Studies in the Dictyotaceae.

II. The Cytology of the Gametophyte Generation.

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With Plates XII, XIII, and XIV.

IN a previous paper the development, germination and cytology of the tetraspore in *Dictyota* and other members of the group were described. In the present paper it is proposed to deal with the development of the oosphere and antherozoid, the fertilization of the ovum and its subsequent segmentation, together with the parthenogenesis of unfertilized eggs—the observations in this case applying to *Dictyota dichotoma* only.

In this plant the male and female gametes are borne upon different individuals. Excepting so far as the reproductive cells themselves are concerned, there is no difference either of form or of structure between the tetrasporic plants on the one hand and the sexual ones on the other. It is true that the latter generally have the branches of the thallus broader than those of the former, but to this rule there are frequent exceptions.

As already described, the discovery of motile antherozoids, first made in 1896, was not confirmed till nearly a twelvemonth after. Subsequently the astonishing fact was established that, unlike the tetraspores, both the oogonia and antheridia are developed simultaneously in fortnightly crops, each crop being initiated a little before the lowest neap tide, and arriving at maturity about the period of the highest succeeding spring tide. The gametes thus produced are liberated during two or three days while the high tides are on the wane. A regular succession of crops continues thus from July to the end of October. So far as I am aware nothing like this remarkable periodicity has hitherto been observed in the case of any other Alga. During the last six summers careful records have been kept of the appearance of these crops, and their relation to the conditions of their environment. The data thus collected, together with a discussion of the factors concerned, will be published in a separate paper. These observations only apply to the plant as growing on the North Wales coast; it

would be instructive to know whether they hold good for other localities also.

As the sexual cells pass through the various stages of development at approximately the same dates, it is far easier to find any required mitosis here than in the gametangia of the Fucaceae or in the tetrasporangia of the Dictyotaceae. After the preliminary trials one has only to consult the tide tables for the particular locality to know within two or three days when certain stages may be found.

Although Thuret and Reinke have given excellent figures of the sexual cells of *Dictyota* no description of their cytology has as yet been published.

I. THE DEVELOPMENT OF THE OOGONIA.

The oogonia instead of being isolated, as is the case with the tetrasporangia, are grouped together in sori, each sorus consisting of twenty-five to fifty oogonia, closely packed together without paraphyses or any other accessory cells. In the case of weak plants, or under adverse conditions, the number may be reduced to half a dozen or fewer. The sori are scattered all over the two surfaces except the basal portion, the apices, and a very narrow band at the margins of the thallus. The sori of the new crop appear between the scars of the older ones, and, in the case of an elongating plant, in acropetal succession on some of the surface cells which have sufficiently matured since the preceding crop. This process may go on till the whole of the available surface has been used up, then the plant dies. In no case are gametangia produced by the stalk-cells left by preceding sori.

As in the case of the tetrasporangium the rudiment of the oogonium is one of the small cells of the assimilating layer, which increases in dimensions till the free part is about three or four times the height of the original cell (Pl. XII, Fig. 1). The nucleus then divides, and a stalk-cell is cut off. The oogonium mother-cell increases somewhat in size, but it undergoes no further division, the nucleus becoming that of the ovum without an additional mitosis. This is essentially different from the state of affairs in the oogonium of the Fucaceae, where eight nuclei are produced even in the cases where only one egg ultimately matures. In the Fucaceae also, where there is no asexual generation, reduction takes place in the first of the three oogonial mitoses; in *Dictyota*, as shown in the preceding paper, it is brought about in the first of the two tetrasporic divisions. In a few days after the separation of the stalk-cell the apex of the oogonium ruptures and the naked oosphere is liberated into the water, where fertilization takes place.

The stalk-cell mitosis is accomplished from one to four days before the highest spring tide, the variation in the time being dependent on the length of the interval between two spring tides, the conditions with respect

to temperature and light prevailing at the time, and the period of the year. The whole process is gone through with considerable rapidity, and all the stages may be found in a single sorus. In the case of the tetrasporangium the stalk-cell separates while the rudiment is still comparatively small; here, however, it is delayed till the cell is nearly fully grown, and liberation follows in about three to five days.

Of the oogonia in a sorus the central ones only are vertical to the thallus; in consequence of their great increase in diameter as compared with the stalk-cells the others lean outwards, the angle being greater as the periphery is approached; this makes it difficult to obtain median sections through the dividing nuclei.

It is generally stated that there are no borders of elongated sterile cells to the oogonial sori, as there are to the antheridial ones. This, however, is not strictly correct; borders, partial or complete, are frequently found and may arise in one of two ways.

1. Some of the outer cells of a sorus may at a comparatively early stage in their development be compressed by the more actively growing inner ones, and prevented from developing further. These are visible in sections as narrow elongated cells, but in a surface view are completely hidden by the outermost of the fertile oogonia.

2. Towards the close of the season, when unfavourable conditions supervene, most of the outermost oogonia fail to mature even after attaining their full size; the cytoplasm is smaller in amount and the nucleus does not divide. These facts suggest the mode in which the antheridial borders may have arisen, and the manner in which borders may also in the course of time be acquired by the oogonial sori. In this connexion it is instructive to notice that the central portions of both male and female sori are more active than the peripheral ones; in the early stages the nuclei are more advanced, liberation generally starts in the middle of the sorus, and sterilization, although it sometimes occurs there, is not nearly as frequent as it is in the marginal regions of the sori.

Fig. 1 shows the structure of the oogonium-rudiment shortly before the separation of the stalk-cell, as seen in a section vertical to the surface of the thallus and transverse to the branch. As in the case of the tetrasporangium-rudiment the basal part of the cell has very large vacuoles, while the remainder is occupied by denser cytoplasm, in which the chloroplasts are much more crowded. The curved, rod-like centrosomes and radiations are very distinct, not only at the distal pole but most commonly at the basal pole as well. The physodes as usual are chiefly located in the peripheral layers of the free surface.

The early prophase stage is very much like that of the stalk-cell division in the tetrasporangium; there is the same coarse chromatin thread, irregular in the arrangement and thickness of its granules, and soon

segmenting into the chromosomes. When, however, the latter make their appearance, they exhibit the longitudinal split far more clearly than do the chromosomes in any other division (Figs. 2, 3). At first the nucleolus has one or more fairly large vacuoles with a number of smaller ones; soon, however, the nucleolar membrane disappears, and the bulk of the nucleolus is seen to consist of a large number of deeply staining fibrillae, together with a single, slightly staining globule (Fig. 3). During this period the amount of nucleoplasm is very small, and the centrospheres are less distinct.

The spindle (Fig. 4) is intranuclear and narrow. There are a few mantle-fibres extending outwards, the spaces between which are occupied by a large number of the deeply stained fibrillae derived from the disintegrating nucleolus. The globule is generally present either on the spindle-fibres or in the immediate vicinity.

The polar view represented in Fig. 5 shows the chromosomes to be curved in form and clearly sixteen in number. The nucleus at this stage is somewhat nearer the base of the cell, and the cordate appearance shown in Fig. 4 is very frequently met with; it is almost certainly due to a contraction of the spindle after fixation. In preparations of segmenting eggs depressions are frequently found at both poles of the nucleus. These facts confirm the observation that the spindle-fibres at this period are in a state of tension and that they contract on fixation.

During the anaphase stage the spindle is still narrow, but the nuclear cavity is wide and the membrane is intact except at the poles. The centrosomes are clearer than during the preceding stage, and in most cases the nucleolar globule is present. When the chromosomes reach the poles of the spindle the membrane disappears, the lower nuclear mass remains in the same position or descends slightly while the upper ascends, thus causing the whole figure to become greatly elongated. In the telophase stage (Fig. 6) of the oogonial nucleus the chromosomes, now greatly increased in size, but still preserving their curved form, are seen to be quite separate from each other and sixteen in number. When they fuse they frequently form a thick deep-staining irregular ring in which at one particular stage of the fusion about eight deeply stained masses may be distinguished (Fig. 7). Something similar was described for the stalk-cell division of the tetrasporangium, but was not observed in any of the other mitoses. The two divisions are strikingly similar also in the fact that only one deeply staining mass is formed in both cases, which ultimately becomes the nucleolus.

If Figs. 1, 10-13 be compared certain peculiarities of cell-wall structure may be observed. Although the drawings are made from preparations fixed and stained by different methods they all agree in showing a distinct difference between the membranes of the parts of the cells embedded in

the thallus and those of the free, reproductive portions. For want of space this topic must be deferred for discussion in another paper. It may be pointed out that we have a provision here for the rapid elongation of the gametangial walls, for the early splitting of the lateral walls, and for their dissolution when the gametes arrive at maturity. It is evident that the basal cells have their walls of different chemical composition, and of more enduring character.

When quite mature the degeneration of the apical walls and the increasing turgescence of the ovum causes the former to burst and to liberate the oosphere, which now lies a naked spherical mass of protoplasm. Fig. 19 shows a section through such an ovum. The nucleus now occupies nearly one-third of the diameter of the egg. It is surrounded by a zone of 'kinoplasm,' outside of which is the somewhat vacuolated cytoplasm with its numerous chloroplasts. In well fixed material these are generally oval or rounded, but when much contraction has taken place they are very narrow and elongated. At the periphery of the egg there is a zone of granular cytoplasm in which are embedded numerous intensely staining physodes.

Very soon after liberation of the oospheres in a sorus the oogonial walls are completely dissolved, leaving only the basal cells, which are marked from the other surface cells of the thallus by their slightly greater width, and particularly by their paler colour. The latter peculiarity is due to their having fewer and paler chloroplasts. As soon as they are exposed to the light by the liberation of the overlying eggs, the chloroplasts begin to multiply and acquire a deeper colour, but the scars of two or three of the most recent crops can be easily distinguished from each other by their different shades of colour.

II. THE DEVELOPMENT OF THE ANTHERIDIUM.

If a fresh plant of *Dictyota* be examined one to five days before the lowest neap tide the rudiments of the antheridia may be distinguished by their slight increase in diameter and their deeper colour, but chiefly by the pale circular area in the centre, indicating the position of the nucleus. Material fixed about this period would show the various stages in the stalk-cell mitosis, and perhaps also in the first division of the mother-cell of the antheridium.

The antheridia, like the oogonia, are arranged in sori, but they are much more numerous than the former, the number in a fair-sized sorus being from one to two hundred. Each sorus has always a well-defined border of sterile cells consisting of three or more rows, the innermost of which are of about the same length as the antheridium, while the others successively diminish in height (Figs. 12-14). Some time after the liberation of the antherozoids the innermost border-cell cuts off a cell near the

top; occasionally this happens also in the case of some of the cells of the next row.

The stalk-cell division takes place when the cell is still very small, consequently the nucleus, though much larger than that of one of the vegetative cells, is considerably smaller than the nucleus of the tetrasporangium or of the oogonium. The result is that the various processes are more difficult to follow, the counting of the chromosomes being in some stages particularly difficult. This has necessitated the comparison of a very large number of figures. It is found that the mode of division closely resembles that which obtains in the stalk divisions already described. While the increase in size is as yet exceedingly slight and there has been but little accumulation of protoplasm, the nucleus and nucleoli are greatly enlarged though the nuclear network is still inconspicuous. Even at this stage the centrosomes are perfectly clear at both poles, and their form is that already described in other cases. As usual the distal pole is distinguished by beautiful radiations, which are nearly always absent from the other pole. There are no new points to be noted with regard to the spirem, the chromosomes, or the spindle. In many instances the lower pole seems to project into the vacuolated part of the cell. Sometimes a few strands of protoplasm bridge over the space, and connect the nucleus with the thin lining of protoplasm at the base. Frequently thick sections show strands that end blindly in the vacuole. These recall the appearance of the cords of protoplasm described by Phillips and seen in the living cells of certain Florideae, and which are in a state of continual motion within the vacuole, either extending or retracting or bending upon themselves. The absence of radiations and the position of the lower pole with regard to the vacuole preclude the possibility of a pull being exerted by any kinesis structures at the base.

During the metaphase and anaphase stages the nuclear membrane is present, in fact it persists in some cases till the very commencement of the telophase stage, then, as in the oogonium, the membrane disappears and the lower nucleus descends into the middle of the vacuolated region.

The reconstruction of the nucleolus sometimes follows the same course as in the two stalk-cell divisions already described. In other cases several masses may be seen which differ greatly in size. These soon coalesce to form the spherical nucleolus, which at first presents a curious appearance, as if it consisted of a globule of pale-staining substance with a number of chromosome-like bodies embedded, chiefly though not solely at the periphery. This appearance, together with the very scanty, slightly staining nuclear network, confirms the idea that in the daughter-nuclei resulting from these three stalk-cell mitoses the whole of the chromatin is located in the nucleolus.

The partitioning of the antheridium into antherozoid mother-cells

is completed a few days before liberation, the whole process occupying from six to ten days. Particular attention was paid to the later cell-divisions to see if any evidence of amitosis could be observed. As far as can be seen, however, all the usual stages of karyokinesis are repeated at each step, and after each cell-division the nucleus assumes the appearance of the resting condition. Several reliable countings of chromosomes were obtained in the earlier mitoses, both in the prophase stage and in polar views of the equatorial plate stage (Fig. 11). All these agreed in giving the number as sixteen. Occasionally a larger number was indicated, but in all such cases it was easy to see that some of the bent chromosomes had their angles cut off, thus giving two chromatic segments instead of one. The first division-wall of the antheridium mother-cell is vertical to the thallus and at right angles to the long axis of the branch, the second is also vertical but parallel to the axis, so that in surface view four cells are seen. The succeeding divisions are not so regular, but a number of tangential division-walls appear before any further vertical ones are formed. Ultimately the antheridium in surface view is seen to consist of sixty-four cells arranged in four groups of sixteen. In vertical section there is great divergence between different antheridia, the number of tiers in some cases being twenty-four (Fig. 14), in others about twenty. The number of successive nuclear divisions must be about twelve, but it is clear that many of the cells fail to divide and others abort. It is instructive to note in this connexion that in many cases when the circumstances are unfavourable it is the lowermost cells in an antheridium that are first retarded; this fact will be referred to again when discussing the importance of light as a factor in the development of the gametangia.

Careful countings of the antherozoid mother-cells in a large number of antheridia show that the average number of sperms produced in an antheridium is about 1,500. It has already been shown that the number of oogonia in a sorus ranges from about twenty-five to fifty, while the number of antheridia is generally from 100 to 300. Taking the lower number in each case we see that for every egg in an oogonial sorus there will be 6,000 antherozoids in a sorus of antheridia. In order to form an approximate idea of the number of gametes produced by a single plant the sori were counted on a specimen twelve inches long. They were found to be over 3,500 in number. Allowing an average of 1,500 antherozoids for every antheridium this would give us a total of over 500 millions for the whole plant, a number which is certainly much too low for a vigorous, full-grown plant. If we consider a single locality, the Menai Straits for instance, where *Dictyota* flourishes in great abundance, and the vast multitude of sexual plants which on certain days every fortnight throughout the summer months fill the water in their vicinity with untold myriads of swarming gametes, we cannot but be filled with astonishment at the

prodigious numbers produced, and yet so far as the production of new plants is concerned nearly the whole of the energy expended and the elaborate mechanism employed has been wasted. This, of course, is an old story, but every new exemplification of it comes with a fresh surprise.

The antherozoids were described by me in a short paper published in 1897. Since then I have had many opportunities of examining these bodies in the active condition, of fixing and staining them by better methods, and of studying their structure by means of superior objectives. Some of the conclusions first arrived at are now found to be incorrect in certain particulars, and several points which were then obscure have now been satisfactorily cleared up.

Taking first the antherozoid as it appears in the living condition, we find that it is pear-shaped while active, but spherical when it comes to rest (Fig. 18). At the broader, posterior end there is a globular colourless part which with chromatin stains takes on an intense colouration, showing it to be the nucleus. The pointed end is occupied by granular protoplasm in which there is always a very minute red eyespot, sometimes two as in the lowest example in the figure. Instead of being situated close to the attachment of the cilium as is usual in other antherozoids and in zoospores, the eyespot here is always at a distance from it, frequently at the pointed anterior end. The cilium is not terminal as was stated in the '97 paper, but lateral as in other Phaeophyceae; this fact can be clearly seen only while the antherozoid is in motion. The cilium is attached at the posterior edge of the cytoplasmic cone, near its junction with the nucleus. Very often there seems to be a slight thickening at the very base, but on this point it is not easy to speak with certainty.

Of the fixed preparations one of the most successful is that shown in Fig. 15, fixed with potassium-iodide iodine in sea-water; this preserves the form and structure very faithfully. Those in Fig. 16 were fixed in dilute Flemming solution, and stained by the iron-alum method. Here the anterior part is much wrinkled, but the nuclei and physodes are very distinct. When fixed with dilute Hermann's solution (Fig. 17) and stained with gentian violet the nuclei stain very deeply, the cytoplasm slightly, and occasional physodes are seen. At the upper right-hand corner is shown a monstrous antherozoid with two nuclei. If doubly stained with brazilin and Hoffmann's blue the nucleus takes on the red colour, while the anterior part stains blue.

The number of physodes present varies greatly, as also does their reaction to a solution of vanillin in HCl. When fresh plants are tested with the above reagent the sori frequently give no trace of the red colour (phloroglucin?) usually given by the physodes of mature cells. At other times every sorus is coloured a deep red; this happens generally when there has been some delay in the liberation of the antherozoids. When

the reaction is partial it generally appears in the central antheridia only, an additional confirmation of the greater vigour of this region.

Much time and trouble have been expended in trying to find whether there is a second cilium or not. In the Fucaceae, as is well known, the posterior cilium is very long but very much thinner than the anterior one. The possibility had to be considered that in *Dictyota* the second cilium is so reduced in length as to be quite rudimentary, or that it is attenuated to such an extent as to be indistinguishable. In the upper left-hand corner of Fig. 17 is represented an apparently biciliate antherozoid, and several more such cases have been observed. After a careful comparison with fixed and stained antherozoids of the Fucaceae I have failed to satisfy myself that these appearances are not due to strands of mucilage. In the living condition I have only once seen anything suggestive of a second cilium. On one occasion, while examining a number of antherozoids by means of Zeiss's D* water-immersion objective, one was observed which had got stuck fast at the bottom of the dish. In almost all cases of the kind it is the end of the long cilium that becomes attached; in this instance, however, the cilium was quite free, and it could be clearly seen violently lashing about in the water. Careful observation showed that the antherozoid was attached to the glass by a filament of much greater tenuity than the cilium, and of about the same length as the body of the sperm. This is the strongest evidence yet obtained for a second cilium, but even this is quite insufficient to enable one to come to a positive decision on the point.

III. THE FERTILIZATION OF THE EGG.

Thuret, Reinke, and other investigators tried to solve the question of the mode of fertilization in *Dictyota*. Their failure to obtain active antherozoids for their experiments prevented them from arriving at a correct solution of the problem. It was further complicated by the fact that many of the eggs segmented in the total absence of antherozoids. Among the various suggestions advanced from time to time in explanation of the difficulty were the following:—

(1) That fertilization is effected before the liberation of the eggs from the oogonia; this seemed to explain the apparent germination of liberated oospheres in the absence of sperms.

(2) That there are two kinds of asexual spores.

(3) That the antherozoids have become functionless and that the eggs are habitually parthenogenetic. To Johnson, in his study of *Dictyopteris*, belongs the credit of having first pointed out the true answer to the question, though his somewhat tentative pronouncements were never confirmed by him.

The present investigation has now conclusively demonstrated that

fertilization is entirely external as it is in the Fucaceae, that unfertilized eggs will pass through a few of the earlier segmentation stages by an abnormal process of parthenogenesis, but that normal germlings can only be produced from fertilized eggs.

The following is the method employed for securing material for this stage. Oogonial and antheridial plants are collected when the sori are quite mature, if possible on the evening preceding the day of maximum liberation. The plants are kept separate in moist chambers till the following morning, pieces are then immersed in glass dishes containing clean sea-water. The oospheres slowly burst through the walls of the oogonia and gradually sink to the bottom of the vessel. Before a sufficient number have been liberated to make it profitable to experiment with, half an hour or more will have elapsed. When the antheridial plants are immersed the antherozoids come out of the restraining cells very quickly, and soon the water is cloudy with swarming sperms. If now some of the latter are added to the oospheres by means of a pipette, it will be seen that instead of impartially surrounding all the eggs, as do *Fucus* antherozoids, many of the eggs are here passed over and entirely ignored, whereas others are covered with antherozoids, which in some cases lie three to twelve layers deep. If the same material be fixed after an interval of twenty-four hours and subsequently sectioned it will be found that the antherozoid-covered eggs have segmented in a perfectly normal manner, whereas the others show the abnormal mode of division described below, a method which is characteristic of the so-called parthenogenesis already referred to.

When pieces of oogonial plants have been immersed for about an hour the eggs set free will have been liberated at various intervals of time, some nearly an hour, others only a few minutes. If now instead of adding antherozoids we test the eggs with distilled water, we find that those that have been liberated for half an hour or more have already acquired walls. This partly explains why they are no longer capable of attracting the antherozoids, and why they cannot possibly be fertilized. From this circumstance it is very difficult to get uniformity of stages in cultures of *Dictyota* oospheres.

Although the mode of fertilization in *Dictyota* is very similar to that in *Fucus* there are important differences. In the former the liberation of the gametes is simultaneous; this tends to give it a great advantage over *Fucus*, where there is no regular periodicity in the production of the sexual cells. On the other hand, the eggs in *Dictyota* very soon lose their capacity for fertilization, whereas those of *Fucus* may retain it for days. This would seem to act as a disability, and so to neutralize the advantage obtained by the simultaneous emission of gametes.

As already explained it is my intention to deal with physiological considerations in a succeeding paper; two points, however, demand mention here.

The first is the relation of the antherozoids to light. It was stated in a former paper that light is essential to their mobility. It is true that light has a very great influence upon the antherozoids, as will be shown again, but if they are really ready for liberation they will on immersion display their activity at any hour, night or day.

The second point relates to chemotaxis in fertilization. Buller has recently performed some experiments on the eggs of certain animals, which lead him to infer that in external fertilization there is no such thing as chemotactic attraction of the spermatozoids. He seems inclined to follow Bordet, and to extend this generalization so as to include the Fucaceae. Thus he says in explaining Bordet's conclusions: 'According to this observer it is simply the ability of the spermatozoa to adhere to surfaces by the tip of one of the cilia which leads to their collection upon an egg, while their meeting with it is a matter of chance. A few observations of my own at Naples upon the fertilization of *Cystoseira barbata* did not reveal to me any certain attraction of the spermatozoids from a distance, but the collection of the spermatozoids upon the eggs in consequence of their ability to cling to surfaces was clearly seen. Nevertheless in view of the positive statement of Strasburger a careful re-investigation of the question seems to be desirable.' It is not my intention at present to discuss the question of chemotaxis in the Fucaceae. As regards *Dictyota*, however, it would be difficult to find anywhere a stronger suggestion of attraction at a distance than is offered by the phenomenon described above, where, out of several hundred eggs crowded together in a small dish, a few here and there are surrounded by several layers of spermatozoids, while the great majority of the eggs have none attached to them. Nor can it be said that in the former case the crowd of antherozoids are entangled in a mucilaginous envelope, for in the first place their movements are absolutely unrestrained, and furthermore, staining with gentian violet fails to demonstrate the presence of any such substance.

During the process of fertilization the eggs of *Dictyota* do not revolve as do those of *Fucus*, but the antherozoids, unless too crowded, slowly gyrate with the end of the cilium resting upon the surface of the egg. At the same time the body of the antherozoid displays a rapid vibratory motion. Frequently the cilium, instead of being straight, presents a wavy appearance (figured in the '97 paper). When the sperm is becoming less active waves can be seen travelling along the length of the cilium.

It has been far more difficult to trace by means of sections the path of the spermatozoid through the cytoplasm to the nucleus than it was in *Fucus*, and the reasons for this are obvious from the description given above. The examples observed are too few in number to enable one to base any conclusions upon them. The case shown in Fig. 20 is interesting for various reasons. The dark object seen to the right

of the egg-nucleus is almost certainly an antherozoid. When compared with those that are lying outside the membrane it is seen to have greatly increased in size and to be somewhat fibrillar in texture. The egg-nucleus is in a prophase stage, but its appearance is more like that of parthenogenesis than of normal segmentation (see Figs. 22, 23). For some reason or other this particular spermatozoid, although it has entered the egg, has failed to reach the nucleus. Fig. 29 is still more curious. Here I regard the egg-nucleus, the one to the right, as having divided parthenogenetically, while the left-hand nucleus is probably an antherozoid nucleus which has failed to fuse with the former, but, at the expense of the egg cytoplasm, has greatly increased in size. On this view this is a stage further than the preceding one.

IV. THE SEGMENTATION OF THE FERTILIZED EGG.

The first reliable evidence of fertilization having been accomplished is the presence in the egg-nucleus of a second nucleolus, which is nearly always smaller than the original one (Figs. 21, 22, 23); this undoubtedly represents the chromatin brought in by the spermatozoid. Very soon the nucleoli present an appearance suggestive of their containing a number of deeply stained granules or chromosome-like masses. At the same time the chromatin thread is very fine and distributed through the whole of the nuclear space. After this the chromosomes are seen as thick curved rods, coarsely and irregularly beaded (Fig. 22). These after a slight increase in size are longitudinally split, and the chromatin disks become more distinct. The crowded appearance of the chromosomes at once suggests that they are more numerous than in the preceding stages: when they are counted the number in each case is found to be thirty-two. The nucleoli at this period are very irregular in form, but though they seem as if going to fragment they keep their coherence till the spindle stage.

Returning now to a consideration of the extra-nuclear structures we find that in the unfertilized newly liberated egg, there is a zone of 'kino-plasm' surrounding the egg, and that outside this the chloroplasts tend to place their axes radially. At this period it is most difficult to find any recognizable centrosomes. In the stage represented by Fig. 21 there is a centrosome and radiations on one side of the nucleus; everywhere else the chloroplasts seem to be disposed without definite order, and in many instances they abut directly on the nuclear membrane. It is difficult to be quite certain about the number of centrospheres, but from all I have hitherto seen I am strongly inclined to think that there is only one.

Fig. 24 shows an appearance which is so common as to lead me to think that it represents the normal mode of development of the seg-

mentation spindle, as it undoubtedly does also in the second division of the tetraspore mother-cell. Two sheaves of fibres make their appearance at points in the periphery not far from each other; the whole figure forming a kind of angular spindle with the fibres not continuous. At the ends of the fibres are the chromosomes and a nucleolar mass. The spindle gradually straightens out till it assumes the form of a normal spindle (Fig. 25). If this view is correct then it furnishes a corroboration of the theory that the spermatozoid brings into the egg something (the centrosome, or the influence which produces the centrosome) that determines the polarity of the spindle.

First we have the newly liberated egg without centrosphere or any evidence of polarity. From the description given below we find that in parthenogenetic figures there is a total absence of directive influence, the figures are always multipolar, and very irregular. In the prophase stage of the fertilized egg there is a single centrosphere which divides into two; as the two separate the angular spindle straightens out until the two poles are exactly opposite each other.

Of the mature spindle it is unnecessary to speak at any length. It presents the usual features, as may be seen from Fig. 25, excepting that there is always less nucleolar matter. An interesting question, which I have not yet been able to solve, is the fate of the male nucleolus; is it all used up in chromosome-formation or does it ultimately fuse with the other nucleolar mass? I have up to the present been unable to identify the two masses during the spindle stage. The next figure shows a good polar view of the equatorial plate, which conclusively shows the chromosomes to be thirty-two in number.

The telophase stage (Fig. 27) is much like that of tetraspore-segmentation, but there is more frequently the rudiment of a nucleolus present; sometimes two may be distinguished. Later on the daughter-nuclei seem to contain several nucleolar masses. Very soon, however, the nuclei assume the appearance of the resting stage, in which there are most frequently two nucleoli. These appear very similar to those shown in Fig. 35 of the tetraspore paper; there, however, only one of the two bodies represents the true nucleolus, the other disappears soon after; here the two bodies are almost certainly the male and female nucleoli.

The centrosomes and radiations are very clear at this stage, and they remain distinct during the early stages of the succeeding division. Many of the later mitoses have been studied, but they present no new features, so it is useless to describe them.

The time occupied by the segmentation process in *Dictyota* is much less than it is in the Fucaceae. In the latter it averages sixteen to twenty-four hours: if fertilized eggs of *Dictyota* be left for this length of time it will be found that many of the germlings are already two to

four-celled. In material which was left for nine hours after the addition of antherozoids the following stages were found:

- (1) Nuclei with two nucleoli and spirem.
- (2) Nuclei with two nucleoli and chromatin segments.
- (3) Spindles.
- (4) Diasters.
- (5) A few binucleate stages.

In addition to these, however, there was a large number of eggs which had been liberated from half an hour to an hour before the addition of antherozoids, and so had become 'stale' and incapable of being fertilized; these showed various stages of parthenogenesis.

V. THE PARTHENOGENESIS OF UNFERTILIZED EGGS.

A very large number of germination experiments was carried out upon the oospheres of *Dictyota*. Most frequently antherozoids were added, but control experiments were also performed with the antherozoids omitted. In all the former cases there was a mixture of germlings which had followed the normal course of segmentation as detailed in the preceding section, together with others which showed the characteristic phenomena of parthenogenesis described below. There is no difficulty in distinguishing between the two sets of figures. Those of the fertilized eggs can easily be recognized by their resemblance to ordinary karyokinetic figures; those of the parthenogenesis are quite abnormal in appearance. That the latter really are parthenogenetic figures is confirmed by the fact that they are very numerous in the control cultures, to which no sperms were added, and that these never show any signs of normal mitotic figures.

Similar irregular multipolar figures are obtained in certain animal eggs, chiefly as the result of artificial stimulation or of polyspermy, and also in malignant tumours. The cases described by Loeb, Morgan, the Hertwigs, Wilson, Galeotti, and a great many others are too well known to need recapitulation. It is important, however, to bear in mind that parthenogenesis in *Dictyota* is not the result of any such unusual conditions; the very fact that they occur side by side with perfectly normal karyokinetic figures is sufficient to exclude any such supposition.

As it is very important to ascertain the exact number of chromosomes in the figures, and to know whether two nucleoli are present or only one, it is evidently essential to compare together all the sections of a nucleus before drawing any conclusions. I have throughout adhered rigidly to this practice, but in dealing with cultures of oospores and tetraspores there are certain difficulties which do not confront one when sectioning the reproductive cells on the plant itself. It is very difficult to get cultures of *Dictyota* eggs free from sand, diatoms, &c., as in

order to get a sufficient number of the ova large pieces of the plants have to be placed in the culture dishes. When such siliceous particles are present they often interfere with the microtoming, and cause the tearing of the ribbons and the consequent loss of sections. Even when the cutting has been successful there are so many eggs in each slice that the identification of the successive sections of any particular one is often a matter of some difficulty. To obviate this trouble it is often a convenient practice to include in the material some other easily cut, and sufficiently distinctive, object to serve as 'landmark' so to speak, by reference to which to locate the others.

There are two ways in which an unfertilized egg-nucleus of *Dictyota* may initiate its division. In both cases the single nucleolus increases in size, and shows a tendency to break up into chromosome-like masses. In the mode shown in Fig. 30 the nuclear membrane disappears, though the limits of the nuclear cavity are still recognizable. A globular mass is found in the centre, which consists probably of both nucleoplasm and nucleolar substance. This is the least common method, but a fair number of examples has been met with. Fig. 31 shows a far more frequent case; here the membrane is still intact, but the nucleolus seems to be fragmenting. The considerations that incline me to the conclusion that the nucleolus breaks up directly into chromosomes are the following:

(a) In many hundreds of cases examined I have never yet seen a spirem.

(b) As soon as the chromosomes are formed the nucleolus completely disappears; neither nucleolar globule nor chromatin fibrillae remain.

While the change in the nucleolus is going on the nuclear network preserves its usual appearance. Outside the nucleus there is nothing suggestive of either centrosome or polar radiation, while the fertilized egg-nucleus on the other hand has a distinct centrosphere (Fig. 21). The cytoplasm and chloroplasts frequently retain the faintly radiate structure shown in Fig. 19. The stage where the chromosomes have been differentiated, and the nucleolus has very nearly disappeared, is seen in Figs. 20 and 32. Not only is this stage distinguishable by the absence of nucleoli, but the chromosomes are only sixteen in number, and they are different from those of fertilized eggs in being thinner and less regular in form.

Fig. 32 shows the only case observed where any fibres had appeared before the dissolution of the nuclear membrane. It is not unlike Fig. 24, but the very rudimentary appearance of the figure, and the fact that there are only sixteen chromosomes in the three sections of the figure, prove that it is merely an exceptionally regular case of parthenogenesis.

It is a very striking fact that while in nearly all the normal mitoses described in these two papers the nuclear membrane persists until a very

late period of the anaphase stage, in the division figures of parthenogenetic nuclei the *membrane is invariably absent*. The disappearance must be very rapid, for in all the hundreds of cases examined I have never met with any where the dissolution was partial. Coincidentally with the disappearance of the membrane the nuclear space, now sharply delimited by the crowded chloroplasts and coarser reticulation, is seen to be occupied with very fine meshed or finely fibrillar 'kinoplasm,' in which long fibres can be distinguished. No two figures are alike in the arrangement of the threads. Some of the most regular are shown in Figs. 33-36. In many the longer threads cross each other in inextricable confusion, so that no two can be seen to converge. In such cases it almost seems an abuse of language to call them spindle-fibres.

A point in which these figures present a sharp contrast to the artificially produced multipolar figures of animal eggs is the total absence of both centrosomes and radiations. The figure is entirely confined to the nuclear space, and no cytasters or any other radiate structures are seen outside. This is the more remarkable as centrospheres are such prominent features in normal mitoses.

The subsequent history of the dividing nucleus is rather peculiar. In the dense 'kinoplasmic' mass a number of approximately spherical lighter areas appear, and in each area one or several curved chromosomes. Very soon after differentiation each area is delimited by a membrane, and the interior of the dense felt of kinoplasm is occupied by a cluster of nuclei, some large and some small, the size generally depending upon the number of the contained chromosomes (Figs. 37, 39). I have been unable as yet to decide whether as a preparation for this stage there is a division of chromosomes. I am inclined to think there is, but the supposed instances of it are not clear enough to enable one to come to a final decision. There is undoubtedly a subsequent increase in the number of nuclei, but not by karyokinesis.

The number of eggs in a group varies: comparing the several sections of a germling together, I found it to contain twenty nuclei; in others the number would not be more than five or six.

After a time the several chromosomes in a nucleus become spherical in form, then fuse together to form a nucleolus; at the same time a faint reticulum appears (Fig. 40), which very often contracts away from the membrane.

It was stated above that no cytoplasmic radiations were ever associated with the multipolar spindles described in the preceding section. Fig. 41 represents a later stage in which radiations occurred, and where also one of the nuclei had a well-formed centrosome and the usual distortion of the membrane opposite to it. Whether this is an instance of the formation of these structures *de novo*, or whether we have here one of those not

infrequent cases of polyspermy already referred to, could not be decided, as I have not yet seen the phenomenon in any of the control cultures.

After a time the nuclei separate into two or more groups. As a general rule there is nothing more to be seen than a constriction of the kinoplasmic mass containing the nuclei, followed by the appearance of two groups instead of one; these groups taking up the positions which in a normal segmentation would have been occupied by the two daughter-nuclei (Fig. 42). The plane of division of the cell becomes almost clear of chloroplasts, and after a time the dividing wall makes its appearance; the early stage of this process is shown in Fig. 42.

In a solitary instance nuclei were observed with connecting fibres between them (Fig. 38); these presented the additional peculiarity of being in the dispirem stage. Although it is very evident that the nuclei increase in number, the chromatin does not increase proportionately in amount. There is always a great difficulty in staining the nuclei, and in the later stages many of them are undistinguishable from cytoplasmic structures, excepting by their form. The multiplication of nuclei must be accomplished by direct division as, after the original multipolar figures, neither chromosomes, fibres, nor any other signs of mitosis are ever seen.

Instead of first dividing into two cells each with its group of nuclei, the original cluster in rare cases separates into three or even four groups. The largest number of cells seen in a germling was six; they were very unequal in size, but every cell contained a cluster of nuclei. After a few divisions the germlings invariably died. It may be suggested that some of them may develop into mature plants in their natural habitats; this is very unlikely, for the fertilized eggs in the same culture grow vigorously.

GENERAL CONSIDERATIONS.

A few general questions suggest themselves for consideration. At the present stage I do not intend to discuss them fully; this will be done when the investigation of other Dictyotaceae has been completed.

1. *The Nucleolus.* That this structure in all the cases discussed contains the bulk of the chromatin is fairly obvious. In most cases there is a quantity of substance which is not employed in building up the chromosomes. How is it that in unfertilized eggs the nucleolus breaks up directly into chromosomes, and how is it that no residual nucleolar substance is ever left over after the differentiation of the chromosomes? Can it be that in the mature egg the nucleolus has lost some of its capacity for metabolism, and that this can only be restored by the advent of the antherozoid?

2. *The centrosome and radiations.* It is clear from the foregoing description that, while the centrosome cannot be regarded as a mere condensation point at the focus of the system of radiations—its peculiar

curved rod-like form precludes that possibility—it cannot, on the other hand, be regarded as a permanent cell-organ, for at several stages it disappears completely. At the same time, the comparison of normal with parthenogenetic segmentation strongly supports the idea that the egg after maturation is far less capable of giving rise to a new centrosphere than before. The unfertilized egg shows no indication of its presence, while in the fertilized ovum there is apparently only one, which subsequently divides to form the two centrosomes of the angular spindle. It is difficult to resist the conclusion that the antherozoid introduces into the egg something which enables it to form a centrosphere afresh. If this be correct, the imported substance can hardly be a centrosome, for no such structure can be recognized in the antherozoid before entry.

With regard to the division of centrosomes, Mottier has described and figured the phenomenon in the early prophase of the second division of the tetrasporangium. I have never been able to satisfy myself that I have actually observed the splitting in the way that he figures it. In certain stages it is quite common to see the two chromosomes close to each other, and to find that during the later stages they travel farther from each other until the spindle, if already initiated, from being angular as at first, becomes quite straight or only very slightly curved. This occurs in the prophase of the first mitosis of the fertilized egg, in the newly formed daughter-cells of both tetraspore and oospore segmentation, as well as in the case described by Mottier, but not in the newly formed nucleus of the tetrasporangium or in the first division of the tetraspore.

The peculiarly irregular appearance of the membrane in the neighbourhood of the centrosome in some stages tempts one to subscribe to the idea advanced by Mathews and others, that the centrosome is a liquefying enzyme, and that in virtue of this characteristic it may thus act not only upon the cytoplasm but upon the polar regions of the membrane also. This, however, is exceedingly doubtful, for not only have investigators failed to extract an enzyme from cells containing centrosomes, but in the parthenogenesis of eggs of *Dictyota* it has been shown that where the centrosome is absent the dissolution of the membrane is sudden and complete, and takes place at an early stage.

3. *The nuclear membrane.* Whatever may be the structure and consistency of the membrane the phenomena just described support the hypothesis that its formation is determined by the metabolic processes going on in the chromatic mass. Evidently the reason for the formation of a number of nuclei in the parthenogenesis here described is that the chromosomes are too widely scattered, so that when a chromosome is isolated it directly or indirectly initiates the formation of a membrane round itself. Juel, in his paper on *Hemerocallis*, describes a stray chromo-

some surrounding itself with a membrane. It is a curious fact that in *Dictyota* a stage like Fig. 5 in the Tetraspore paper (Pl. IX) is followed by one similar to Fig. 33 of the same series, or Figs. 6, 27 accompanying the present paper, where the chromosomes are less crowded than before. Whatever be the cause of the former condition, it probably facilitates the formation of a common membrane, and so prevents the separation of the chromatic elements.

It has been shown that the thickness of the membrane varies considerably in different stages: we find it to be thinnest, for instance, during the synapsis stage of the tetrasporangium nucleus. This has probably some significance from the point of view of nutrition. The further fact that at certain periods, in some newly formed nuclei for example, the nuclear network contracts away from the membrane far more easily than at other stages, also suggests that the relation of the network to the membrane, and probably also to the cytoplasm, varies from time to time.

4. *Segmentation in parthenogenetic germings.* Is the separation of the nuclei into two groups due to inherent repulsion between the nuclei of the separating groups, or is it due entirely to the action of the cytoplasm? If the former, then there must have been some sort of division of the chromosomes, or, failing that, subsequent differentiation in the characters of the nuclei must have taken place. At present the matter is obscure, and must be left over for further investigation.

SUMMARY.

1. The sexual cells, unlike the tetraspores, are produced and liberated simultaneously in fortnightly crops. Fertilization is external. Eggs not fertilized within about half or three-quarters of an hour after liberation become invested with walls and germinate parthenogenetically. Freshly liberated oospheres strongly attract the antherozoids, are fertilized and segment in a normal manner.

2. The oogonium and antheridium are produced by the increased growth of surface cells, which, after cutting off a stalk-cell, form respectively a single egg, or over 1,500 antherozoids.

3. There is no division of the nucleus in the oogonium as there is in that of *Fucus*. All the divisions of the antheridium as well as the stalk-cell division of the oogonium are homotype, and are very similar to the stalk-cell division of the tetraspore, except for the fact that there are only sixteen chromosomes.

4. The antherozoid has the cilium lateral. There may be a second very much reduced cilium, but this is difficult to demonstrate. The nucleus is in the thicker end of the pear-shaped antherozoid; the eye-spot is very small, and instead of being at the base of the cilium is generally near the anterior end of the 'beak.' The antherozoids crowd

round the newly liberated eggs in preference to the others, probably in consequence of a chemotactic attraction.

5. When an egg has been fertilized the nucleus generally has within it a second smaller (male) nucleolus. Later on thirty-two chromosomes appear, but the two nucleoli are still present. There is at first a single centrosome which divides into two; as the two separate the two spindle-cones also diverge, till ultimately they form a normal spindle.

6. When an egg has not been fertilized the nucleolus breaks up into chromosomes; leaving no residual nucleolar matter to be extruded into the cytoplasm as occurs in other mitoses. The mitotic figure is very irregular and multipolar; there is no nuclear membrane, and a cluster of nuclei is formed each containing sometimes one, sometimes several chromosomes. These separate into two or more groups, and partition walls are formed between them. The process may go on a little further, but very soon it stops and the germlings die.

7. When normal germination is compared with parthenogenesis we find that the entry of the antherozoid into an egg produces the following results:—

(a) It causes a centrosome and radiations to appear in the cytoplasm.
 (b) Sixteen additional chromosomes are introduced into the nucleus.
 (c) The metabolism of the nucleus is far more active, as is shown by the greater amount of nucleolar matter and the increased size of the chromosomes in the prophase stage.

(d) In the mitosis it introduces a directive influence which is completely absent from the parthenogenetic figure. This prevents the scattering of the chromosomes and the consequent formation of a large number of nuclei.

(e) It prevents the early disappearance of the nuclear membrane.

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EXPLANATION OF FIGURES IN PLATES
 XII, XIII, AND XIV.

Illustrating Mr. Lloyd Williams's paper on the Dictyotaceae.

For convenience of comparison the same scale of magnification has been preserved throughout, all the figures having been drawn with the camera lucida under the Zeiss apochromatic oil-immersion objective 3.0 mm., aperture 1-40, with ocular 8.

The development of the Oogonium.

- Fig. 1. The rudiment of the oogonium before stalk-cell division.
 Fig. 2. Prophase. The spirem has segmented, and the nucleolus is becoming vacuolated.
 Fig. 3. A stage further; the chromosomes are longitudinally split, and the nucleolus is breaking up into fibrillae and the usual globule.
 Fig. 4. Stalk-cell division spindle. The cordate form is due to contraction, the nucleolar globule and fibrillae are present.
 Fig. 5. Polar view of the equatorial plate, showing the curved form of the chromosomes; the number is sixteen.
 Fig. 6. Dispirem of the nucleolus of the oogonium, showing the increase in size of the chromosomes before fusion.
 Fig. 7. Telophase stage of the nucleus of the oogonium, showing a number of deeply stained masses embedded in lighter staining substance.

The development of the Antheridium.

- Fig. 8. Rudiment of the antheridium, the nucleus in the prophase of stalk-cell division.
 Fig. 9. Telophase of the stalk-cell division; the stalk-cell nucleus has descended into the vacuolated basal region.

Fig. 10. Vertical section parallel to the long axis of the thallus. The antheridium has already divided transversely.

Fig. 11. Vertical section transverse to the axis of the thallus. First division of the antheridium, polar view of the nuclear plate, showing the number of chromosomes.

Fig. 12. Vertical section showing border-cells.

Fig. 13. Vertical section transverse to the thallus. Division more advanced. The inner border-cell is seen to the left.

Fig. 14. Vertical section through a mature antheridium; the inner border-cell is still undivided.

Fig. 15. Antherozoids fixed with potassium-iodide iodine in sea-water and stained with methylene blue. Nuclei and physodes shown.

Fig. 16. Antherozoids fixed with dilute Flemming's mixture and stained by the iron-alum-haematoxylin method.

Fig. 17. Antherozoids fixed with dilute Hermann's solution and stained with gentian violet. At the top left-hand corner is an apparently biciliate, and at the right-hand side of the figure a binucleate antherozoid. In Figs. 15-17 the small black dots are physodes.

Fig. 18. Antherozoids drawn while motile. The small black dot is the red eye-spot, the colourless part is the nucleus; some show the presence of physodes.

Fertilization and segmentation of the Egg.

Fig. 19. Section of newly liberated egg fixed with dilute Flemming. There is a zone of 'kinoplasm' round the nucleus, and a radiate arrangement of the chloroplasts. The physodes are at the surface of the egg. Of the numerous antherozoids in the neighbourhood three have been drawn.

Fig. 20. Delayed fertilization. The antherozoid, greatly increased in size, is seen to the right of the nucleus, a few are shown outside the membrane. The egg-nucleus is in the prophase of parthenogenesis.

Fig. 21. Fertilized egg-nucleus with male and female nucleoli and a single centrosphere.

Fig. 22. Prophase of the first segmentation mitosis; the chromosomes are thirty-two in number, the remainder being in two other sections.

Fig. 23. A later stage; the chromosomes are split and the nucleoli very irregular in form.

Fig. 24. Early spindle showing two cones at an angle with each other. The faint radiations are better seen in the succeeding section.

Fig. 25. The completed spindle.

Fig. 26. Polar view of the nuclear plate, showing the thirty-two chromosomes.

Fig. 27. The two daughter-nuclei in the dispirem stage; the nucleoli beginning to differentiate.

Fig. 28. The daughter-nuclei completed; each has two nucleoli as in the prophase of the preceding mitosis.

Fig. 29. Abnormal fertilization; a stage later than Fig. 20.

Parthenogenesis of unfertilized Eggs.

Figs. 30, 31. Two modes of initiating the parthenogenetic figure. In both the nucleolus seems to be directly converted into chromosomes.

Fig. 32. An exceptionally regular early spindle. Here again the chromosomes seem to be directly converted into chromosomes, of which there are eleven in this section and five in the next.

Figs. 33-36. Four examples of the parthenogenetic 'spindle.' It is very multipolar and exceedingly irregular, there is no trace of nuclear membrane or of nucleolar substance, and the chromosomes are invariably sixteen in number.

Fig. 37. Vesicles (nuclei) containing one or several chromosomes beginning to differentiate in the kinoplasmic mass.

Fig. 38. An exceptional figure showing the separation of two groups of nuclei with a few connecting fibres.

Fig. 39. Newly formed nuclei with chromosomes.

Fig. 40. A few nuclei out of a group of twenty; nucleoli beginning to appear.

Fig. 41. The only instance observed of a group of nuclei with centrosome and radiations.

Fig. 42. Parthenogenetic germling in which the nuclei have separated into two groups; a septum is beginning to form between the two halves of the cell.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 8.

Fig. 9.

Fig. 11.

Fig. 12.

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Fig. 4.

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Fig. 18.

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Fig. 34.

Fig. 35.

Fig. 36.

Fig. 37.

Fig. 38.

Fig. 39.

Fig. 40.

Fig. 41.

Fig. 42.

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