On the Life-History of *Rhabdonia tenera*, J. Ag.

BY

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With Plates XX and XXI.
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RHABDONIA TENERA, J. Ag., is a characteristic species of the warmer waters of the eastern coast of the United States, and is found abundantly in sheltered bays and coves. The abundance of the plant in certain localities near Woods Hole, Mass., together with the fact that certain peculiarities in the structure and development of the cystocarp and the proliferation of the tetrasporic plant seemed to be little understood, led the writer, while studying at the Marine Biological Laboratory at Woods Hole, to begin the investigation of the life-history of this plant with the hope of throwing some light upon these and other points.

The species was first described by J. G. Agardh ('41) as *Gigartina tenera*, from specimens from the West Indies. Later ('51) he referred it to Harvey's genus *Rhabdonia*. Harvey ('52) described it anew, and referred it to the European *Solieria chordalis*. J. G. Agardh ('71) subsequently stated that Harvey's plant is his *Rhabdonia tenera*. Farlow ('75 and '81) has given the best account of the cystocarp. Schmitz ('89) separated the species from the genus *Rhabdonia*, and placed it in a new genus *Agardhiella* belonging to the Cystoclonieae. As the description of the new genus has not yet appeared, I have preferred to retain the older name.

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METHODS.

The best results were obtained from material which was fixed in a saturated solution of picric acid in sea-water, washed fifteen to twenty hours in running sea-water and preserved in sea-water, to which was added plenty of camphor broken up into small pieces. Material so treated is still in excellent condition after standing for over a year. Material killed in chromic acid (1 % in sea-water), or picric acid and preserved in formalin (2 % in sea-water), is also very satisfactory, while material placed directly in 2 % formalin without previous treatment retains its colour for some time and is kept in excellent condition. The addition of 1 % chrome-alum to the formalin seems to be an advantage.

At the commencement of the work hand-sections were made with a razor; but as it soon became evident that some more efficient method of sectioning was necessary, the material was embedded in paraffin and serial sections were cut. The shrivelling of the tissue in all except very young parts of the frond was so great that this method was abandoned. Freezing by means of ether was then tried, and excellent results were obtained; but this method of freezing proved so troublesome, that in looking about for a better one, the writer began experimenting with ice and salt, with the result that an apparatus was finally devised which could be operated with very little trouble and which proved very efficient, a description of which appeared in the *Botanical Gazette* for May, 1896 (vol. xxi. p. 195). It was found that large frozen sections when cut very thin, easily broke in pieces in the region of the medulla. To avoid this the material was placed before sectioning in 50 % gelatine¹ for a short time;

¹ This is prepared as follows: The best gold-label gelatine is carefully brushed clean and used according to the following formula:—

Gelatine	100 gm.
Distilled water	100 c.c.
Thymol	Trace.

Mix over a water-bath, and add the white of one egg to clarify.

the gelatine was then removed from the exterior by washing in warm water, and the object was placed on the freezing chamber in a gum-arabic solution and cut. Most excellent results were obtained by this method, and all delicate parts were held in place. The sections were removed from the knife, placed in a watch-glass in 20 % glycerine, which was allowed to stand until it became considerably more concentrated; enough 50 % gelatine was then added over a water-bath to make a good glycerine-jelly, and the sections were placed on a slide in a drop of this and mounted. The whole process can be carried out on the slide, but it is much easier to treat a great many sections at once in a watch-glass. If it is not desirable to mount them at once, it is only necessary to protect the glycerine-jelly from evaporation, and it can be kept indefinitely. In many cases it was found convenient to prepare thick sections in this way, and mount them between two covers so that they could be studied from both sides. If it is desirable to fix such a preparation permanently to the slide, Canada-balsam makes a sufficiently good cement.

Sections made from material not previously infiltrated with gelatine were mounted in glycerine-jelly or glycerine. In the latter case it was found advantageous to mount in 20 % glycerine, and after the latter had become more concentrated by standing, to ring the preparation with a syrupy solution of gum-arabic. This forms around the edge of the cover a glycerine-gum which becomes hard in a short time, and the preparation may then be ringed with Canada-balsam.

Numerous stains were tried, of which Heidenhain's iron-alum haematoxylin proved to be by far the best. Anilin stains and the ordinary haematoxylin mixtures have the disadvantage that they stain the gelatinous wall and intercellular substance deeply. On the other hand a purely aqueous haematoxylin and Schneider's aceto-carmin are free from this objection, but they stain all protoplasmic structures rather diffusely. With Heidenhain's method the gelatinous

wall and intercellular substance, as well as the cytoplasm, can be washed out so as to be entirely unstained, while the nucleolus, chromatin, linin, chromatophore, and the refractive plates accompanying the pits stain dark blue or black. Material stained *in toto* may be subsequently boiled, and employed in making crushed preparations without impairing the stain. Such material should remain from one to several hours in the haematoxylin.

EXTERNAL ANATOMY.

The plants are often two to three feet long, varying from a light yellowish red to deep red, cylindrical, tapering at the base, and attached by a discoid holdfast. The branches are numerous, alternately decompound, long and straight, and tapering at the base and apex. The antheridia, cystocarps, and tetraspores are borne on distinct plants. The three kinds of plants resemble each other closely, but the cystocarpic plants are as a rule more robust, and are easily distinguished by the prominent swellings on the branches, which are produced by the immersed cystocarps projecting on one side of the branch. The older tetrasporic plants bear on their older portions numerous short, simple proliferations, which bristle out from the frond in all directions. The antheridial plants resemble the tetrasporic, but lack the proliferations.

HISTOLOGY.

The cells at the growing-point are small and closely crowded together, which makes a study of their arrangement rather difficult. By clearing slightly with potash, and by contracting the cell-contents with glycerine, one can make out in most cases a central filament of cells, which gives off on all sides lateral branches which grow obliquely upwards, making an acute angle with the central filament. Fig. 9 shows the

central filament *c*, *c*, *c*, &c., and the lateral branches. It is not always possible to decide whether there is one or several central filaments. A careful study, however, of the tips of ordinary branches, and especially of the proliferations on the tetrasporic plants, strongly favours the conclusion that only one such filament is present. Each lateral branch ramifies so as to produce a corymbose cluster of branchlets; these clusters are firmly held together by a common gelatinous intercellular substance, so as to constitute a firm parenchyma-like tissue. Fig. 10 shows a section passing through two such clusters.

Cell-division is almost exclusively confined to the terminal cells of the branches and their branchlets; the frond therefore grows only at the surface. The terminal cells divide longitudinally, transversely, or obliquely, or they cut off from the distal end segments on all sides by means of oblique walls. The method of branching is in consequence very irregular. The vegetative growth of the thallus conforms to the rule laid down by Schmitz ('83 and '92), according to which the Florideae possess thalli which are composed of branching filaments, and only the terminal cells of these filaments ever divide transversely or in such a way that the dividing-wall lies in the organic long axis of the cell. Exceptions to this rule are found in the Corallinaceae (Schmitz, '92), in the Delesseriaceae (Schmitz, '92; Naegeli und Schwendener, '77; Kny, '79; and Reinke, '80), and in the cells which form the pericarp in *Rhabdonia*. In all these cases the same individual shows a transition, in some or all of its parts, from a filamentous to a non-filamentous mode of growth. This transition forms an interesting parallel to the similar development which must have taken place in the Chlorophyceae, if they represent the ancestral stock from which the Liverworts are descended.

A median longitudinal section of the frond passing through the apex, shows that the cells of the central filament, as well as those which surround it, become more and more elongated the further they are removed from the apex. At the same time they tend to assume a direction parallel to the long

axis of the frond. At a short distance from the growing-point it is impossible to distinguish the central filament from the cells which surround it. The cells occupying the interior of the frond finally become long, narrow tubes, much curved and bent, resembling those of the medulla of *Fucus*. The highly refractive plates between the cells become developed to an unusual degree (Fig. 10 at the left). In this manner a medulla arises composed of a varying number of tubular longitudinal filaments, which anastomose laterally in an irregular manner, and lie imbedded in a gelatinous substance which fills the central portion of the frond. The medulla traverses the frond throughout its entire length.

The cells lying externally to the medulla constitute the cortex. Those forming the exterior of the frond are relatively small and elongated in the radial direction (Fig. 10). They are densely filled with protoplasm, and have large, deeply-coloured chromatophores. They form the most actively assimilating layer. Passing inward towards the medulla, the cells of the cortex grow rapidly larger, becoming rounded or ellipsoid. At the same time they grow poorer in protoplasmic contents, and the chromatophores become paler and more reduced the nearer the cells lie to the medulla.

Physiologically considered, the relations between the cells are not unlike those of a radial leaf, especially one with a poorly-developed central-bundle system. The actively assimilating outermost layer, composed of cells directed perpendicularly to the surface, corresponds to the palisade-layer. The rounded cortical cells have large intercellular spaces filled with water containing the necessary gases for the metabolism of the innermost cells of the interior, much as the spongy parenchyma provides for the circulation of gases in the leaf. The inner cortical cells also serve to convey elaborated products to the elongated cells of the medulla, which probably convey them to the growing-point and also to the holdfast, where they are stored up as Floridean starch. Wille ('85) mentions *Rhabdonia* as having

a mechanical system with the mechanical elements arranged in a ring around the conducting system. The numerous types into which he has sought to separate the Florideae on anatomical-physiological grounds have so little value, that I have not considered it necessary to refer *Rhabdonia* to any one of them. In his work on the storage-system of Florideae (Wille, '87), *Rhabdonia* is not mentioned.

The structure in the region of the holdfast corresponds in general to that described above, but the intercellular spaces are almost entirely lacking, and the closely packed cells, which are arranged so as to give the greatest mechanical strength, are square or angular in section. Just above the holdfast the distinction between medulla and cortex entirely disappears. All the cells of the holdfast and the region directly above it are densely filled with Floridean starch. It is not impossible that in winter the plant may die down to the holdfast, which may in the spring regenerate the lost portions.

The cortical cells near the tip of the frond often bear long unicellular hairs, which soon drop off and leave the surface of the frond perfectly smooth.

The cells are connected by primary pits which arise at division, and by secondary ones which are formed subsequently. Shortly after the formation of a pit, the cell-wall immediately around it becomes swollen and highly refractive; and, as the cell grows older, forms a fairly thick, highly refractive circular plate (Fig. 10 at the left). In the oldest cells it often happens that such plates become separated into two, which are connected by a delicate strand of apparently protoplasmic substance (Fig. 1, between *st* and *cpg*).

If the connecting substance is really protoplasm, it is without doubt continuous through the plates with the protoplasm of the cells lying on either side. According to Wille ('85) such plates in *Cystoclonium purpurascens* and some other Florideae are pierced by numerous pores like the callus-plates of Laminariaceae.

Both kinds of spores are uninucleate. The younger

vegetative cells are as a rule uninucleate, while the older ones are multinucleate. The nuclei contain fairly large nucleoli with a coarse linin-network, on which the chromatin is arranged in coarse, scattered granules.

The chromatophore in the youngest cells is a plate which almost entirely covers the wall on the inside. As the cells increase in size, the chromatophore becomes band-shaped, with long, irregular serpentine branches, which wander about over the inner surface of the cell-wall, and occasionally anastomose (Fig. 16). In both kinds of spores the chromatophores are of this character. The cells in the interior of the oldest portion of the frond gradually lose all trace of chromatophore and become colourless. Starch-grains, which turn reddish-brown and finally violet with iodine, are abundant in the cells in the region of the holdfast, but in other parts of the frond they seem to be entirely lacking.

ANTHERIDIA.

The antheridia, which have not been hitherto described, occur on all parts of the male plants, forming larger or smaller patches or even a continuous covering extending over considerable portions of the plant. They originate from the outermost cells of the cortex, which by oblique divisions cut off at the distal end small cells often to the number of four or five (Fig. 16). Each of these cells then cuts off a single small cell by a transverse wall, or two small cells by oblique walls. These either become antheridia, or divide in the same way, and bear the antheridia in their turn. The antheridia are very small elongated cells filled with clear, colourless protoplasm, which is sometimes more or less granular and vacuolate. They are borne either singly or in twos; in the latter case they stand side by side. The escaped antherozoid is a small, slightly elongated, naked mass of clear protoplasm.

STRUCTURE OF MATURE CYSTOCARP.

The cystocarps are immersed in the frond, which they cause to swell out on one side in a striking manner. A cross-section of the frond passing through the centre of the cystocarp shows that just outside the latter the cortex is thicker, and composed of smaller cells than it is elsewhere, and that an opening, the carpostome, leads from the outside to the cystocarp. The central part of the latter, commonly called the 'placenta,' is spherical in shape, and is composed of sterile cells which have no chromatophores. They are very irregular in size and shape. Surrounding this sterile mass is a sporiferous layer composed of radiating filaments of spores from two to five cells in length, and often bearing two to four-celled branches. The sporiferous tissue is interrupted here and there by strands of elongated sterile cells proceeding from the 'placenta' to a fairly thick pericarp, which surrounds the spores, and is composed of radiating filaments of cells which are smaller than those of the 'placenta' and elongated in the tangential direction. This elongation is especially marked in the outermost cells, which come to resemble the cells of the medulla with which they form secondary connexions by means of pits.

The pericarp forms a complete investment to the cystocarp, except at the carpostome, and the application of reagents brings out the fact that it is enclosed in a special gelatinous envelope, which separates it from the medulla on which it abuts.

In the older cystocarps spores are often found which divide irregularly and produce rhizoids, but their development is apparently soon arrested.

DEVELOPMENT OF THE PROCARP.

Procargs originate as a rule very close to the growing tips of the frond, but sometimes a few scattered procargs are formed in somewhat older portions of the plant, where the majority of the procargs are ready for fertilization. They

arise as follows: one of the lateral branches composing the cortex cuts off, usually midway between the medulla and the surface, a single cell which is situated on the upper side of the branch. This cell divides in a plane parallel to the long axis of the branch which bears it, and the uppermost cell divides in its turn, so that a three-celled branch is formed, which is directed obliquely upward and inward (Fig. 10). Occasionally four- and five-celled procarpic branches are found. The development of the trichogyne begins with the formation of a small papilla filled with protoplasm, which is clear and free from granules (Fig. 15). It elongates slightly, and then recurves abruptly on the cell which bears it, the trichophore, so that the tip is directed towards the surface of the frond (Fig. 10). It grows directly outward towards this, occasionally making a more or less complete spiral turn, and finally reaches the surface and protrudes through the gelatinous covering of the frond (Fig. 11). In the meantime it has elongated considerably at the base, so that the point at which it recurves is further from the trichophore than it was originally (Figs. 10 and 11). The whole trichogyne is at this time filled with clear protoplasm nearly free from granules, and with few, if any, vacuoles. The wall is very delicate, and is surrounded by a gelatinous layer, often as thick as the trichogyne (Fig. 11). During its growth the trichogyne has a more or less swollen tip, but when it reaches the surface the tip appears no longer swollen but of about the same diameter as the rest of the trichogyne and slightly tapering.

The protoplasm of the trichophore, which is at first free from granules and vacuoles, acquires vacuoles as the trichogyne develops, and becomes more or less granular. The cells of the procarpic branch below the trichophore contain reduced chromatophores with sometimes a mere trace of colour, but are usually without any colour whatever. Their protoplasm very early becomes vacuolate and granular. Occasionally one of them (in the majority of cases the one next the trichophore) bears a lateral cell.

FUSION OF THE ANTHEROZOID.

Only a few cases of the fusion of the antherozoid were found, but in all of these the fusion occurred very near the tip of the trichogyne. Wherever trichogynes could be traced with certainty to the surface, they did not project much beyond the gelatinous coating of the frond (Fig. 11). Trichogynes with attached antherozoids were found only in crushed preparations in which it was not possible to determine how far the trichogyne projected beyond the jelly. From the facts observed, it seems probable that the trichogyne does not project very much beyond the jelly, though this point is one of some uncertainty.

CONJUGATING-TUBE.

After fertilization, the contents of the trichophore lose all connexion with the contents of the trichogyne, being separated from them (Fig. 13) by a considerable interval filled by the gelatinous wall which encroaches on the lumen of the trichogyne, and finally completely fills it. The trichophore then puts out on one side a short projection filled with clear protoplasm, which grows into a long, delicate tube, tapering gradually towards the end (Fig. 13, *ct*). The contents of the tube are slightly granular and at first free from vacuoles, but these gradually make their appearance, being as a rule small and scattered. The tube grows towards the tip of the frond, keeping in the medulla and avoiding the cortex. Shortly after the tube begins to grow out from the trichophore, a second (Fig. 13), and often a third, makes its appearance, and grows in the same general direction as the first. It is less common to find only a single tube proceeding from the trichophore, but such cases are not difficult to find. These tubes, to which the name 'conjugating-tubes' will be applied, often reach a surprising length, and become very much attenuated towards the end. Occasionally a tube is found with one or more globose or ovoid swellings somewhere in its continuity. At a point opposite to an auxiliary cell, the

tube bends outwards and makes its way through the cortex to the auxiliary cell. As the conjugating-tube develops, the trichogyne is displaced to one side and gradually becomes disorganized, the disorganization proceeding from the tip towards the point of attachment (Fig. 13 *tr*).

ORIGIN OF THE AUXILIARY CELLS.

Certain lateral branches of the cortex begin very early to have a different appearance from the ordinary branches which surround them on all sides. Their cells become more densely filled with granular protoplasm, and in many of them the chromatophore becomes paler. The basal cell of every such branch rests on one of the elongated cells of the medulla, is usually much elongated and has less protoplasmic contents than the other cells of the branch (Fig. 2, *a*). The next cell is larger (Fig. 2, *st*) and has fairly dense contents, and frequently bears one or more lateral branches. The next cell of the branch is commonly somewhat constricted in the middle; this third cell in the branch is the auxiliary cell (Fig. 2, *cpg*) with which the conjugating-tube later unites. The auxiliary cell always bears three large cells, one at the top (Fig. 2, *b*), and two just below it on the outer side (Fig. 2, *c* and *d*). The auxiliary cell, together with the three cells which are borne on it, and the cell upon which it rests, form a group of five large cells, which possess colourless chromatophores, and are densely filled with granular protoplasm. Each of the three cells borne upon the auxiliary cell gives rise to several branches which branch more or less freely, and form a mass of small cells lying externally to and around the auxiliary cell (Figs. 3, 4, 5 and 1).

These possess for the most part well-developed chromatophores, which are deeply coloured as those of other cortical cells of the same size which are borne on purely vegetative branches, but the first-mentioned cells are distinguished from the last-mentioned by their position and by their richer protoplasmic contents.

FUSION WITH THE AUXILIARY CELL.

The conjugating-tube, which proceeds from the trichophore, appears in all cases to unite with the auxiliary cell near its base (Figs. 12 and 14); at the point of union the conjugating-tube is more or less swollen and closely pressed against the auxiliary cell. The wall between them is absorbed, and the contents of the conjugating-tube unite with those of the auxiliary cell (Fig. 14).

The number of auxiliary cells is very small compared with the number of procarps, not over two per cent.; consequently they are quite certain to be found by some of the numerous conjugating-tubes.

SEGMENTATION OF THE CARPOGENIC CELL.

After conjugation the auxiliary cell cuts off a segment by an oblique wall near the top and on its inner side (Fig. 3). The cell thus cut off divides and gives rise to a mass of comparatively small cells densely filled with protoplasm (Figs. 4 and 5). By growth and repeated division the mass enlarges and finally completely surrounds the auxiliary cell, and also the three large cells which are borne upon it (Fig. 1). It is then possible to see that the whole mass is made up of radiating filaments of cells, the gonimoblastic filaments, which branch in a somewhat irregular manner, and at the ends bear short branched filaments of small cells, densely filled with protoplasm, which become spores (Fig. 1). Thus there arises an irregularly spherical mass of cells composed of gonimoblastic filaments radiating in all directions from the carpogenic cell (Fig. 1. *cpg*) and bearing spores at the surface of the mass, the interior of which (the 'placenta') is composed of sterile cells which have enlarged considerably and lost most of their contents, with the exception of a delicate film of protoplasm lining the cell-wall. Imbedded in this mass are the three cells which were mentioned before as being borne on the auxiliary cell, and which are now very large and almost entirely destitute of protoplasm.

Many of the terminal cells of the gonimoblastic filaments which radiate from the carpogenic cell, instead of changing into spores, grow out into sterile filaments, often branching dichotomously, and composed of a few elongated cells (Fig. 1, *x, x, x*, &c.) These reach the pericarp and attach themselves to it, forming connexions by means of secondary pits with the cells of its innermost layer.

DEVELOPMENT OF THE PERICARP.

The sterile cells of the branch containing the auxiliary cell give off branching filaments of cells, and perhaps a few such filaments occasionally proceed from the auxiliary cell; these divide very irregularly, not following the rule for the division of ordinary vegetative cells, and by the time that conjugation with the auxiliary cell takes place, they form a pericarp investing on all sides the auxiliary cell and its four large accompanying cells, already mentioned (Figs. 2, 3, 4, and 5). In that portion of the pericarp lying towards the interior of the frond the divisions take place in such a way that numerous rows of cells are formed, radiating from the centre of the mass, which is occupied by the auxiliary cell and its accompanying cells (Fig. 4). Nearest the centre of the mass the cells of each filament are small, and there is a regular increase in the size of the cells going towards the periphery; this is accompanied by an elongation of the cells in the tangential direction. In the case of the outermost ones this elongation is so great that they come to resemble the elongated cells of the medulla of the frond (Figs. 4 and 1). The portion of the pericarp lying outside the auxiliary cell forms the cortex of the frond at that point (Figs. 2-5). It is composed of branching filaments like the ordinary cortex, but the cells are from the first more densely filled with granular protoplasm. These cells begin, shortly after conjugation with the auxiliary cell takes place, to grow and divide rapidly, forming a mass which, as compared with the ordinary cortex, is much thicker and composed of smaller cells. The entire pericarp thus develops almost

exclusively from the sterile cells of the branch containing the auxiliary cell.

DEVELOPMENT OF THE CARPOSTOME.

The development of the carpostome begins quite early, and by the time conjugation with the auxiliary cell has taken place a considerable depression has been formed in the exterior of the frond just over the auxiliary cell (Fig. 3). It arises as follows: some of the filaments proceeding from one of the two lateral cells borne by the auxiliary cell cease to grow, and finally the terminal cells (i. e. the outermost) perish (Figs. 2, 3, 4, 5 and 1). These cells are easily recognized in preparations of living material by their greenish colour. The cells of the filament then die as far back as the large cell which is borne on the auxiliary cell, and an opening is thus made to the exterior. In the meantime the filaments in the immediate neighbourhood have commenced the rapid growth already mentioned, and those nearest the carpostome produce around it a lining of very small cells (Fig. 1). A surface view of the carpostome shows the cortical cells of the pericarp arranged in rows, which radiate from the carpostome in all directions.

THEORETICAL CONSIDERATIONS.

Whether the cystocarp of the Florideae represents a sporophyte or not, must remain for the present an open question. The fact that we are not able to say whether the trichophore or the auxiliary cell, or both, are fertilized does not affect the question, so long as the sexuality itself is not called in question. The grounds on which the sexuality of the Florideae has been questioned, are the result of erroneous or incomplete observation. The statements of Bornet and Thuret to the effect that the cystocarp often originates from cells which are separated from the trichophore and which do not fuse with it, were made under the impression that fusion was not necessary to ensure fertilization. They consequently

gave the matter of fusion no special attention. These statements have been corrected by later investigation. Davis ('96) has recently questioned the sexuality of *Batrachospermum*, but my own observations, not yet published, so far from confirming his results, show conclusively that a true fertilization takes place. Wille ('94) has also been able to demonstrate fertilization in *Nemalion*. Until further and conclusive evidence to the contrary has been brought forward, we must assume that the cystocarp is the result of a sexual act. But, as De Bary ('70) long ago pointed out, the cell from which it originates does not become morphologically separate from the mother-plant. In Mosses, Ferns and Flowering-plants, the sporophyte-generation begins with a cell which is morphologically separate from the gametophyte on which it is borne. Too much stress need not be laid on morphological separation, since in the Phanerogams the mother-cell of the embryo-sac, which is generally assumed to be the starting-point of the female gametophyte-generation, shows no more morphological independence than the trichophore of the Florideae. The question cannot be decided until it has been determined at what point in the life-cycle the reduction to one half the number of chromosomes takes place. The general behaviour of the cystocarp points to the conclusion that an alternation of generations is really present.

Whether this be really the case or not, it is interesting to notice how the development of the cystocarp finds a striking parallel in that of the sporophyte of Liverworts. In its most elementary form it consists almost entirely of sporogenous tissue (*Callithamnion*) like the sporocarp of *Riccia*. In its most highly developed form it consists of more or less sporogenous tissue borne on and nourished by a mass of sterile tissue (the 'placenta' of *Rhabdonia*), corresponding to the foot and columella of *Anthoceros*. Between these extremes numerous intermediate forms are present in both groups. Positive proof of the homology of the two structures is at present lacking, but the analogy, if it be nothing more, is most striking.

It is interesting to note how the Liverworts, responding to terrestrial conditions, have in the higher forms raised the sporophyte above the thallus to secure a better dissemination of the spores, while in the Florideae the opposite tendency has been manifested; protection has been the determining factor, and the cystocarp has, in the higher forms, been buried beneath the surface of the frond much as the sporophyte is in the lower Hepaticae. The formation of a protective covering by the sporophyte is peculiar to the Liverworts; and, while not a character of fundamental importance, shows a higher degree of development.

TETRASPORANGIA.

The tetrasporangia develop directly from the outermost cells of the cortex (i.e. from apical cells of the cortical filaments). They become enlarged, and densely filled with protoplasm; the nucleus divides into two and then into four; the protoplasm by 'free cell-formation' separates into four masses somewhat rounded off from each other, but not separated by cell-walls. In this condition they remain until they escape from the tetrasporangium or commence to germinate in position.

The tetrasporangium, during its growth, encroaches on the surrounding cells, and distorts them; when mature it often has a double wall (Fig. 6), the inner layer of which is thinner and somewhat denser than the outer. The tetraspores escape by the rupture of the gelatinous wall of the sporangium; they remain for a short time enclosed in a delicate membrane; this is then dissolved, and the spores separate from each other, become spherical, and finally become attached to the substratum and germinate. The germination proceeds exactly like that of the carpospores, the first walls being laid down without any regular arrangement.

GERMINATION OF TETRASPORES.

The older tetrasporic plants commonly bear on the older portions of the plant numerous short simple proliferations,

which are not found on the male or female plants. They often reach a length of an inch and a half, and bristle out from the frond in all directions. These proliferations are found to originate from tetrasporangia, which germinate inside the frond, the whole contents of a sporangium taking part in the formation of each proliferation. The process begins with the division of all the spores by means of oblique walls which do not occur in regular succession (Fig. 7). For a time it is possible to see distinctly the four separate masses of cells which result from the process, but as the process continues the boundaries between the originally distinct masses become obliterated, and one finds only a mass of cells occupying the place of the sporangium and preserving its general outline (Fig. 8), but already encroaching decidedly on the surrounding cells, and also projecting from the frond. The mass grows most rapidly in that portion lying nearest the outside of the frond, from which it gradually grows out to form the proliferations just described. As it does so the cells of the tip begin to show a definite arrangement into a central filament, which bears numerous short appressed branching filaments (Fig. 9), the terminal cells of which form the exterior of the frond. In the meantime the cells at the base of the proliferation grow out into rhizoidal filaments (Fig. 9), which make their way between the cortical cells of the parent frond, and finally reach the elongated cells of the medulla and form secondary connexions with them.

Some of the proliferations remain sterile, but in the majority of cases they bear reproductive organs often before they are more than a quarter of an inch in length. Antheridial proliferations are very common, while tetrasporic and cystocarpic ones are rather rare; but the three kinds often occur growing side by side.

The union of several spores to form a single individual is by no means an isolated phenomenon, though it cannot be said to be a common one. Proliferations resembling those of *Rhabdonia* occur on the tetrasporic plants of *Cystoclonium*,

Gracilaria, and other higher Florideae, and it is probable that they originate in a similar way.

According to De Bary ('84, p. 2) the germinating spores of *Nectria Solani* unite (in groups of from two to eight) by means of short tubes to form a single individual. Woronin ('95) describes a similar case in connexion with the germinating spores of *Sclerotinia Padi* and *S. Aucupariae*. These cases are homologous with that of *Rhabdonia*, if we consider that the tetraspores which are contained in the same sporangium are morphologically independent. This seems to be the case, since they are capable under favourable circumstances of separating from each other and germinating independently without having in the meantime undergone any modification other than that of escaping from the sporangium. When they remain enclosed in the mother-plant, they undergo a most fundamental modification, in that each spore, instead of developing into a separate individual, produces only a small portion of one, and so perfect is the correlation that the outermost spore produces merely a growing-point, whilst the innermost develops rhizoids. Whether the case in question belongs to the category of strictly epigenetic phenomena or is an inherited character, I do not attempt to decide.

Two views are easily possible as to the physiological conditions obtaining previous to germination. It may be assumed that physiological and morphological independence are acquired at the same time, and that the former is gradually lost as the result of the fact that the spores are unable to escape from the sporangium and separate. In the case of abnormal eggs of *Ascaris*, described by Strassen ('96), two eggs remain in contact by means of a broad protoplasmic connexion, and throw off the first polar body independently; but they unite to make a second polar body common to both eggs. Later they develop into a single embryo of unusual size. The separate individualities are apparently gradually merged into one as the result of continued union; it must, however, be remembered that the

case in question is an abnormality of rare occurrence, which has not been thoroughly investigated, and that it is moreover quite susceptible of a different interpretation. The above-mentioned cases of *Nectria* and *Sclerotinia* show that spores may fuse and give up their individuality completely, but these are exceptional cases. In the majority of cases individuality once established is not subsequently surrendered as the result of fusion (apart from the case of gametes), as is shown by the conidia of Ustilagineae, which after fusing put out separate germ-tubes exactly as though no fusion had occurred. On the whole, therefore, the assumption that physiological independence is first established and subsequently lost, rests on no direct evidence, and finds its chief justification in the general consideration that physiological and morphological independence usually go hand in hand.

On the other hand it is possible to assume that the spores contained in a single sporangium probably form a physiological unit so long as they remain in contact, or at least that they do not acquire complete physiological independence. The whole contents of the sporangium are so polarized that at one end they produce a growing-point, at the other end rhizoids which penetrate to the medulla of the mother-plant. The polarity of the individual spores is not developed, or at any rate does not manifest itself, until they become separated from each other so as to germinate separately, which, as stated above, not unfrequently happens. The separate spores may be compared to a magnet composed of four separate pieces placed end to end. As long as they remain in contact there are but two principal poles for the whole, but as soon as they are separated, each is polarized independently. Or they may be compared physiologically to an animal egg in the four-cell stage, each of whose cells if separated from the others produces an embryo. A still better comparison is furnished by the embryo-like structure in Dicyemidae, which develops from an egg, but at a certain stage of its development falls into separate cells, each of which (with the exception of one)

develops into an embryo (Whitman, '83). On this view, the case of *Rhabdonia*, though homologous with that of *Nectria* and *Sclerotinia*, is not analogous with them.

The purpose of this peculiar method of development is much easier to understand in the case of *Sclerotinia* than in *Rhabdonia*. The spores of the former germinate on the stigma of the host-plant, and, according to Woronin, are unable, when they germinate independently, to produce a germ-tube long enough to traverse the entire length of the style and reach the ovary. By uniting they are able to produce a much more vigorous germ-tube, which reaches the ovary with no difficulty. In the case of *Rhabdonia* the object seems to be entirely different: the plants produced by the union of four spores remain extremely small in comparison with those which in all probability are produced from a single spore. Here the object is perhaps to produce a large number of antheridia (the proliferations most commonly bear antheridia), and so to compensate for the small number of antheridial plants.

In the case of *Nectria* it seems natural to suppose that the union of several spores produces a plant which at the start has an advantage in competition with those plants which are produced from a single spore. This is probably also the case in the Ustilagineae. It is doubtless of advantage in the Florideae, and certainly seems to be so in *Sclerotinia*. Exactly what the nature of this advantage is, is not clear, but it may be that it claims a distant kinship with that which results from the fusion of two gametes, and perhaps affords a hint as to the mode of origin of the sexual act.

RESULTS.

The frond is composed of branching filaments which grow by division of the terminal cells. At the growing-point there is a single central filament which gives off branches on all sides.

The procarp is usually a three-celled branch borne on one

of the lateral branches of the cortex. The terminal cell of the branch produces an abruptly recurved trichogyne, which makes its way to the surface, and the antherozoid unites with it at the tip. The trichogyne then loses its connexion with the trichophore, and the latter puts out one or two conjugating-tubes which make their way through the medulla toward the tip of the frond and unite with the auxiliary cells. The latter are cells in the continuity of certain specialized lateral branches of the cortex; each specialized branch contains a single auxiliary cell; the remaining cells of the branch (with the exception of the basal one) develop sterile filaments which surround the auxiliary cell and form the pericarp. The auxiliary cell, after the conjugating-tube reaches and conjugates with it, gives rise to a mass of radiating filaments which bear at the ends short branching filaments of cells which become the spores. The basal portions of the radiating filaments remain sterile and constitute the placenta. Some of the filaments grow out into chains of sterile cells which reach the pericarp and connect the placenta with it. In the meantime a carpostome has been formed by the disorganization of some of the filaments lying externally to the cystocarp.

The occurrence of numerous short proliferations on the tetrasporic plant is known in several of the larger Florideae, and naturally excites some interest. In the case of *Rhabdonia* these proliferations are independent plants, arising from the germination of tetraspores in position in the parent plant on which they occur. A point of interest is that it is not a single tetraspore, but the whole contents of a tetrasporangium, which is required to produce one of these plants.

The formation at the base of the young plant of rhizoids which make their way to the medulla of the parent plant and form protoplasmic connexions with its cells, suggests that the young plants are probably partly parasitic on the parent.

Another point of interest is the occurrence on these young plants, often before they are more than a quarter of an inch in length, of antheridia (very commonly), and occasionally of

tetraspores and cystocarps. Two kinds of reproductive organs never occur on the same proliferation.

In conclusion, I wish to express my most sincere thanks to Prof. W. A. Setchell, at whose suggestion the work was undertaken, and whose direction and assistance have been of the greatest value throughout its progress.

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EXPLANATION OF FIGURES IN PLATES XX AND XXI.

Illustrating Mr. Osterhout's paper on the Life-History of *Rhabdonia tenera*, J. Ag.

All the drawings were made from nature by the author with the aid of the Abbé camera.

Figs. 1-5 are from material killed in picric acid and preserved in camphor water, subsequently infiltrated with gelatine and cut when frozen.

Fig. 1. Median longitudinal section of a young cystocarp: *cpg.*, carpogenic cell (i.e. auxiliary cell); *cs.*, carpostome; *st.*, stalk-cell; *b* and *c*, accompanying cells; *ee* &c., *ff* &c., *gg* &c., *hh* &c., *ii* &c., gonimoblastic filaments proceeding from the carpogenic cell and bearing spores at the ends. The spores are shaded in the figure: *x, x, x*, &c., sterile filaments connecting the placenta with the pericarp. $\times 196$.

Fig. 2. Median longitudinal section of frond showing auxiliary cell, *cpg*, before the conjugating-tube has united with it. *a*, basal cell of branch; other letters as in preceding figure. The carpostome, *cs*, has just commenced to develop, as is indicated by the external depression in the frond. $\times 274$.

Fig. 3. Median longitudinal section of frond. The auxiliary cell, *cpg*, has cut off a small cell (shaded in the figure) which has divided into four. Another, a sister-cell, which lies above it and which has not yet divided, is not shown in the figure. The carpostome, *cs*, is further developed than in the preceding figure. The cells external to the cell *h* (which lies under the cells immediately surrounding it in the figure) have died away, causing a depression in the surface of the frond. The pericarp consists of a few radiating filaments of cells. Letters as in Fig. 1. $\times 384$.

Fig. 4. Median longitudinal section of frond. The small cells which are shaded in Fig. 3 have begun to grow out into gonimoblastic filaments which here consist of a mass of small crowded cells (shaded in the figure). The carpostome, *cs*, and pericarp are further developed than in the preceding figure. Letters as in Fig. 1. $\times 384$.

Fig. 5. Median longitudinal section of frond. The gonimoblastic filaments (shaded in the figure) begin to show their filamentous character. The carpostome, *cs*, has the position and shape which it possesses in the mature cystocarp. Letters as in Fig. 1. $\times 384$.

Figures 6-9 are drawn from living material.

Fig. 6. Transverse section of frond, showing mature tetrasporangium with a thinner inner wall and a thicker outer one. The transverse lines in the tetrasporangium indicate the surfaces of contact of the spores, which are masses of protoplasm not separated by cell-walls. $\times 280$.

Fig. 7. Transverse section of frond, showing the spores dividing inside the tetrasporangium. $\times 280$.

Fig. 8. More advanced stage of same; the lines of separation between the four masses of cells resulting from the division of the tetraspores are no longer visible. $\times 280$.

Fig. 9. More advanced stage than Fig. 8. The tip of the young plant is seen in median optical section, showing a central filament (*c*, *c*, *c* &c.) and the branches which proceed from it. At the base of the plant rhizoidal filaments, *rh*, are beginning to grow out. $\times 280$.

Fig. 10. Median longitudinal section of frond, showing the elongated cells of the medulla with highly refractive plates between the cells. At the right, two lateral branches of the cortex; from the lower arises a young procarp. $\times 420$.

Fig. 11. Median longitudinal section of frond, showing trichogyne reaching surface. $\times 474$.

Fig. 12. Trichophore, *tr*, putting out a conjugating-tube which has reached an auxiliary cell, *cpg*, and conjugated with it. *o*, a second (abortive?) conjugating-tube. Other letters as in Fig. 2. From a crushed preparation. $\times 128$.

Fig. 13. Trichophore sending out two conjugating-tubes *ct*; the trichogyne, *tr*, is pushed to one side and is disintegrating at the end. From crushed preparation. $\times 535$.

Fig. 14. Part of Fig. 12 enlarged; letters as in Fig. 12. The conjugating-tube, *ct*, proceeding from the trichophore shown in Fig. 12, *tr*, is seen conjugating with the auxiliary cell. From crushed preparation. $\times 550$.

Fig. 15. Procarp showing the beginning of a trichogyne. From crushed preparation. $\times 770$.

Fig. 16. Transverse section of male plant, showing antheridia. From living material. $\times 1160$.

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