The Spermatogenesis of Anthoceros laevis, L.

BY

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With Plate VI.

INTRODUCTION.

THE spermatogenesis in *Anthoceros* has been broadly treated by Campbell (1), who, owing to the minuteness of the spermatogenous tissues, gives little cytological detail. Professor Farmer suggested that a detailed study of the spermatogenesis in *Anthoceros* might throw some light on the affinities of the Anthocerotales to other members of the Hepaticeae. This investigation was accordingly taken up at his suggestion, and the writer wishes to express thanks for his advice and guidance during the course of the investigation.

METHODS.

A part of the material was fixed in acetic alcohol in the field, other material was kept in the laboratory growing on large sods under a bell-jar, and was fixed from time to time in various strengths of chromo-acetic, Flemming's, and Hermann's solutions. Chromo-acetic of medium strength and Flemming's weak solution proved quite good, but much better results were obtained from material fixed in half-strength Hermann's fluid for ten hours. The material was very quickly taken through grades of xylol to paraffin, so that the time taken for the entire process up to embedding was reduced to six hours. Even then great difficulty was experienced in cutting sections, as the hot xylol makes the antheridia very hard and brittle; sections were cut from 3 to 8 μ. The process of division of the spermatogenous tissues has been studied from sections of 4 to 6 μ and the development of the spermatozoids has been described from sections of 3 μ. In staining, Flemming's triple stain, polychrome-methylene blue, and Heidenhain's iron-haemotoxylin, followed by Bismarck-brown (1 per cent.

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Bismarck-brown in 15 per cent. alcohol) as counter-stain, were used. The haematoxylin was carefully washed away, leaving the delicate cell-wall colourless, when a careful touch of Bismarck-brown gave a satisfactory contrast. The cell-wall took a bright brown colour, while the cytoplasm, mucilaginous layer, and the pectic membrane separating the spermatozoids took a much lighter colour with just a shade of difference between them. The figures are all drawn from Heidenhain-stained preparations, but the results were all checked by Flemming's and polychrome-stained preparations.

The Nature of the Spermatogenous Tissues.

The cells of the spermatogenous tissues are seldom seen to divide simultaneously, though in some mature antheridia one finds several stages of development. This tissue is arranged in the antheridia in form of a regular pyramidal cone or a rectangular patch, obviously related to the early segmentation of the antheridium during the metaphase and telophase divisions. The spermatogenous cells present a more or less uniform stage when they advance towards the formation of spermatocytes. The antheridium attains its mature size before the formation of spermatocytes from the spermatogenous tissue is complete. The cells of the spermatogenous tissue divide very rapidly, with the result that the spermatid mother-cells, which are packed in the form of regular cubical cells, decrease to almost one-fourth of the original size. The cells of the antheridia retain their cubical shape throughout the division. The amount of mucilaginous substance in the cells increases as the cells advance towards the final divisions leading to the formation of the spermatocytes.

The Prophase in the Antheridial Nuclear Divisions.

The resting nucleus of the young antheridial cell contains finely granular cytoplasm and a large and prominent nucleolus which is rather eccentrically situated (Pl. VI, Fig. 1). Several highly refractive granules are noticed outside the nuclear membrane. The nuclear membrane is at first very fine and almost hyaline, but it becomes considerably thicker as the nucleus advances towards the spireme stage (Fig. 2). The cytoplasm becomes much coarser during the early prophase, and there is a gradual accumulation of granular chromatin which later on takes the form of a delicate thread (Fig. 2). This is very clear in a thick tangential section which shows the chromatin thread in the form of a concave reticulum along the nuclear membrane, while a thin median section shows only the cut ends of the reticulum along the membrane. The thread is drawn out at several points in the nuclear area. This concentration of chromatic material gradually increases at those points, so that after a time there appear several thick

knots within the nuclear space (Fig. 3). These knots are connected with the nucleolus by fine filaments, while a number of loose ends radiate from the other side of the knots towards the nuclear membrane. The nucleolus in the meanwhile loses its round shape and appears to be rather elongated (Fig. 3); and this phenomenon might be due to the pull of the chromatin reticulum. Soon after this the whole mass of chromatin fragments into four distinct groups (Fig. 4), which finally give rise to four slightly curved, rod-shaped chromosomes of two different sizes, two large and two small (Fig. 5).

The Nature of the Spindle.

The spindle is organized by numerous delicate fibrils which slowly accumulate along the nuclear membrane during the prophase. Neither polar caps nor any specialized part of the cytoplasm was observed to be connected with its formation. The spindle is very regular in the early stages of division, and its axis coincides with the long axis of the cell (Fig. 5), as in an ordinary division. The threads of the spindle are clear, but the poles generally vary in shape from sharply pointed cones to flat ones in which the fibres run almost in parallel bundles. The indication of the advance division is given by the oblique position of the spindles. The final division of the spermatid mother-cells, which are very small, is easily recognized by the greater number of oblique spindles, though even here a great many of them are in the long axis of the cell, and not oblique to it (Fig. 8). But cases are also seen (Fig. 9) where the finally dividing cells have all the spindles arranged diagonally, and this has been described by many authors as characteristic of the final division in the spermatogenesis of the Hepaticeae. Unlike the earlier spindles those of the final division have sharp-pointed poles. Granules are noticed at the poles, but, being of very rare occurrence, have been omitted in the diagrams.

Telophase and the Formation of Spermatids.

The chromosomes lose their individuality as soon as they reach the poles, where they are reduced to a narrow, crescent-shaped band of chromatin (Fig. 10). The spindle fibres become much finer and ultimately break away in the middle, and the two parts recede towards the poles, so that a clear space is formed at the equatorial region (Fig. 11). As the result of this peculiar kind of division no actual cell-wall is formed between the two daughter nuclei, and the spermatids are only separated by a delicate pectic membrane. The nucleus keeps the elongated shape of the original chromatin for a short time, but it rounds itself off and appears as a faintly-stained body when the spermatids undergo a period of rest. The young spermatids (Fig. 12) have a very characteristic appearance. They present either a triangular or

a semicircular outline, depending on the original position of the spindle, i. e. whether the latter was oblique or longitudinal in position. The nucleus always lies near the broadest side of the spermatids which are facing each other. The spermatid contracts away from the cell-wall, leaving a clear space all round, which is filled up by more hyaline mucilaginous substance. The cell-wall undergoes the greatest degree of mucilaginous disintegration at this stage, and the middle lamella is almost indistinguishable.

The origin of the mucilaginous substance and its gradual increase from the early divisions to the formation of spermatids are not only due to the disintegration of the cell-wall but also to the metabolic activity of the young spermatids, which seems to a certain extent responsible for its formation.

Blepharoplast and the Formation of Spermatozoid.

Special attention was given to the exact processes concerned in the formation of blepharoplast and of spermatozoids, which varies considerably in the Hepaticeae; even in the case of a single species such as *Marchantia polymorpha* there is a difference of opinion, and consequently controversy has arisen. Unfortunately the present material does not provide much scope for a detailed study of the spermatozoid formation. Repeated attempts were made to detect the centrosome or any structure like a centrosome, but without any success. It has been already pointed out by Davis (2) that centrosomes are entirely absent in *Anthoceros*. The blepharoplast arises as the result of fragmentation of the main mass of chromatin, and it lies for a short time at one end of the narrow strip of chromatin (Figs. 15 and 16). The blepharoplast thus appears rather late in the history of spermatogenesis of *Anthoceros*, and it disappears as soon as the cilia are visible around the young spermatozoid. The spermatid has now changed to an almost circular shape, and the nucleus is reduced to a faintly-stained body (Fig. 17). The nucleus lies eccentrically in the spermatid. The body of the spermatozoid is formed by the elongation of this nucleus and by further condensation of nuclear material along the outer contour of the spermatid (Figs. 18 and 19). The cell-wall is gradually dissolved, while the membrane keeps longer intact. Finally, the membrane is lost sight of as the spermatozoids become embedded in a loose matrix of mucilaginous substance and occupy a quarter of the antheridial cavity. The mature spermatozoid (Fig. 20) has a curved and linear body with a slightly club-shaped head. The cilia are almost of the same length as the body of the spermatozoids.

Discussion.

There have been a considerable number of investigations on the development of the spermatozoids in the Hepaticeae, and they all tend to show that the final division of the spermatid mother-cell conforms to the

diagonal type. In *Anthoceros*, however, the final spindle is both oblique as well as longitudinal, and these two arrangements occur side by side and both give rise to spermatids.

The origin and function of blepharoplast are two interesting points in the history of the spermatogenesis. Ikeno (6) first pointed out the presence of centrosomes in the cells of the spermatogenous tissues of *Marchantia polymorpha*. According to his very careful investigations they appear in the later dividing cells of the antheridia, and are always present during the final division of the spermatid; the single centrosome migrates to the acute angle of the spermatid, and forms the blepharoplast from which the cilia arise. Ikeno points to the probable homology of the two organs—the centrosome and the blepharoplast—in a phylogenetic sense. The existence of centrosomes in *Marchantia* and their subsequent change to blepharoplasts, the cilia-bearers, has been denied by Mottier (8) and more recently by Escoyez (4). Escoyez maintains that the corpuscles which appear in the spermatogenetic cells during the early divisions, and which can be traced from one division to another, are mere cell-structures, and are not genuine centrosomes, while he argues that the others, which always appear in the final and diagonal divisions, are really blepharoplastic in nature. Lewis (7) regards these bodies as centrosome-like rather than true centrosomes, but he seems to favour Ikeno's (6) view of the homology of the two organs in question.

It is out of place here to go farther into the details of this controversy, as the centrosome is entirely absent in *Anthoceros*. Several slides were obtained which showed the 'aster' and 'diaster' stages of the final division (Figs. 7, 9, and 10), which, according to Ikeno, are the most critical stages where the centrosomes are invariably present in *Marchantia*, but even at these stages persistent search failed to reveal any structure which could be positively identified as a centrosome. In this respect *Anthoceros* behaves much like *Fossombronia longiseta*, where Humphrey (5) could not trace the origin of blepharoplast from a centrosome. But in *Anthoceros* the origin of blepharoplast, at any rate, which has not hitherto been clearly seen, and has up till now been a subject of speculation, is very clear. It is formed by fragmentation from the main mass of chromatin of the nucleus. Even in the same slide one can find all the stages from its beginning in the main body of the chromatin (Figs. 13 and 14) to the lateral migration of the small fragment as a blepharoplast (Figs. 15 and 16), while the bigger body rounds itself off and takes the central position as the nucleus (Fig. 17).

It is interesting to note that the origin of blepharoplastic corpuscles in the spermatids of *Anthoceros* shows some similarity to the formation of the 'chromatin bodies' which have been observed in animals and plants, both in vegetative and reproductive cells. The history of the development of these bodies has been studied in *Galtonia candicans* by Miss Digby (3).

They originate either from the nuclear framework or from the nucleolus. They may lie in the nuclear cavity or they may invade the neighbouring cells where they remain in the cytoplasm. But the further history of blepharoplastic corpuscles in *Anthoceros* is not so simple as that of the 'chromatin bodies' of the higher plants. In *Anthoceros* and in other members of the Hepaticeae they are always connected with the formation of the motile organs (cilia), while in *Galtonia* and in other higher plants they are destined to undergo an extremely rapid degeneration.

The contraction of the chromatin reticulum as knots at several points of the delicate spireme in the evolution of chromosomes is a regular phenomenon in all the successive divisions (Figs. 3, 4, and 8). The number of chromosomes is four, which agrees with the number given by Davis (2).

Summary.

1. The gametophytic number of chromosomes is four.

2. The centrosome is entirely absent in the spermatogenesis of *Anthoceros*.

3. The final division of the spermatid mother-cell is both oblique as well as regular, and the two kinds of divisions take place side by side and both give rise to the spermatocytes.

4. No true cell-wall is formed between the spermatids, which are separated by a hyaline membrane of pectic substance.

5. The blepharoplast arises from the main body of chromatin as the result of fragmentation. It disappears as soon as the cilia are visible round the spermatid.

6. The spermatozoid has a linear body which is a little broader towards the head, with the cilia of almost of the same length as the body.

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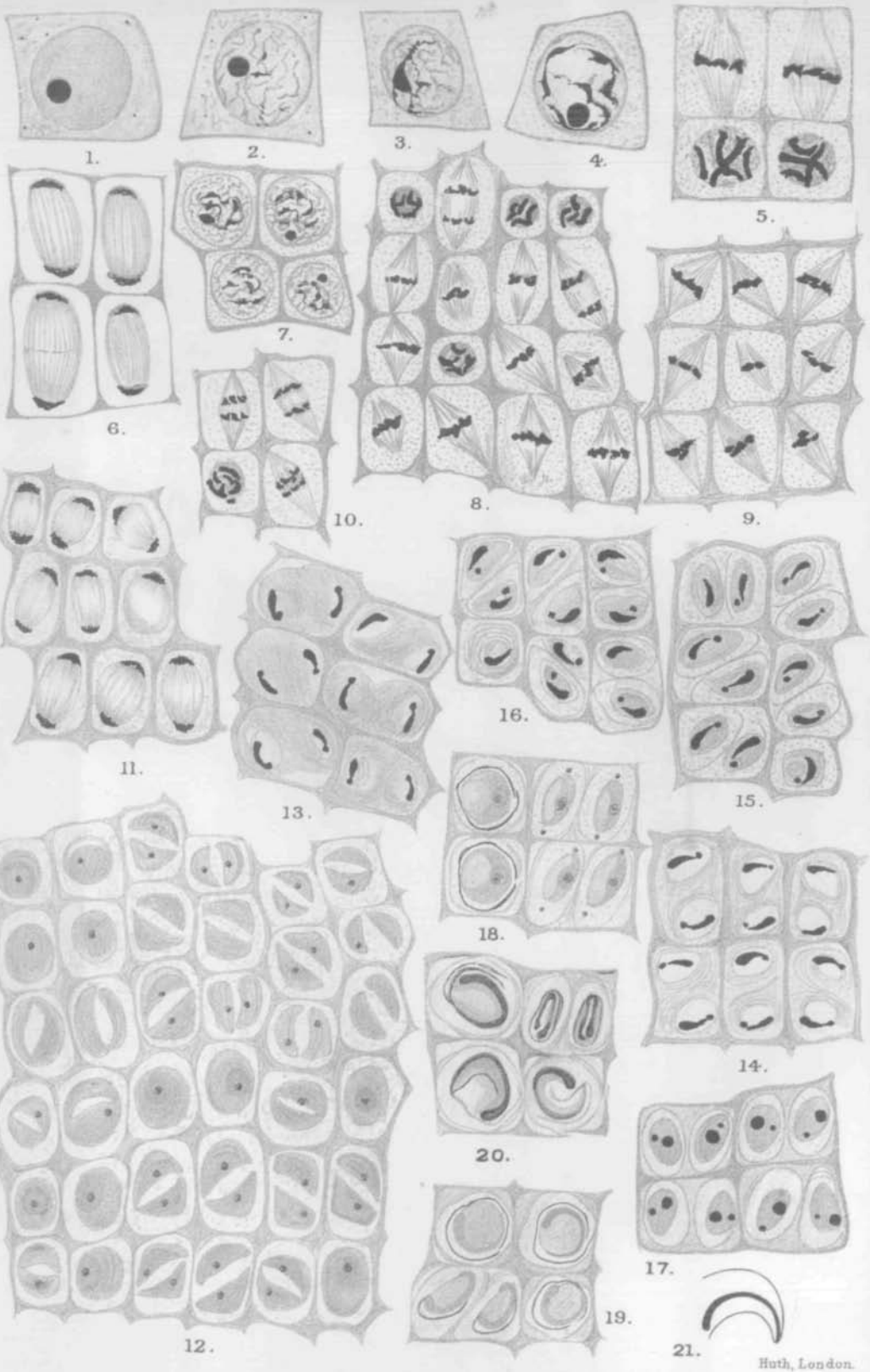
EXPLANATION OF PLATE VI.

Illustrating Mr. Krishnadas Bagchee's paper on the Spermatogenesis of *Anthoceros laevis*, L.

All the figures, with the exception of Figs. 20 and 21, were drawn with the camera lucida under 1/15 semi-apochr. Koristka with comp. oc. 18 (× 2550).

Figs. 20 and 21 were drawn with camera lucida under 2 mm. apoc. imm. Zeiss N.A. 1·40 with comp. oc. 18 × 2250.

Fig. 1. Resting stage of nucleus in the cells of spermatogenous tissue of very young antheridium.
Fig. 2. Delicate spireme stage of nucleus of almost the same age as Fig. 1.
Fig. 3. Formation of thick spireme from several knots.
Fig. 4. Fragmentation of spireme into four groups of chromatin.
Fig. 5. Aster stage of mitosis of very young antheridium.
Fig. 6. Diaster stage of almost the same age as Fig. 5.
Fig. 7. Spireme stage of nucleus from which the spermatid mother-cells arise.
Fig. 8. Final division in spermatid mother-cells showing mixed spindles.
Fig. 9. Final division with oblique spindles.
Fig. 10. Anaphase stage of mitosis in spermatid mother-cells.
Fig. 11. Late telophase of final division, showing the formation of clear space in the equatorial region of the spindle.
Fig. 12. The resting spermatids within the spermatid mother-cells.
Fig. 13. The appearance of constriction in the main mass of chromatin after the late telophase.
Fig. 14. Formation of blepharoplast.
Figs. 15 and 16. Lateral migration of blepharoplast.
Fig. 17. Lateral migration of blepharoplast and the formation of nucleus by rounding off of the main mass of chromatin.
Fig. 18. Formation of cilia and disappearance of blepharoplast.
Fig. 19. Condensation and elongation of spermatozoids.
Fig. 20. A later stage in the growth of spermatozoids.
Fig. 21. A free spermatozoid.



BAGCHEE — ANTHOCEROS.