A few Observations on the Encystation of Actinosphærium eichhorni under different conditions of Temperature.

By
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With Plate 24 and 1 Text-figure.

Previous to Richard Hertwig's exact study "Über Kernteilung, Richtungs-Körperbildung und Befruchtung von Actinosphaerium eichhorni" (1898), our knowledge of the life-cycle in this heliozoon was still very incomplete. Hertwig's work laid clear for the first time the complicated series of changes that take place within the Actinosphærium cyst, and his subsequent work, Über Physiologiche Degeneration bei Actinosphaerium eichhorni" (1904), threw further light on the conditions affecting the life-processes in this organism, both free-swimming and encysted.

In the last few years cytologists have found in Actinosphærium a highly favourable object for experimental cell-study. Mr. Geoffrey Smith, of New College, Oxford, working in Munich in 1902, made interesting comparisons between cultures brought to encyst at different temperatures. While in Professor Hertwig's laboratory in Munich, early in 1907, I undertook, at his suggestion, a series of similar experiments, to determine, if possible more exactly than previous results

suggested, the effects of high and low temperature on the nucleo-cytoplasmic proportions in the Actinosphærium cyst.

Briefly stated, the normal course of encystation, as observed by Hertwig, is as follows:—Encysting Actinosphæria withdraw their pseudopodia, adhere to the substratum, and take on an opaque, greyish appearance. This is the stage of the mother-cyst; during it a silica coat begins to form, and about 95 per cent. of the nuclei present in the unencysted state disappear. The mother-cyst divides up into a varying number of primary cysts, each containing one nucleus: that is to say, the number of the primary cysts formed by a given Actinosphærium depends on the number of nuclei remaining over from the mother-cyst reduction. Each primary cyst gives rise by division to two secondary cysts. From the nucleus of each of these, two polar bodies are given off. The secondary cysts then fuse, two and two, each pair of nuclei forming together the single nucleus of a conjugation-cyst. From this conjugation-cyst the free-swimming Actino-sphærium is finally liberated. Encystation can be artificially induced by starving Actinosphæria that have previously been feeding well.

In April I prepared starvation-cultures from fresh Actinosphærium material fetched from a pond at Possenhofen, near Munich. I chose for my purpose 90 or 100 individuals of approximately similar size, and kept half of them in closely covered watch-glasses of clean culture-water at a temperature of 10° C., the other half at 25° C. I allowed them no food, and merely from day to day removed impurities, and put in fresh water. When encystation had occurred, as happened usually within three or four days, I killed them off at the primary-cyst stage, and substituted fresh cultures. (In the beginning I kept a third line of cultures going at an intermediate temperature of 17° C. Soon, however, as the weather grew warmer, it was impossible to keep the room where the cultures stood at this low temperature, and I gave up the attempt.)

At first, though encystation took place rapidly and nor-

mally, I did not obtain satisfactory material. I either allowed the cultures to develop too far, or I killed off different, and, therefore, non-comparable, stages in the two temperatures. I also lost much good material through subsequent inexperienced handling with reagents. Owing to the thickness and opacity of the silica coat the cysts are troublesome objects to stain and clear. Finally, when I had attained some proficiency in treating my material, the cultures began to encyst abnormally. I did not succeed in preparing perfectly parallel stages from both sides of a normal culture till the beginning of June, and then from Possenhofen material fetched in on the 9th of May. After that depression set in, and subsequent cultures from the same source were again abnormal.

The material for the April cultures I named A, that used from the 10th of May till the 4th of June, B. The following table illustrates the course of B cultures:

Culture.	First day of starvation.	Fate in warmth (25° C.).	Fate in cold (10° C.).
B 1	10th May	Attempt at encystation.	Die off without encysting.
B 2	11th ,,	Satisfactory encystation.	Majority encyst, but with abnormalities.
B 3	14th ,,	15 animals encyst out of 40.	20 animals encyst out of 40.
B 4	15th ,,	19 ,, ,, ,, 45.	10 ,, ,, ,, 45.
B 5	18th ,,	7 ,, ,, ,, 23.	5 ,, ,, ,, 23.
B 6	27th ,,	12 ,, ,, ,, 25.	Attempt at encystation.
B 7	29th ,,	None encyst.	None encyst.
B 8	31st ,,	A few primary cysts formed.	8 encyst.
B 9	1st June	Satisfactory encystation.	Satisfactory encystation.
B 10	2nd ,,	Encyst abnormally.	Die off without encysting.
B 11	4th ,,	Encyst, but with some abnormalities.	Attempt at mother cysts.

That is to say, all cultures except B 9 showed some abnormality in the warmth or in the cold, and sometimes in both. For my purpose it was essential that the cysts should be normal. B 9 was the only culture where I obtained completely satisfactory final results.

Study of this table shows that during my observations Culture B underwent two periods of deep depression, when no cysts were formed. From such a depression the culture recovered till the maximum of "encystability" was reached, when it again degenerated.

Abnormality almost always showed itself first in the cold side of the culture; which suggests that a lowered temperature weakens the resistance of the organism to unfavourable internal conditions. That lowered temperature has some paralysing effect on the ordinary cell-functions is further borne out by the much greater length of time required to elapse before encystation begins in a cold culture, and by the relative slowness with which the successive stages are reached in it. The following table illustrates this:—

Culture.	First day of starvation.	Date of beginning of encystation. In the warmth.	In the cold.
B 3	14th May	15th May	20th May
B 5	18th ,,	20th ,,	24th ,,
B 6	27th ,,	29th ,,	5th June
B 8	31st ,,	1st June	4th ,,

That is to say, encystation usually sets in within forty-eight hours in the warmth, but not until four or five days later in the cold.

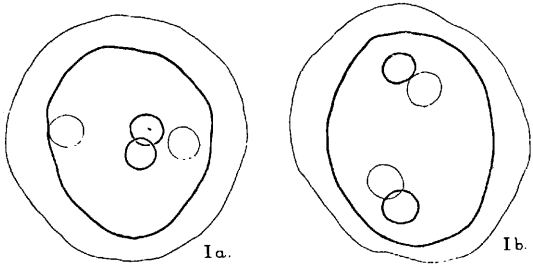
The primary cysts afforded the most favourable material for observation, as in this stage the silica-coat had not yet developed to great thickness. I fixed the primary cysts then with picro-acetic acid, stained them with borax carmine, and cleared them with clove-oil. In nearly every case in culture B 9, which was the one I used for taking measurements, the primary nuclei had already divided into secondary nuclei, though division into secondary cysts had not yet begun: frequently, however, the nuclei had become heteropolar. Successful preparation showed the cytoplasm stained a faint rose-colour, while the outlines of the nuclei, and even their finer internal structure, shone quite clearly through the silica coat. When the cysts were no longer normal, the excessive development of silica made this clear view impossible.

I examined the cysts while they were in clove oil, and took measurements of their length and breadth—these measurements I found to be very constant so long as the culture was normal. Even to the eye there was at once a quite striking difference between cysts from the warm culture and those from the cold. Those from the warm were noticeably the larger, and of more oval form, with a thin coat of silica: those from the cold were smaller, and more rounded in outline; they lay together more compactly, and showed a tendency to greater production of silica. My measurements brought out the size-difference strikingly, the average length in the warmth being 118·5 μ, and the breadth 87·5 μ, while in the cold the average length was 83·5 μ, and the breadth 77·5 μ.

I had not taken the pains to measure the Actinosphæria used for these cultures, nor had I observed whether fusion took place between two or more during the starvation period previous to encystation. I therefore cannot say that, in the compared cultures, the groups of cysts arose from exactly equally-sized individuals. Still, in a general way, I had chosen Actinosphæria of about the same size. Therefore, it is at least interesting to note that the average number of primary cysts formed by one individual was in the cold culture 5, as compared with 3 in the warm. Seven or eight cysts in a group was not uncommon in the cold, but in the warmth was rare. In the warmth sometimes only two cysts, and twice only one cyst of large dimensions were formed by one individual. Now if, as has been stated, the number of cysts formed depends on the number of nuclei remaining over from the reduction in the mother cyst, then this larger number of cysts formed at a low temperature must mean that, in the cold, a larger number of nuclei escape destruction. The smaller size of these cysts is the necessary result of their greater number, there being less cytoplasm available for each. Mr. Smith's experiments gave the same general result. ('Biometrica,' vol. ii, 1903).

To obtain measurements of the nuclei in the primary cysts, I made sections, 10 μ thick, of the cysts that I had previously

measured. (Before sectioning, I drew the outlines of the cyst with a Zeiss drawing-apparatus, and filled in also the nuclear outlines, when I could see them distinctly. Frequently I measured the nuclei at this stage, to act as a check on later measurements in section). The nuclei, when sectioned, I measured under oil-immersion. As in most cases the primary karyokinesis had been finished and the nuclei had begun to become heteropolar, I took measurements in two directions. This brought out an average, in the warmth, of 16·6 $\mu \times 13{\cdot}8\ \mu$, and, in the cold, of 15·2 $\mu \times 14{\cdot}8\ \mu$. That



TEXT-FIGS. 1*a* AND 1*b*.

Four primary cysts from culture B 9, arranged so as to show the comparative dimensions of cyst and nucleus in the warmth, 25° C. (darker line), and the cold, 10° C. (thinner line). Drawn with Zeiss drawing apparatus, oc. 3, obj. 7 (microscope tube of normal length), at the level of the work-table.

is to say, the mean diameter in the warmth was 15·2 μ, and in the cold 14·8 μ. Nuclei measured previous to the primary karyokinesis gave, in the warmth, an average diameter of 20·2 μ, and in the cold of 19·5 μ. In fact, the difference in size between nuclei from warm and cold cultures was scarcely appreciable; those from the cold culture were very slightly the smaller. In comparison with the considerable size-differences between the cysts themselves, this agreement in the dimensions of their respective nuclei is rather striking.

Though fairly exact correspondence in nuclear dimensions

seems to obtain, irrespective of temperature, yet I incline to think that, in the cold culture, the chromatin content of the individual nuclei is greater than in the warmth. Mr. Smith, in his article already quoted, also finds that "in cysts built in the warmth the amount of chromatin is absolutely, as well as relatively, less than in the other cases." He disregards, however, the comparative sizes of the nuclei in his cultures, considering that "such change of size" (of the nucleus) "could be brought about by an alteration in the conditions of tension in the cell, without any deeper changes in the physiological relations."

That the matter can be so lightly dismissed I do not believe, from what I have seen of the constancy in the relative nuclear-cytoplasmic proportions in my warm and cold cultures. An explanation is suggested by Professor Hertwig's Kern-plasma Relation. I must here recapitulate somewhat. According to Professor Hertwig, an encysting Actinosphærium loses about 95 per cent. of its nuclei in the earliest stages of encystation. Probably only 5 per cent. of the original nuclei will survive till the end of the mother-cyst stage, and pass on to further development. The manner of this nuclear elimination is not yet clear. Hertwig considers it unlikely that the reduction is usually the result of fusion; and he also holds it for improbable that, under normal conditions, nuclei are thrust out bodily. To this latter point I shall return later. What one actually sees is a gradual shrinkage of the nucleus; its membrane hangs round it like a loose sac, and it dwindles from about 14 μ to 6 μ or less. At the same time the central chromatin-rosette becomes less and less distinguishable, and the nucleus stains almost uniformly. In this "dead" condition it may remain for some time, but, under normal conditions, it disappears entirely before the primary cysts are formed.

Professor Hertwig's theory of "Kern-plasma Relation" gives a possible reason for this nuclear reduction. He supposes a sort of mutual antagonism to exist between nucleus and cytoplasm, such that, during high function in the cell,

the nucleus grows at the expense of the cytoplasm, while, during periods of rest, the cytoplasm exerts a "reducing" influence on the nuclear mass, bringing it back to its normal proportions. Hertwig sees in the maintenance of a certain definite proportional relation between nucleus and cytoplasm, fixed for each kind of cell, an absolute essential for the continuance of healthy cell-life. Anything that disturbs the equilibrium unduly, such as long continued feeding (when the nuclear element becomes abnormally enlarged), or starvation (when shrinkage of the cytoplasm is the disturbing factor), produces an injurious state of things that will result in death to the cell, if it be not corrected by nuclear reduction, and a consequent return to normal proportions. Consider the conditions used artificially to produce encystation—abundant nourishment, followed by complete starvation. Nothing could be more favourable to a great nuclear preponderance, and for this state of "depression" the organism seeks a remedy in encystation. As the nuclear growth had previously been excessive, so now the "reducing" power of the cytoplasm comes into play to an equally abnormal degree. Ninety-five per cent. of the orginal nuclei are "absorbed," and still further reduction is effected by subsequent formation of polar bodies.

In the cold culture in my experiment an undue proportion of nuclei survived the eliminating process, and, further, they were markedly rich in chromatin. A lowered temperature may be considered to have an inhibiting effect on nuclear reduction. This is probably also the cause of the relative slowness of the early stages in the cold culture. In the cold, nuclear reduction is slow and incomplete.

But there is every reason to believe, from the experiments of Hertwig and his students, that the "Kern-plasma Relation" is lowered or raised by the altering of the temperature conditions to which the cell is subjected. What would be excess of nuclear mass at a high temperature might not be in the least unfavourable to further development at low temperature.

The strongly-marked chromatin "haloes," however, round most of the nuclei in the cold culture, both before and subsequent to the primary karyokinesis, struck me as a later attempt to reduce the great nuclear preponderance to some extent by extrusion of a fine chromatin dust.

In one cyst from the cold culture I detected two nuclei. These are not the result of the primary karyokinesis, but are both primary nuclei, of unequal size (22·5 μ and 18 μ respectively). They lie closely apposed near the centre of the cyst, and are both very rich in chromatin (fig. 2). I regard this as still further proof of the unmanageably large number of nuclei remaining over in a case where lowered temperature has caused incompleteness in nuclear elimination.

Here and there I noticed the inclusion within the cyst-group of intensely-staining, round bodies, usually about 6 μ in diameter (fig. 3). At first sight these bear resemblance to polar bodies, but their occurrence at this early stage, the hint of a central chromatin rosette and of a shrunk nuclear membrane, decided me to class them as out-thrust dead nuclei from the mother-cyst stage. It is quite conceivable that, with the nuclear-absorbing power so much lowered by the cold, "dead" nuclei may easily escape complete dissolution in the mother-cyst, and may linger on through subsequent stages.

To sum up:

I. At a low temperature, Actinosphæria form small and numerous cysts, with nuclei scarcely below normal size, but markedly rich in chromatin.

At a high temperature, the cysts formed are large and few in number, with nuclei scarcely larger than those of the cold cultures, but poor in chromatin.

II. Lowered temperature paralyses the cell-functions to some extent. Nuclear elimination is slow and incomplete, as indicated by—

(1) The large number of nuclei retained from the mother-cyst reduction to act as centres for primary cysts.

(2) The superabundance of chromatin in these nuclei, as suggested by "haloes."
(3) The occurrence of two nuclei in one primary cyst.
(4) The occurrence of occasional "dead" nuclei within the groups of primary cysts.

Hitherto I have described the effect of cold on a culture of Actinosphærium that encysted quite normally. The majority of my cultures, however, encysted during the oncome of a "depression"-wave, and showed many abnormalities of structure. A few points noticed seem to be worthy of brief description. Full account of the effect of temperature on cultures of degenerate Actinosphærium will be given by my fellow-student, Miss Boissevain.

Where there was a tendency to abnormality it made itself noticeable in the cold culture first, as a rule—probably the next set of cultures would show abnormalities in the warmth. Finally, a stage would be reached when both sides of the culture would entirely refuse to encyst, or would encyst so abnormally and feebly that further development was impossible, and the cysts disintegrated in that stage.

Examination of non-encysted members of such cultures gives a clue to this condition. The organisms are undergoing a period of "depression." Prolonged function has led to an overwhelming nuclear preponderance, and, though by out-thrust of chromatin and nuclear fusion, attempts are being made to bring the relation back to the normal, yet the nuclear mass is so unmanageably great that the "reducing"-power of the cytoplasm is insufficient to cope with it, and, in extreme cases, the limits within which satisfactory encystation is possible, are never reached. Further, if it be granted that abnormal nuclear dimensions are injurious to cell-life, then the very presence of this pathological condition in the cell must render the cytoplasm still less equal to meet the heavy demand put on it.

Such individuals as succeed in establishing a certain amount of nuclear reduction may encyst; the rest of the culture will dwindle and die. The cysts formed show certain outward signs of abnormality, such as great unevenness in size within one cyst-group, irregularity of form, excessive development of silica.

Treated with reagents, and sectioned in the usual way, they are generally found to show corresponding abnormalities in the minute structure. Such abnormalities are exactly those observed by me in the cold "normal" culture B 9, but on a very much exaggerated scale. In both cases they may be looked upon as the result of a lowered vitality. In the normal culture this was induced by lowered temperature. In the other cultures it was the result of physiological degeneration.

Extreme examples of the condition may be induced by the co-operation of both factors, as is, indeed, shown by such of my degenerate Actinosphæria as encysted in the cold.

My culture series was too incomplete for me to make satisfactory comparisons from successive warm and cold cultures during the depression. I shall content myself with arranging the described abnormalities in the same order as that given at the end of my remarks on the normal cultures, adding a brief remark on the fate of the culture and its appearance in the other temperature. Such a scheme is, of course, rather artificial, but makes comparison easier, and brings my results together more compactly.

In an encystation culture of depressed Actinosphæria nuclear elimination tends to be incomplete.

(1) Too many Nuclei are retained to form Centres for Primary Cysts.—This means that the cysts formed tend to be smaller than the normal. I found, for instance, that 65 μ × 55 μ was a very common measurement in the cold. There were, however, wide deviations from this, and, in the warmth especially, I noticed considerable irregularity of size. In such cysts the nuclei were of normal size or rather larger. The disproportion between the small cysts and their nuclei

was generally much greater than at first appeared, for the cytoplasm was often reduced to a minimum by extreme vacuolisation and by the great thickness of the silica coat.

The nuclear mass retained may be so much in excess of the available cytoplasm that development can proceed only up to a certain point.

(*a*) Disintegration may occur in the primary cyst. Fig. 4 shows one of a group of two primary cysts from the cold culture of B 8. The cyst pictured was of large size, 129 $\mu \times 79\,\mu$, but the silica was more than 10 μ thick, and the cytoplasm much vacuolated. So that the dimensions of the nuclei (the primary karyokinesis had already taken place), 19·5 $\mu \times 19{\cdot}8\,\mu$ in one case, and 18 $\mu \times 22\,\mu$ in the other, are excessive. The chromatin has been thrust out on all sides into the cytoplasm, and lies there in irregular strings, blotches and specks. The largest chromidia do not radiate from the nuclei, but tend to lie closely apposed to their surfaces in a tangential direction. The nuclei are almost devoid of chromatin, and show signs of shrinking. Probably further development was impossible.

Of the other cysts formed in this culture, one group made primary cysts with very large nuclei, and two more formed small vacuolated secondary cysts. The warm culture died off in the mother-cyst stage.

(*b*) Fig. 5 represents a cyst from the cold side of culture B 11. Here are nine nuclei (only six appear in the section figured) of very uneven size,—one is 32 $\mu \times 23\,\mu$, others measure 26 $\mu \times 15\,\mu$, 20 $\mu \times 17\,\mu$, etc., and two are under the normal size for this stage, 16 μ and 15 μ respectively. They are all rich in chromatin, and show vacuolated nucleoli. In contrast to the available mass, the cytoplasm is so scanty as to be almost absent round some of the nuclei. Silica has been excessively developed, and in such a way as to indicate that an attempt has been made to map out primary cysts, of which these nuclei are the centres. The power to separate these definitely from one another has been lost, and further development is scarcely possible. Several of the nuclei are surrounded by chromatin haloes, in which chromidia radiate

outwards, and in one case, that of the largest nucleus, the nuclear membrane seemed to have disappeared at one point, and the whole nuclear content is streaming out.

(*c*) No other member of this culture, B 11 (cold), got beyond the mother-cyst stage. In fig. 6 is shown one of these cysts. This shows elimination to have been so imperfect that a quite excessive number of nuclei remain; in the cyst figured there were 278. These have all shrunk and died, but remain as darkly-staining spots (6·5 μ to 9 μ in diameter), not near the borders of the cyst, but aggregated in little strings and groups. Each group lies in an "island" of normally-staining cytoplasm surrounded by non-staining brownish material, in which are shrinkage rents. This is an instance of "pycnosis," when the nuclei die off without being further disposed of, until the whole cyst disintegrates.

(The corresponding warm culture of B 11 showed such abnormalities as great fluctuation in size of cysts, and frequent occurrence of non-absorbed nuclei.)

(2) Occurrence of more than one Nucleus in a Cyst.—In Culture A 2 I noticed a few abnormalities in the warm and room temperature cultures, while the cold culture encysted only very feebly. In one cyst from the warm culture I counted as many as six nuclei (fig. 7). The cyst was the only one formed by the individual, and measures 120 $\mu \times 111\,\mu$, much of the bulk being due to the silica, which is about 18 μ thick. The nuclei are arranged in a group round a central point. They are of much the same size, 16 $\mu \times 14\,\mu$, and of markedly elongated form. On one side, generally that towards the exterior, they have shrunk away a little from the cytoplasm. At first, I took them to be heteropolar, but subsequent use of Delafield's hæmatoxylin failed to bring out any such arrangement of the chromatin. The nuclei are, in fact, singularly devoid of chromatin, and the nuclear reticulum is very faint, and free from chromatin aggregations of any size. In the cytoplasm lie also fourteen smaller darkly-staining bodies, measuring about 4 μ in diameter, and each surrounded by a vacuole. These remained unaffected by the hæmatoxylin

stain. In appearance they bear a very strong resemblance to polar bodies, but their number, fourteen, does not fit in with the number of the nuclei, which is six. Possibly, however, some of them may be the last remnants of "dead" nuclei. The Delafield's hæmatoxylin brought out clearly the broad zone of "Dotter-plättchen," just within the silica coat.

I was unable to decide as to the stage that this cyst has reached. I incline to think it a mother-cyst in which the nuclei have swelled out to the size of primary-cyst nuclei; but the power to form separate cysts round these has been lost.

(3) The Retention within the Cyst Group of Dead Nuclei from the Mother-cyst.—This occurs freely in most of the cases already described. In both warm and cold cultures the cyst-groups are very commonly accompanied by such nuclei in various stages of "shrinkage."

Fig. 8. Here is a group of seven primary cysts from B 2 (cold). The nuclei are in process of the primary karyokinesis. Twelve out-thrust nuclei remain round the group, and show still quite clearly their original structure.

Fig. 9 is a section from a group of twenty-four secondary cysts and conjugation-cysts from the room-temperature culture of A 2. Most of the cysts are in process of polar-body formation, but are not very clearly marked off from one another. Within the common cyst envelope are as many as forty-six nuclei of still quite appreciable dimensions.

I failed to observe out-thrust nuclei surviving till later stages than this.

I am inclined to think that nuclei are thrust out bodily from the early stages in the mother-cyst as soon as, from either of the causes suggested (i. e. lowered temperature and degeneration), nuclear absorption by the cytoplasm has been weakened.

In unencysted Actinosphæria Hertwig observes that, from such as have suffered hyperplasia or hypertrophy of the nucleus, parts of the organism, containing numerous nuclei, are thrust out bodily ('Physiolog. Deg. bei Actinosph.')

He speaks of nuclear out-thrust as occurring only "very abnormally" in the reduction in the mother-cyst. From what I have seen in my cultures I believe that, though certainly not quite normal, such out-thrust methods tend to come into play very quickly as partial substitute for a weakened power of nuclear elimination by the usual method. In all my cultures, except B 9 warm, I met with out-thrust unabsorbed nuclei till a certain stage in the degeneration was reached, when even this more drastic measure seemed unavailable, and the nuclei died off in the mother-cyst, remaining there unabsorbed until the whole gradually disintegrated.

I wish to express my warm thanks to Professor Hertwig and his assistants for their unfailing readiness with advice and practical help during my work in Munich; and also to Miss Boissevain for her many valuable suggestions and personal assistance.

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December, 1907.

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EXPLANATION OF PLATE 24,

Illustrating Miss Doris L. Mackinnon's paper on "A few Observations on the Encystation of Actinosphærium eichhorni under different conditions of Temperature."

(For Fig. 1 see text, p. 412.)

Fig. 2.—Occurrence of two nuclei in one primary cyst previous to primary karyokinesis. (B 9, cold.)

Fig. 3.—To illustrate occasional retention of dead nuclei in the common cyst-envelope. (B 9, cold.)

Fig. 4.—Chromidia in primary cyst after primary karyokinesis. (B 8, cold.)

Fig. 5.—Unsuccessful attempt to form primary cysts. (B 11, cold.)

Fig. 6.—Mother-cyst dying off, with 278 "dead" nuclei. (B 11, cold.)

Fig. 7.—One cyst containing six nuclei and 14 "dead" nuclei (?). (A 2, warm.)

Fig. 8.—Group of primary cysts, in course of primary karyokinesis, showing ejected nuclei. (B 2, cold.)

Fig. 9.—Group of conjugation-cysts and secondary cysts, in course of polar body formation, showing ejected nuclei. (A 2, room temperature.)

All, except Fig. 8, are drawn from sections 10 μ thick.

Fig. 8 is from a preparation still in toto.

All were fixed with picro-acetic acid and stained with borax carmine. 6 and 7 were afterwards stained with Delafield's hæmatoxylin.

All figs. are enlarged 193 diameters.

Fig. 2.

Fig. 3.

Fig. 4.

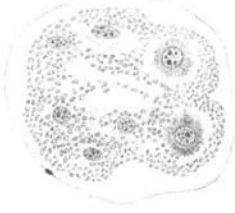

Fig. 5.

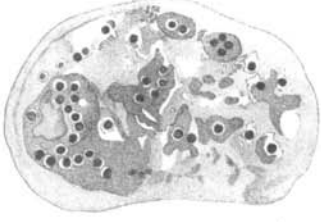

Fig. 6.

Fig. 7.

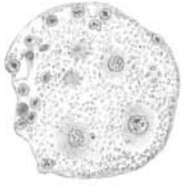

Fig. 8.

Fig. 9.

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