well shown in figures 6 to 16. The shape of the nucleolus is apparently of no significance, but the small spheres or spirals or the irregular masses as shown in figure 11 and 12 would present a greater surface than a spherical body. This may be a matter of some importance.

Of much greater interest is the condition shown in figures 7 to 9, in which there is seen a sort of ring of finely granular matter in the nucleus near and inside the nuclear membrane. Let it be noted that the groundwork of the nucleus is reticular and the ring of granular matter is not reticular nor arranged with any reference whatever to the reticulum and it will then be evident that the nuclear reticulum and the ring of granular matter are not the same thing and are probably not directly related. Rather it is just the condition that we might find if there were a wave of matter spreading outward from the nucleolus toward the edge of the nucleus; this material, being somewhat different chemically from the nuclear sap and reticulum, it would present a different staining reaction. Figure 9, indeed, is proof that some such thing is happening, for, in addition to this ring of granular material in the nucleus, there is in the cytoplasm a radial arrangement of the small granules as though a similar current were passing outward from the nucleus into the cytoplasm. This same radial arrangement of cytoplasmic granules just outside the nucleus of the growing egg is very characteristic and continues up to a very late stage, indeed it is present just as long as there is nucleolar

matter left in the nucleus (figs. 10, 12, 13, 15, 17, etc.). This last condition is strong evidence that the suggested interpretation is correct; certainly the fact that breaking up of the nucleolus and streaming currents from the nucleus into the cytoplasm co-incide; the fact that when the nucleolus has disappeared there is no longer such an arrangement of the cytoplasmic granules near the nucleus (i.e., no strong currents going from the nucleus into the cytoplasm) can not indicate other than a causal relation.

The nucleolus entirely disappears in the process just described. First of all, there is a very great increase in size of the nucleolus during the early growth of the egg, as a comparison of figures 3, 4 ($\times 1900$) with figures 5, 8, 9, 10 ($\times 1228$) shows, an increase which is much greater in amount than the increase in volume of the nucleus itself. Very soon, however, the nucleolus begins to undergo the modifications figured. It is certainly significant that a rapid increase in the body of the egg should be evident at the time when the nucleolus becomes vacuolated and breaks up, and streams of matter are going from the nucleus into the cytoplasm (figs. 8 to 10, 16). These are evidences that changes are occurring within the nucleolus, apparently quite rapid and considerable. In figures 6 to 11 there appears to be a change in shape of the nucleolus only, with little breaking into fragments, but a great vacuolization (these fragments, vacuoles, etc., are visible in the living egg). In later stages (figs. 12 to 17) the entire nucleolus breaks into many pieces, usually quite small, which, by the time the stage shown in figure 17 is reached, have almost entirely disappeared and a little later nothing of the nucleolus is left.

Another phase of these same activities is indicated in the character and appearance of the material which is leaving the nucleus and getting into the cytoplasm. In figure 6, as already noted, there are a few small deeply staining granules in the cytoplasm, close against the nuclear membrane; in figure 7 this is more evident. In the stages which follow this the same thing is seen, sometimes very plainly, at other times not so clearly (figs. 7 to 15). What becomes of the material when it gets into the cytoplasm? Let it be noted, as figures 3 to 7 show, that the cytoplasm is at first finely granular, in very early stages very closely packed

(figs 3 to 5), but a little later (figs. 6 and 7) becoming vacuolated and alveolar. In the periods represented by figure 8 (which may be variable as far as the size of the egg cell is concerned) for the first time there appear deutoplasmic bodies in the cytoplasm, and, since this coincides with the great activity of the nucleolus, it seems that there must be some connection between the two conditions.

If it be objected that figure 8 shows the deutoplasm closer to the periphery of the egg than to the nucleus and therefore there can be no connexion between nuclear emissions and yolk formation, let the following also be noted. First it is not assumed nor believed that all the *material* which forms yolk bodies comes from the nucleus; on the contrary, I believe a greater amount of it comes into the cytoplasm from the food stream in the enteric cavity of the gonophore and never enters the nucleus. This would probably be more abundant near the periphery of the egg than elsewhere. In the next place, the first emissions from the nucleus occur quite a while before yolk bodies form (figs. 6, 7). It is believed that at least the first emissions are of a ferment nature and not until they get into the cytoplasm is it possible for the material there to be synthesized into reserve food. It is even conceivable that the first nuclear contributions to the cytoplasm and the later ones are the same. And it is conceivable and possible that the same substances will at first hydrolyze the food material coming into the cytoplasm, hence there will appear no yolk bodies, and later the same substance synthesizes the dissolved food and the result of this synthesis is yolk bodies. The reversible action of enzymes is too well known to call for any particular explanation in regard to the relation of enzymes and yolk formation. If this possibility be granted, there is no difficulty in accounting for deutoplasmic formation near the periphery rather than near the nucleus; indeed it would take place where the concentration of the hydrolyzed substances was greatest and this would be near the place the original material entered the egg, namely, near the periphery. Furthermore, the presence of yolk near the periphery is only at the first; the yolk is formed so quickly when it once starts that it fills the whole of the cytoplasm and is especially

prevalent near the nucleus (figs. 9, 10; fig. 9 is of the same stage as fig. 8).

With regard to the direct connection between the nucleolar matter and the yolk bodies, it is true that the nucleolus dissolves within the nucleus and there is certainly some commingling of nucleolar matter and nuclear sap, probably also a mixture of some of this matter with the chromatic network. But the growth of the nuclear reticulum and the dissolution of the nucleolus do not go on together, the reticulum showing almost no change until the nucleolus has disappeared. Again it is found, as figures 13 to 17 show, that the nucleolar fragments are always surrounded by a space; that is, they lie in a vacuole. Further, the smaller particles, when abundant, are often arranged in a rather definite row close to the nuclear membrane (figs. 14 and 15) as though it were here that dissolution was most rapid and the current outward had carried them here. These facts show that the dissolving nucleolar material is not being added directly to the nuclear reticulum, but, since the nucleus is increasing in size during this period, it is probable that the nuclear sap is increased very much in amount by the dissolved substance. Figure 16 shows well that the nucleolus is dissolving little by little; in this case the different particles showing different staining reactions, since the lightly stained nucleolar fragments show small drops of the material passing into the vacuole surrounding the fragment. The most satisfactory and crucial evidence of the connection between the nucleolus and yolk lies in this: during all the time that yolk bodies are being formed there is evidently a considerable exchange of material between the nucleus and the cytoplasm as shown by the currents already mentioned. During this same time (figs. 7 to 12) there is no change in the nuclear reticulum, which remains very fine-meshed, exceedingly finely granular and staining very faintly; that is, the reticulum is apparently unchanged and unmodified by any of the striking and active modifications that are going on. Since the nucleolus is the only portion of the nucleus showing signs of activity during this period and since there is clearly great activity going on in the cytoplasm in the synthesis of food matter, there appears to be no other possibility than to

conclude that the nucleolus actually stands in some causal relation to these changes.

Now plainly the whole egg during the period of growth is in a most active state of metabolism. In addition to the ordinary functions which it has to conduct in order to remain alive there is the further task of preparation for the cleavage period which is to come, in which it will not receive food from the outside. This rests principally in the storing of food as reserve for that period of activity. Even if we assume that the food which enters the egg from the enteric cavity has already been digested, there is the necessity for a great amount of it to be synthesized into the stored products needed later, and also a lot to be assimilated and used for the present needs of growth. Perhaps this is the most strenuous period of activity of any single cell in the life story of the cells of the body. Apparently, then, the nucleolus stands in some rather close relation to this activity of the egg cell during the active growth period. If not actually taking part in the transformation of the food itself it is closely related in some other way. The odd shapes assumed by the nucleolus may, therefore, be for the purpose of securing as much exposure of surface as possible. The nucleolus certainly aids in transforming some of the material, since this body alone is not sufficient to account for the increase in substances within and without the nucleus.

The origin of the nucleolus in the egg cell was not definitely determined, for in the earliest recognizable egg the nucleolus was already present. But the behavior is sufficiently clear. In the young eggs (figs. 2, 3, 4) the nucleolus is very small, smaller even than the nucleolus of the neighboring ectoderm and entoderm cells, though the nucleus is larger in the egg cell. But coincident with the disappearance of the spireme in the young egg cell, which takes place very quickly, the nucleolus enlarges considerably, even before the body of the egg increases. The nucleolus arises then, within the nucleus and evidently from the chromatic spireme (at least in part), but all of the chromatin does not enter the nucleolus, for in addition and at the same time a chromatic reticulum is formed in the nucleus; also the staining reactions show that the nucleolus contains a lot of non-chromatic matter.

The fragmentation of the nucleolus and the transference of its substance into the cytoplasm is, therefore, in correspondence with the staining reactions, for the material which is emitted from the nucleus has the same staining reaction as chromatin and the origin of the nucleolus from the nuclear spireme would explain the appearance. It has been shown that the chromidia, so-called, in the cytoplasm have come from the nucleolus and their chromatic relation is thereby explained, since the chromatin earlier entering the nucleolus leaves it later and goes into the cytoplasm to serve a particular purpose.

It may be added that *Gonothyrea lovenii* from Naples was sectioned and showed almost the same relations of nucleolus as *Campanularia flexuosa*. There was perhaps a little less variation in the form of the particles (none of them showed the arborescent-like forms shown in figs. 11 and 12) but there was always a fragmentation about the same period, and it continued about as long, so the agreement is very close. Bergh ('79) described the breaking up of the nucleolus into many pieces of various sizes and shapes and observed it in the living eggs of *Gonothyrea*.

The nucleolus, then, as its activities and functions have been conceived and outlined in the foregoing, would appear to be a 'trophonucleus' in the sense of Goldschmidt ('04). As is well known, this author conceived the nuclei of all animal cells to be double, a somatic (or better, vegetative) and a reproductive nucleus, which were usually united within a single nucleus which he called the 'amphinucleus.' He found from his own investigations that in the egg cells a separation came when a portion of the nuclear matter passed into the cytoplasm in the form of grains, to which were given the name of 'chromidia,' and this emission from the nucleus came only during the time of yolk formation. His point of interest was, not whether this extruded chromatin went to form yolk or whether it was a sort of regulative process for the reproductive chromatin, but that this process did establish a close relation of chromidia formation with a specially active somatic function. For the chromidia were determined to be isolated particles of the chromatin of the nucleus and they were in the place of highest somatic functioning, that is, in the cyto-

plasm of the cell. The behavior of *Campanularia flexuosa* seems to agree very closely with this theory, the actual facts being almost the same as those that Goldschmidt observed. But there is an incidental difference in that the chromidia proceed directly and immediately from the nucleolus, though this nucleolus, as shown, originated in part from the chromatic spireme and in its dissolution still showed reactions similar to those of the rest of the chromatin in the nucleus.

As early as 1895, Van Bambeke found in one of the fishes (*Scorpaena scrofa* L.) that chromatic substance passed through the wall of the germinal vesicle, but in this case the nucleolus had nothing to do with the process. Lubosch ('02) found what he called 'by-products' of the nucleus to pass into the cytoplasm and there take part in yolk formation. He further states that the phenomena of growth of the cell suggest that material taken in from the cytoplasm is synthesized in the nucleolus and transformed into chromatin. Henschen ('04), in *Helix pomatia*, finds a migration of chromatin from the nucleus into the cytoplasm and thinks it may have some relation to yolk formation. Brooks and Rittenhouse ('06), in the coelenterate *Turritopsis*, found the yolk to form close to the germinal vesicle as a result of nuclear activity. Popoff ('07) says chromidia come from the nuclear chromatin and in egg cells chromidia formation is least active in the first phase of growth and most active when the yolk is forming; the richest chromidia formation agrees with the strongest cell activity. The wide distribution of chromidia in strongly functioning tissue cells is suggestive of a physiological condition of the cell, and Popoff says: "I consider the chromidia as morphological consequences of cell growth and cell activity" (p. 104). In 1910 this same author makes the general statement that chromidia, mitochondria, and so forth, are different stages in the same genetic series, originating in nuclear chromatin, and the various appearances are due to differences in diffusion currents, peculiarities of the cytoplasm and the like. Yolk may form from these but "chromidial formation is only an expression of purely physiological cell conditions and these can in no way be specific for the germ cells alone" (p. 41). Others have found chromidia related

to yolk, as Jorgensen ('10) in sponges, Schaxel ('10, '11) in various Hydrozoa and in Ascidia. Nowikoff ('09), in *Haliotis tuberculata* tissue cells, found the nuclear chromatin assembled into nucleoli, worked over there and extruded into the cytoplasm in the form of chromidia.

The described behavior of the nucleolus is also characteristic of other forms of the Coelenterata since Trinci ('06) found, in members of the family Eucopidae, the nucleolus dividing into many and variously formed bodies in constant transformation. Merejkowsky ('83) saw the same thing in *Obelia*, as I have also: Wulfert ('02) noted similar conditions in *Gonothyraea*, as did Bergh earlier. On the other hand, Harm ('03), in *Clava squamata*, and C. W. Hargitt ('06) in *Clava leptostyla*, say the nucleolus sometimes migrates bodily into the cytoplasm. The latter believes the nucleolus has nothing to do with yolk formation.

c. History of the nucleus in the germ cells. When the egg cells are first distinguishable the nucleus is characterized by the presence of a chromatin skein. This skein appears to be a series of chromatin loops, more or less centralized at one pole of the nucleus (figs. 2 to 4), a condition similar to that found by Bigelow ('07) in eggs of *Gonionemus* and by the author ('09) in *Tubularia crocea*. In both these cases the arrangement occurred in oocytes after the last oogonial division and at the stage just before growth started; the author interpreting this as the synaptic stage or period of the conjugation of the chromosomes, which, as Montgomery ('04) says, takes place in metazoa in the early portion of the growth period of both oocytes and spermatocytes. Certainly in the cases just cited, this condition was not a prophase of division, and in *Campanularia flexuosa*, although no divisions have occurred previously, this condition of the chromatin does not lead to division, and at the proper time the reduced number of chromosomes appears in the first maturation spindle. It seems safe to interpret this condition, therefore, as the stage of the 'reduction,' so-called.

The next stage in the egg is that of the migration of the egg into the gonophore, a stage marked by certain peculiarities of the nucleus, as well as of the cytoplasm. The loops of chromatin very

soon disappear and the nucleus contains only a very fine meshed and slightly staining reticulum (figs. 5, 6). Since the reticulum is present only after the loops have disappeared it is evidently formed from the loops, though the nucleolus secures some of the material. After the egg reaches its place in the gonophore a rapid and marked growth takes place (fig. 1). It is during this period that the peculiar nucleolar changes occur which lead to yolk formation. Let it again be noted that the dark bodies within and just without the nuclear membrane in figure 7 to 9 are bodies which have left the nucleolus and are passing through the nuclear wall into the cytoplasm. These bodies are chromatic in character, since the nucleolus has been formed from the dissolving chromatin loops of the earlier spireme, and therefore they should present essentially the same staining reactions as the rest of the chromatin, and such we find to be the case. The nucleolus, therefore, appears to be a synthetic or transforming center of the nucleus where the chromatin is to be changed somewhat for the function it is to perform in the cytoplasm. The fact that these bodies do not belong in the reticulum, as an integral part of it, is shown by figures 7 to 9; they do not lie in the reticulum itself and the latter shows plainly as a delicate and finely granular affair.

In the late growth period of the egg (figs. 11 to 17) the nucleolus breaks into parts of a greater or less size and, as these are surrounded by vacuoles, it is evident that they are not a part of the nuclear reticulum. And while it may be that the dissolving nucleolus adds some material to the reticulum, this does alter the general appearance or behavior to stains which the reticulum has shown during the earlier part of its growth. Figures 11 and 12, for example, show the same sort of a reticulum as is shown in figures 6 to 8, though a considerable portion of the nucleolus has disappeared. The point made here is simply this; the chromatin in the nucleus is of two sorts or if not actually different in composition, at least it serves two different functions in the cycle of the cell. A certain portion of the chromatin (that which has gone, or goes, into the nucleolus), after some probable transformation within the nucleolus, passes out of the nucleolus into the cytoplasm, there to serve a particular purpose, and this portion does

not take part in the reproductive activity of the cell. The second portion of the chromatin plays little part in the described activities of the growing egg, but at a certain period is essential for the reproductive phase. It is not considered that the chromatin intended for differing functions is fundamentally different, for, as already noted, both the reticulum and a portion (if not all) of the nucleolus has come from the same original source, namely, the chromatin skein in the young egg cells. Nor is it conceived that the chromatin, thus having one function, does not take any part in the other function or receive additions from the other portion of the chromatin. There is certainly an interaction between the nucleus and the cytoplasm, as well as between the nucleolus and the rest of the nucleus; the whole cell is a unit and it so works. But there appears to be some division of labor, and in analyzing conditions, functions, and substances, it is convenient to think of them separately.

To summarize the previous paragraphs: There has been no apparent modification in the nuclear reticulum by the dissolving nucleolus, nor is the preparation of the nucleus for division dependent upon a certain stage of nucleolar dissolution, for, as figure 13 shows, a large number of nucleolar fragments are still present and the chromatin of the reticulum is beginning to condense into strands at certain places. On the other hand, some nuclei, not included in the figures, show the nucleolus practically gone and there is only a faint reticulum. But there comes a time, at the end of growth, when the chromosomes begin to form. This may be by the formation of strands in the nuclear reticulum, as shown in figures 13 and 16, or it may be initiated by the condensation of the chromatin at the nodes of the reticulum (figs. 14, 17). In some cases the whole reticulum appears to become coarser, the grains composing it larger and staining more deeply (fig. 15). In many nuclei all these methods are active. But figures 13 to 17 show clearly that at the end of growth the nuclear reticulum, hitherto very delicate and lightly staining (figs. 7 to 12), shows the beginning of a segregation and a condensation of its substances which go to form the chromosomes, and the latter form only from the reticulum. This appears to involve the nuclear reticulum

alone, since the nucleolar substance remaining continues its dissolution and discharge into the cytoplasm. However, there is in no case any indication of the formation of a spireme previous to the formation of the chromosomes. Nor is there such a spireme in the eggs of *Tubularia crocea* or *Pennaria tiarella*, the chromosomes coming from the delicate reticulum of the nucleus.

Sections of eggs of *Gonothyraea lovenii* from Naples, show the same general relation of the reticulum, the nucleolus, and the nucleolar fragments, and the same position and behavior of the chromosomes as already described for *Campanularia flexuosa*. Whether this applies to all the details of the behavior of the eggs and their ingredients has not been determined, but there is a very close similarity in general.

4. *Polar body formation*

In whatever manner the chromatin of the nuclear reticulum condenses, there comes a time when the nucleus is without any trace of a nucleolus and the chromatin within is grouped into grains arranged in a very close reticulum—a stage between figures 17 and 18. When this condition is reached the nuclear membrane breaks, the chromosomes form and enter the spindle, and the divisions into the polar bodies and the egg occurs. Figure 18 shows the formation of the polar spindle outside the nucleus, the nuclear membrane broken and the chromatin granules escaping into the cytoplasm. In this figure, and in the egg from which the figure was made, the chromosomes were not yet formed. Whether it is usual for the chromosomes to delay their actual formation till after the rupture of the germinal vesicle I do not know, but such was the case in this particular egg. A point of significance should be noted in figure 18, namely that some of the chromatin granules, are escaping from the germinal vesicle through the broken wall and may be seen in the cytoplasm, while others are evidently attracted toward and are arranging themselves along the fibers of the developing spindle. This means that, of the chromatin which has as its function the division of the egg, there is only a portion needed for the new cells, the rest is superfluous and passes into the cytoplasm. In spite, therefore, of the large amount

of chromatin matter which has already passed from the nucleus into the cytoplasm during growth, there is still a super-abundance at the end of the cycle and only a portion is handed on to the next generation of cells. In figure 19, in which the chromosomes of the spindle are dividing, this extra chromatin is seen in the cytoplasm of the region as dark granules. These granules are the same as the granules which are found when the membrane first breaks as shown in figure 18. The very fact that there is a superfluity of chromatin after the considerable emission of chromatin during the growth period, is an indication that there has been new chromatin formed. For all the chromatin the egg had to start with came from the entoderm cell which was its progenitor, and this was approximately the same amount as is needed for the formation of the chromosomes. To have the amount necessary to go through two divisions (in the formation of the polar bodies) with a superfluity of apparently double this quantity, and, in addition, the extrusion of a large amount during the whole of the growth period, there must have been the formation of an enormous quantity of new chromatin during the growth period. This synthesis of chromatin I judge to be one of the functions of the nucleolus.

The objection has been raised and will doubtless again be offered that the chromosomes present in the first maturation spindle (fig. 19), which correspond closely in amount to the chromatin received by the primordial germ cell from its entodermal progenitor, do precisely represent these chromosomes. The aim of the objection is to force the conclusion that there has been a direct continuity of the chromosomes of the cell giving rise to the germ cell, and the chromosomes of the mature egg cell. This claim would thereby ascribe to the chromatin emissions no significance as far as relation to the chromosomes is concerned, and the chromatin left over after the chromosomes had formed would be more or less foreign or extraneous matter, or chromatin-like substance, of a different origin and fate but predestined to have no part in chromosome formation.

This does not appear to be a fair position to take, for with the foregoing insisted on as a premise, the significance of the loss of

chromatin from the germinal vesicle could not be properly considered. It is assumed, to start with, that the chromosomes of one generation are the same chromosomes as of the previous generation. Therefore, the chromatin emitted from the nucleus during growth and that remaining unused when the chromosomes are formed—in short all that does not enter into the chromosomes—is of a different sort, had a different origin and cannot be considered as related to the chromatin of the chromosomes. In essence the argument then proceeds, that since the chromatin which leaves the nucleus during growth has a different fate from that entering the chromosomes, it belongs in a different category, and this is evidence of the genetic similarity of the chromosomes of the two generations. We thus arrive at the same point from which we started, the whole argument being based on the a priori assumption that the chromosomes of one generation continue essentially unchanged to the next generation. Even if it should be granted that there is evidence of the continuity of chromosomes in molluscs, echinoderms, insects and so forth, it should not be forgotten that certain cellular activities, as cleavage in some of the coelenterates, do not follow the plan of cleavage of the molluscs and others. Since this difference has been established, it might be expected that differences would exist in other processes. We must, then, *examine the evidence* in *Campanularia* and not *reason* insect conclusions into our data.

The facts are these: The earliest recognizable egg cell has all its chromatin arranged in a spireme. This is relatively small in amount, for the primordial germ cell and its nucleus is little different in size from any other body cell. This spireme of chromatin entirely disappears and there is present in the nucleus a reticulum and a (partly chromatic) nucleolus. During the growth of the egg there is a very considerable loss of chromatin from the germinal vesicle into the cytoplasm; all of the chromatin of the nucleolus goes into the cytoplasm; whether much, little, or any of the chromatin of the reticulum is lost now is not possible of demonstration; it is assumed that little if any is lost. Here is perhaps, the first place for disagreement, the claim being made that the chromatin in the nucleolus is of a different sort from that

in the reticulum, and the latter is retained while the other goes into the body of the egg. But the chromatin of the assumed two sorts came from the same original source, the chromatin loops of the conjugation phase (synapsis) of the primitive egg cell. Is it sufficient to say that, since there was a different ending, there must have been a different source? To claim this would be assumption, not observation, for there is no way of demonstrating a difference in the chromatin of the reticulum and of the nucleolus. But let us grant for the sake of argument that this reticulum retained in the germinal vesicle is the essential chromatin. We have the chromatin scattered over a very fine-meshed, extensive reticulum in the minutest grains. There is no difference in any part of the network so far as can be discovered by differential staining or by the use of apochromatic lenses. Later there appear larger masses of chromatin at the nodes of the network, or the whole reticulum appears to condense into larger masses of chromatin. These appear the same everywhere; again no difference can be discovered by staining methods or by careful use of apochromatic lenses. But some of these chromatin masses enter into the chromosomes of the maturation spindle and some escape into the cytoplasm in the form of grains when the membrane breaks, not having been used to help form the chromosomes. Here the claim will again be made that the chromatin which escapes is of a different sort from that which goes into the chromosomes. But once more, the claim has no basis at all in *observation*; it is simply a position assumed by the necessity of making the facts agree with the theory which is held to apply in this case.

My position is that we can depend on facts more than on interpretation of those facts, particularly in our attempted generalizations of interpretations. So, in the absence of any evidence whatever that the chromatin in the nucleolus is different from that in the reticulum of the germinal vesicle, that the chromatin of the reticulum which forms chromosomes is different from that which remains unused, it must be granted that we are forced to the conclusion that all the chromatin is of the same sort, and not that a portion is fore-doomed to be cast into the cytoplasm and another part destined to form the chromosomes. This would not, of

course, be in agreement with those who hold that the chromosomes are continuous entities from one generation to the other. But the facts do not seem to warrant any such conclusion.

It can not be claimed that, because some chromatin comes to an end different from other chromatin, there is in this very fact an indication of its essential unlikeness. Some of the young egg cells of *Campanularia flexuosa*, in moving from the pedicel into the body of the gonophore, pass by the only place where there is room for them to develop, so they continue their migration (as shown in fig. 1) into the distal end of the gonophore, where they come to naught but degeneration. Can we say that since this egg came to a different end from the one which entered the gonophore where there was more room, it was from the first predetermined for failure? In other forms—as *Tubularia*, *Pennaria*, *Clava* and so forth—it has been clearly shown that two eggs have behaved alike for a considerable time, and it is only the chance of better position as regards food and room and the like which shall determine which has the opportunity to develop into a mature egg and which shall degenerate.

The chromosomes formed in the manner described in a previous paragraph, arrange themselves in a spindle in the usual way. They are ten in number, the reduced or haploid number, and appear, as figure 19 shows in the metaphase, as single bodies. No centrosomes and little indication of polar asters are present in the spindle. Two polar bodies are formed (fig. 20 shows one of them somewhat degenerate). The spermatozoon appears to enter by an attraction cone but leaves no path to indicate its movement into the egg. Figure 20 shows the fusion of the two pronuclei and the formation of the cleavage spindle. Figure 21 is a section through a second cleavage spindle, the right end of which contains all the chromosomes, which are seen to be twenty in number; the spindle having been cut somewhat obliquely, only a part of the chromosomes of the left end of the spindle were present in this section.

Briefly summarizing the nuclear history of the egg cells, we find that after the division of one of the epithelial cells of the entoderm of the stem of the gonophore (or by the transformation of a single entire cell) the basal half has its chromatin arranged in a

spireme or into loops more or less regularly and definitely grouped together. All the chromatin in this new cell came from the old cell. These chromatin loops are transformed into a nuclear reticulum and a nucleolus. There is a great increase in the size and volume of the nucleolus at an early period; later it is believed there must be a synthesis of new chromatin, since the original chromatin of the nucleus is not sufficient to account for all that is used during the growth period by being passed into the cytoplasm, and that which still remains over when the chromosomes of the maturation spindle are formed. When the nucleolus has disappeared the reticulum undergoes a condensation of its chromatin into large grains and eventually these form chromosomes. Only a small portion of the chromatin which remains in the nucleus at the time of polar body formation is actually used in the formation of the chromosomes and the rest is scattered in the cytoplasm and there dissolved. This sort of thing, which from published accounts is not limited to the hydroids or to the coelenterates but is more or less common, leads us to consider whether the matter of the continuity of the germ-plasm, the individuality and continuity of the chromosomes, the alleged supremacy or uniqueness of the chromosomes in heredity and so forth, are not after all mere names or phrases. There should be more careful thought as to whether the things connoted by these names are not also without real meaning or significance. Once they were undoubtedly useful and served a valuable purpose, but are we not allowing ourselves to be unduly handicapped and hemmed in by these older conceptions? Must we not come to look more to the ultimate chemical composition and constitution and not to morphological entities really to harmonize and explain the various and varying functions and activities of all cells, somatic as well as germ cells?

CONCLUSIONS

The egg cells of *Campanularia flexuosa* arise in the entoderm of the pedicel of the gonophore, by the transformation of a single epithelial cell, or from the basal half of a divided cell, the distal half of which remains an epithelial cell and retains its epithelial functions. Therefore the egg cells have come from differentiated body-cells (so-called) and there is no differentiation of the germ-plasm in the sense that germ cells are early differentiated and set aside and do not participate in the body functions. Any cell of the entoderm of *Campanularia flexuosa* may become an egg cell if it is in the position of the developing gonophore. There is no division of the primitive egg cell but each transforms directly into a single mature egg cell.

The chromatin of the primitive egg cell, at first arranged in definitely arranged loops, disappears, forms a fine-meshed delicate reticulum and a nucleolus (the latter also contains non-chromatic matter).

The nucleolus becomes greatly vacuolated, breaks up into fragments of various sizes and shapes, and the chromatin contained in these passes through the membrane of the germinal vesicle to form the chromidia in the cytoplasm. Co-incident with this chromatin emission, the rapid growth period of the egg begins. So long as the dissolution of the nucleolus continues there is a considerable outflow of material from the nucleus, shown by currents in the cytoplasm. The chromatin particles in the cytoplasm become, or have something to do with the formation of the yolk bodies. Yolk formation, chromatin emission, strong currents from the nucleus, and growth of the egg cease when the nucleolus has disappeared. The nucleolus is, then, a dynamic center, concerned primarily with the nutritive activities of the egg cell. It also aids in the formation of new chromatin.

The nuclear reticulum is apparently unchanged by the dissolution of the nucleolus, but when the nucleolus has disappeared, or nearly so, the chromatin of the reticulum forms the chromosomes. There is not the formation of a spireme and not always the formation of strands in the reticulum, but the chromosomes may form by the segregation of the chromatin granules of the

reticulum. The first maturation spindle is formed outside the membrane of the germinal vesicle, the membrane breaks and the chromosomes enter the spindle.

Not all the chromatin of the germinal vesicle enters into the formation of the chromosomes, but the apparently larger amount escapes into the cytoplasm when the membrane of the germinal vesicle breaks. The chromatin which escapes is of the same sort and has the same history as the chromatin granules which form the chromosomes. This is evidence against a continuity of chromatic material from generation to generation.

There are ten chromosomes in the maturation spindle, the reduction apparently having taken place at the time of the polar arrangement of the chromatin loops in the primitive egg cell. Two polar bodies are formed.

On account of the evidence against the continuity of chromatic matter from one generation to another, and because there is shown to be no difference between the germ plasm and the body plasm until after the egg has begun to grow, it is suggested that we must come to look to the ultimate chemical composition and constitution for explaining cellular activities and relations.

January 15, 1913.

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PLATE 1

EXPLANATION OF FIGURES

All figures have been drawn with the aid of the camera lucida. The magnification indicated is the original magnification, the figures as they appear in the plate have been reduced to three-fourths the original size.

2 Primitive egg cells in the pedicel of the gonophore, arising from entire entoderm cells. $\times 1900$.

3 and 4 Egg cells in the pedicel of the gonophore. These have been formed from the basal half of a divided epithelial (entoderm) cell. The chromatin of the nuclei arranged in loops. $\times 1900$.

5 Egg cell passing along the pedicel into the gonophore. The chromatin has lost its polar arrangement and has formed a reticulum in the nucleus. $\times 1228$.

6 Egg cell in position in the gonophore. Beginning of chromatin emission. $\times 715$.

7 Egg grown to about one-fifth its mature size. Nucleolar fragmentation shown, chromatin emission taking place. $\times 1228$.

8 Egg about one quarter grown. Shows nucleolar fragmentation and vacuolization; chromatin emission; beginning of yolk formation. $\times 1228$.

9 In addition to the features shown in figure 8, this egg shows the granules of the cytoplasm arranged in radial lines, an indication of outgoing currents from the nucleus. $\times 1228$.

10 Growing egg. Vacuolization of the nucleolus very marked. Chromatin emission. $\times 1228$.

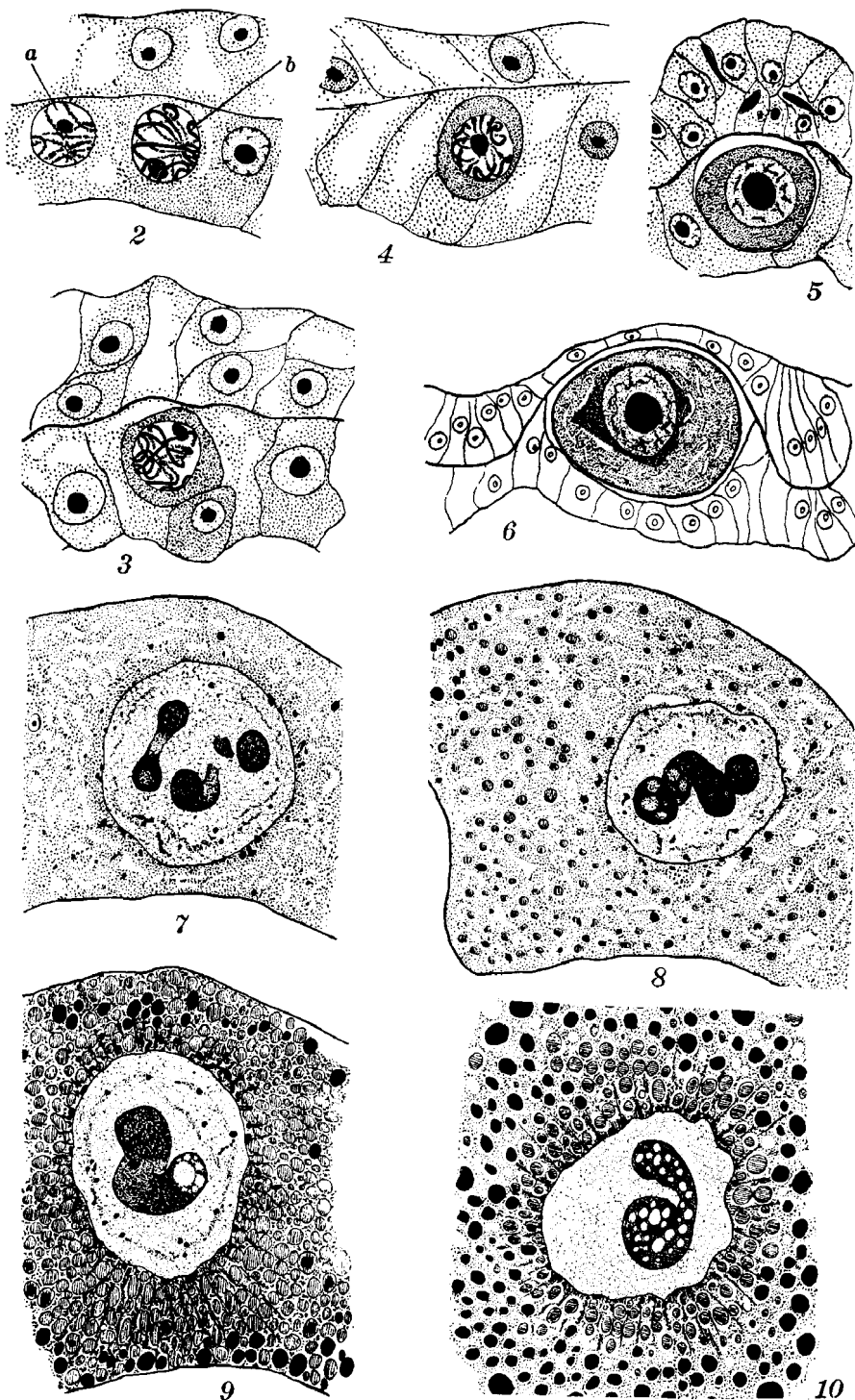


PLATE 2

EXPLANATION OF FIGURES

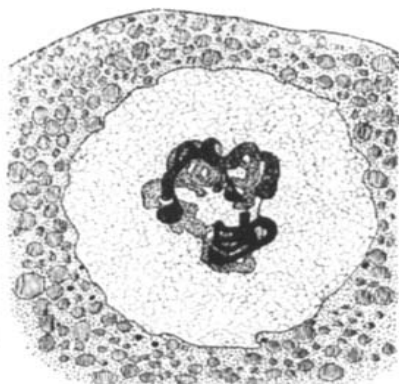
11 and 12 Growing eggs showing extreme nucleolar fragmentation. Note the delicate, fine grained reticulum in the nucleus. The two eggs were treated by different killing fluids and stained in different ways. $\times 1228$.

13 Large growing egg. Nucleolus represented only by small fragments lying in vacuoles in the nucleus. In certain parts of the nuclear reticulum the chromatin is forming strands. $\times 1228$.

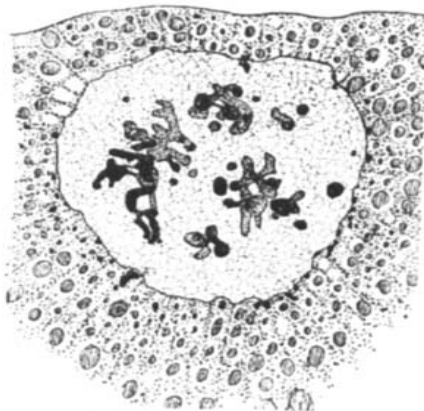
14 Egg near the end of the growth period. Nucleolus in fragments, the nuclear reticulum growing denser at the nodes. $\times 1228$.

15 Egg nearly mature, the nucleolar fragments mostly in a ring near the periphery of the nucleus. The whole nuclear reticulum is becoming denser and more deeply staining. $\times 1228$.

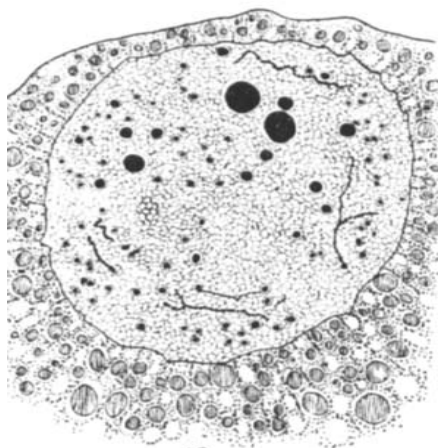
16 Egg at the end of the growth period. The nucleolar fragments, within vacuoles, are of different composition, as shown by the different reactions to stains. Chromatin in the reticulum forming strands. $\times 1228$.



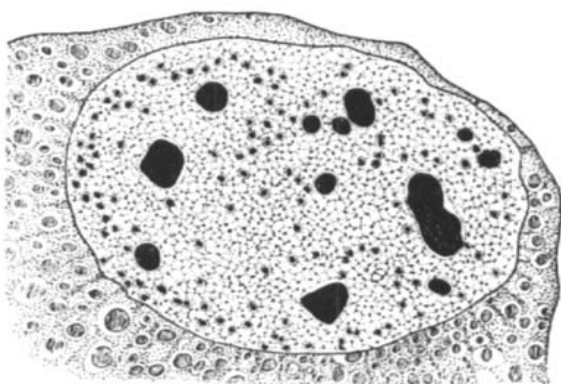
11



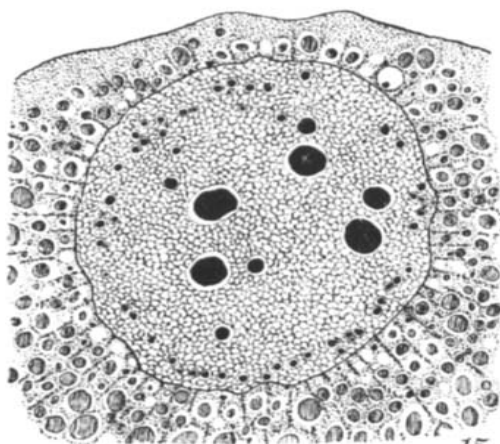
12



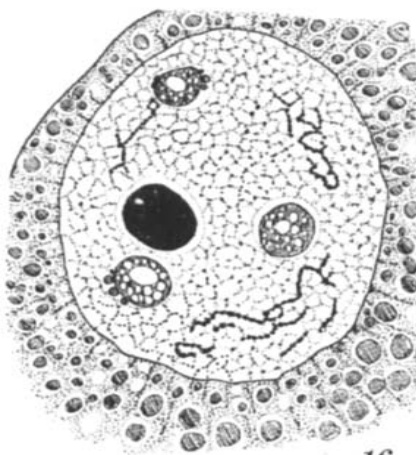
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PLATE 3

EXPLANATION OF FIGURES

17 Germinal vesicle of egg preparing for maturation. The nucleolus is represented by only a few small fragments. Chromatin of the nuclear reticulum segregating into granules at the nodes of the net. $\times 1228$.

18 First maturation spindle forming outside the germinal vesicle. The chromatin granules are arranging themselves along the fibers of the spindle or are escaping into the cytoplasm through the broken membrane. $\times 1228$.

19 Metaphase of the first maturation spindle, with the chromosomes splitting. Note the granules of chromatin in the cytoplasm in the region of the spindle. $\times 1900$.

20 Copulation nucleus, first cleavage spindle forming. Remains of one polar body present. This drawing is compiled from two sections. $\times 1900$.

21 Telophase of the second cleavage spindle. The section is cut somewhat obliquely. At the right end of the spindle all the chromosomes present in the spindle are shown (20 in number). Not all of the chromosomes are present in the left end of the spindle in this section, but are found in adjacent sections. $\times 1900$

