CENTRIOLES AND CHROMOSOMES IN THE ATYPICAL SPERMATOGENESIS OF VIVIPARA

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It has been shown that in many genera of prosobranchiate snails two types of sperms are produced. One of these, the typical or eupyrene, is normal in structure and development; in no way different from that characteristic of Mollusca in general. The other, the atypical, is usually larger, and is very abnormal; the number of axial filaments is increased and the chromatin is either absent (apyrene condition) or greatly reduced in amount (oligopyrene condition). In Vivipara (Paludina) the atypical spermatozoa are oligopyrene. The development of these was worked out in great detail by Meves,[1] 1902, in *V. vivipara*. Considering the remarkable nature of his observations it is surprising that the work has attracted little general attention, being cited chiefly as an example of independent multiplication of centrioles that supports the Henneguy-Lenhossék hypothesis of the nature of the basal bodies of cilia. The fact that in all cases of atypical gasteropod spermatogenesis the increase in number of centrioles is accompanied by very abnormal meiotic behavior and the eventual disintegration of a large number or all of the chromosomes has received little notice. The author is now studying atypical spermatogenesis in an unidentified species of Vivipara (not the same as that studied by Meves) with the object of comparing the behavior of centrioles with that of chromosomes. The results to date confirm all the main features of Meves's account, and they furthermore reveal a strikingly definite numerical relationship between centrioles and chromosomes, the possibility of which is vaguely suggested by Meves's data. The phenomena are so remarkable and are of such general significance that the following brief note seems warranted in advance of publication of full details.

In this species the diploid number of chromosomes is 18. During the growth period of the normal spermatocytes the usual polarized leptotene, zygotene and pachytene stages occur. Nine tetrads are seen at diakinesis and at metaphase of the first maturation division.

The atypical spermatogenesis is shown schematically in figure 1. The atypical spermatocytes grow to several times as large as those of the normal line, the extra size being mostly in cytoplasmic volume. Slender leptotene-like threads may be identified, and these eventually condense to form compact chromosomes that assume a position at the nuclear periphery, like that characteristic of normal diakinesis. The chromosome threads are never polarized toward the idiozomal region; and there is nearly complete

absence of synapsis. The lack of synapsis is confirmed by the observation that at diakinesis there appear to be always a few more than 18 chromo-

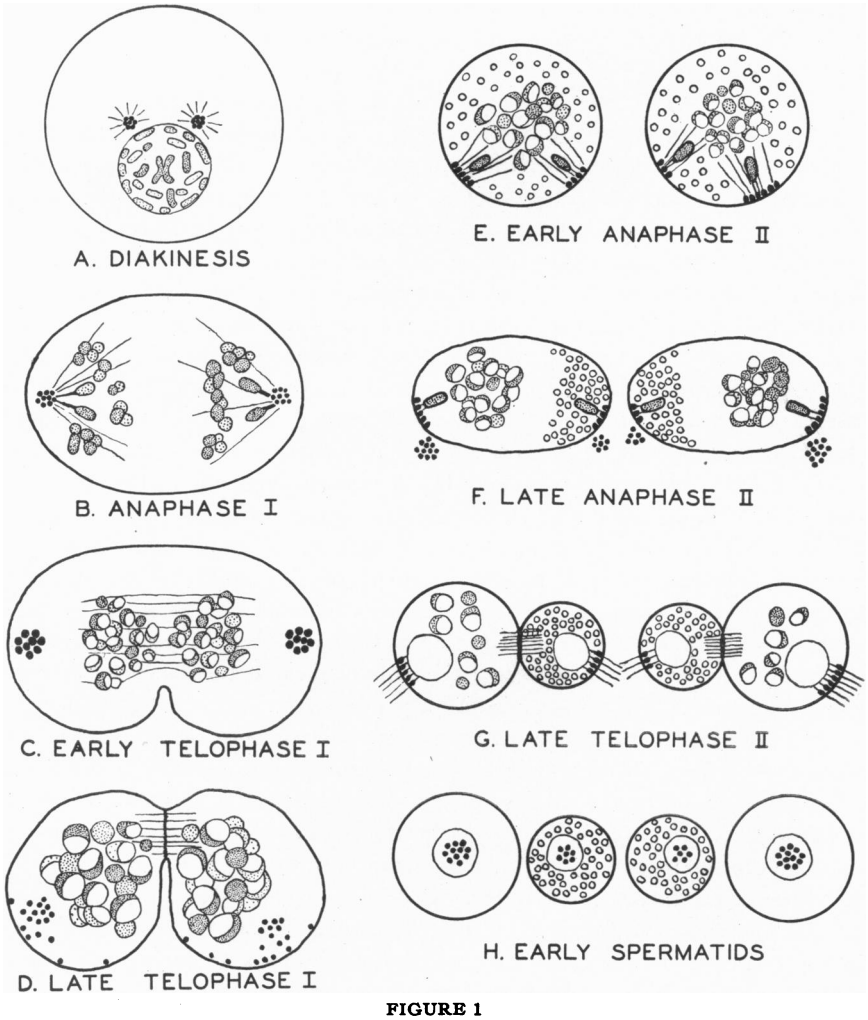

FIGURE 1

Schematized figures of successive stages in atypical spermatogenesis of Vivipara. A–G are similarly oriented. H shows spermatids in position for accurate counts of centrioles (see table 1). Centrioles are solid black, chromosomes stippled, chondriosomes (in E–H) are small circles.

somes; counts showing up to 23. With the exception of one probable tetrad these are simple rods like the chromosomes that are seen in spermatogonia (Fig. 1A). The presence of chromosomes in excess of the dip-

loid number is presumably due to precocious separation of some of the sister chromatids.

In the early atypical spermatocytes two centriole-like bodies may be distinguished in the idiozomal region. Both these increase in size, and near the end of the growth period they are large spheres, over one micron in diameter, and exactly equal in size. At diakinesis (Fig. 1A) the spheres appear mulberry-like, as Meves described them; they are near the nucleus; and each is at the center of a small aster. The spheres are some distance apart though never at opposite poles of the nucleus. As the nuclear membrane breaks down for the first division the chromosomes draw together into a compact ball, into which a number of lines (spindle fibres?) extend from each of the spherical groups of granules, which from now on will be referred to as centrioles. There is no metaphase plate stage. The two groups of centrioles move to opposite poles of the cell, and the chromosomes, most of them sticking together in several (4–8) clumps, accumulate near the centrioles (Fig. 1B). To each centriole group one or two separate chromosomes, isolated from the clumps that include most of the chromosomes, are connected by a definite, robust spindle fibre. Many fibres, however, extend from the centrioles in the general direction of the opposite pole without connecting with any chromosome or group of chromosomes. Just before cell constriction all of the chromosomes move away from the poles and the clumps break up into separate chromosomes. A preliminary small number of counts indicate that there are 34–36 chromosomes at this time. This is a close agreement with what is expected if one assumes that the separation of sister chromatids, apparently begun in diakinesis, has now been completed. The lower number agrees with expectation if the single tetrad noted at diakinesis has given rise not to four chromatids but to two dyads. In spite of these apparently rather disorderly mitotic phenomena each secondary spermatocyte receives very nearly the same number of chromosomes, and the cells formed by telophase constriction are about equal in size.

In late anaphase or early telophase (Fig. 1C) the individual centrioles grow, the groups of centrioles loosen up somewhat and it can be determined that the number in each cluster is almost certainly 9. During telophase each chromosome independently develops into a vesicle, with the chromatin concentrated in a thick cap on one side. These vesicles grow considerably during the interphase. In late telophase (Fig. 1D) each centriole group is located not far from the cell membrane, in a position asymmetrical with respect to both the spindle remnant and the plane of cell constriction, which has proceeded more rapidly from one side. At this time the number of centrioles is much greater than at earlier telophase; counts ranging from 14 to 16 are common. Since this is a stage before which each centriole of a typical normal spermatocyte would have divided it seems likely that in

these atypical cells likewise each of the 9 centrioles noted slightly earlier has now divided, and that the full number of centrioles in each daughter cell at late telophase is actually 18. The fact that the group of centrioles is breaking up and the individual centrioles are becoming dispersed throughout the cell probably accounts for failure to locate the full number. Most of the centrioles presently take up positions on the inner surface of the cell membrane, where they are seen during the second division, and until spermiogenesis is under way.

In the prophase of the second division the centrioles form two groups on the cell periphery, between 90 and 180 degrees apart. The large chromosomal vesicles decrease in size, and fibres may be seen penetrating from each centriole group into the cluster of these vesicles, which is in the approximate center of the cell. Next, two single chromosomes simultaneously leave the central mass, one migrating to each centriole group (Fig. 1E). These two chromosomes are not vesicles but are as fully condensed as those of the first division, and they appear perfectly normal. They are much alike in size and shape, and it is very plausible to regard them as mates. The other chromosomes do not condense beyond the small vesicle stage. Although they move slightly nearer to one pole they never approach it as closely as does the single chromosome that migrated earlier.

Just before cell constriction develops, small granular chondriosomes that were hitherto scattered throughout the cytoplasm become accumulated in the end of the cell opposite that where the chromosomal vesicles are located.

In many of these second maturation division figures one may observe that the number of centrioles in the two groups is markedly unequal; and in later stages the chondriosomes are massed near the smaller centriole group, while the chromosomes are closer to the larger (Fig. 1F). Cell constriction produces two unequal spermatids. The larger contains the greater number of centrioles and all but one of the chromosomes; while in the smaller cell are a single chromosome, the smaller centriole group, and nearly all the chondriosomes (Fig. 1G). On account of this last fortunate circumstance, throughout spermiogenesis it is easy to positively identify the smaller spermatids, not only by their smaller size but because of the heavier stain in the cytoplasm. Spermiogenesis differs in no important feature from that described by Meves in *V. vivipara*. The small nucleus in each spermatid is formed by the single chromosome that behaved normally in the second maturation division. All the abnormal chromosomes soon disappear. All the centrioles function as blepharoplasts, each producing a single axial filament.

The occurrence of the unequal second maturation division adequately accounts for the fact noted by Morita,[2] 1932, that in Japanese species of

Vivipara there are two sizes of atypical spermatozoa, which are always present in ratio of 1 : 1.

As the figures in table 1 indicate, the count of spermatid centrioles, which can be made with absolute certainty in cells that are favorably oriented (Fig. 1H), confirms the observation that the smaller spermatid is organized around the lesser number of centrioles, at least in 95% of the cases. The table is based on counts of 113 small cells and 128 large cells, being all those in which the number of centrioles could be determined in seven adjacent sections on one slide.

TABLE 1

NUMBER OF CENTRIOLES IN SMALL SPERMATIDS

Number of centrioles	*3*	*4*	*5*	*6*	*7*	*8*	*9*
% of cells	3.6	5.3	17.7	21.2	27.4	19.5	5.3

NUMBER OF CENTRIOLES IN LARGE SPERMATIDS

Number of centrioles	*15*	*14*	*13*	*12*	*11*	*10*	*9*
% of cells	7.0	6.3	12.5	23.4	28.9	19.5	2.4

If it be assumed that the number of centrioles apportioned to each two sister spermatids, i.e., the number in each secondary apermatocyte, is constant—which is probable from the previous history of the centrioles—then it is obvious that the data in table 1 agree best with the view that this constant secondary spermatocyte centriole number is 18. For convenience, in the table the complementary low and high numbers that add together in pairs to equal 18, have been placed in the same vertical column. The percentage of each should be equal to that of its complementary cell of the other size; and there is good agreement with this expectation, especially in the groups that include more cells where the numbers are large enough to be significant. The accuracy of this fit of the data to the number 18 will be emphasized if the reader tries fitting them to other possible numbers, e.g., 16 or 20, when it will be immediately apparent that one or more of the expected centriole counts is represented by a greatly deficient percentage of cells.

Analysis of the data in table 1 in a slightly different way is even more conducive to the same conclusion as to the centriole number in the secondary spermatocytes. If it were an invariable rule that a constant even number—an odd number is ruled out because of the occurrence of centriole division at the end of the first maturation division—of centrioles of the secondary spermatocytes were divided unequally between the large and the small spermatids, then the largest number of centrioles occurring in any of the small cells should be at least two less than the smallest number found

in any large cell. In the series of complementary numbers which added together in pairs equal the constant (secondary spermatocyte) number there should then be one number missing; and this missing number would be that which is just half of the constant total being divided (since by assumption there are no equal divisions of the group). In table 1 there is not actually any such gap, but there is a marked deficiency in the class containing 9 centrioles. The members of this class are so few that it is justifiable to regard them as resulting from equal allotment of the centrioles (a fact that is, of course, evident also from the circumstance that this is the only class represented by both large and small cells); and hence these cells are exceptions to the general rule of unequal apportionment of centrioles to the spermatids; and they may be disregarded, i.e., considered as amounting in effect to the gap in the series, as postulated above. Nine must then represent one-half of the total number of secondary spermatocyte centrioles, and we accordingly again conclude that that number is exactly 18.

Since the data in table 1 thus fully agree with the number that is expected if each of the 9 centrioles of the first spermatocyte group divided once in late telophase—which direct observation supports—it seems beyond reasonable doubt that the number of centrioles in each secondary spermatocyte is always exactly 18; that in four sister spermatids there are 36 centrioles, which in the course of spermatogenesis were derived by a single division of 18 centrioles that were earlier present as two equal groups of 9 each in the primary spermatocyte.

With the centriole and chromosome numbers that have been indicated above it is possible to make a highly suggestive comparison between the behavior of centrioles and that of chromosomes. In the typical normal first spermatocyte there are *two* centrioles. These normal centrioles divide once in the first maturation to give 4, one of which enters each spermatid and there functions as a blepharoplast. Comparing the atypical first spermatocyte with the typical normal one, in the former there are *18 centrioles*, in other words, there are *16 extra centrioles*. The haploid number of normal chromosomes is 9, the diploid 18. In the normal late spermatocyte there are 9 tetrads—equivalent to *18 normal dyads*, or to 36 normal chromatids. In the atypical line, by contrast—as indicated by the very specific chromosome behavior in the atypical second maturation division, whereby each spermatid receives one chromosome (single chromatid) —there are but 4 of the original 36 chromatids that function normally in the maturation divisions and remain intact in the spermatid. This idea of four normal chromatids is further supported by the observation of a single tetrad at diakinesis, and possibly also by the events at anaphase of division one. *This leaves then 32 chromatids, originally in the form of 16 dyad-chromosomes, that are abnormal.*

This coincidence of the occurrence of *16 abnormal dyads* and the simul-

taneous presence of *exactly 16 extra centrioles* is too striking to be lightly dismissed, especially since this is only one of a large number of cases where it has been demonstrated that increase in centriole number is accompanied by this same sort of abnormal chromosome behavior. Expressed very simply it looks as if 16 chromosomes have each lost something, while the cytoplasm has at the same time gained 16 units, the extra centrioles. That the centriole is intimately involved with the chromosomes has long been evident from the fact that centrioles grow and divide only immediately after they have been in close relation with the chromosome complement, i.e., located at the poles of the spindle—a generalization about centrioles to which these cases of atypical gasteropod spermatogenesis have hitherto seemed to offer the only real objection. Schrader,[3] 1936, and Darlington,[4] 1937, have recently called attention to numerous similarities between the centrioles and the kinetochore, or spindle fibre attachment point, of the chromosome. The failure to assume normal relation with the spindle is a feature of these abnormal chromosomes in the atypical spermatogenesis of Vivipara and of other prosobranch snails, which is precisely the sort of behavior that would be expected if there were a marked disturbance (complete absence?) of the normal kinetochore function. The increase in number of centrioles and the abnormal chromosome behavior in the atypical spermatogenesis of Vivipara are both adequately explained if one assumes that the kinetochore masses of 16 dyad-chromosomes have been transferred from nucleus to cytoplasm, where each has given rise to a perfectly normal centriole that is in no way distinguishable from the two already present there and always present in each typical normal cell.

[1] F. Meves, *Arch. f. mikr. Anat.*, **61**, 1–84(1902).
[2] J. Morita, *Fol. anat. Jap.*, **10**, 35–51(1932).
[3] F. Schrader, *Biol. Bull.*, **70**, 484–498(1936).
[4] C. D. Darlington, *Recent Advances in Cytology*, Blakiston, Philadelphia (1937).

AN ERGODIC THEOREM FOR n-PARAMETER GROUPS

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Let G stand for either the whole of Euclidean n-space or those points $\alpha = (a_1, \ldots, a_n)$ for which $a_i \geqslant 0$. Let T_α $(\alpha \in G)$ be a linear transformation in a Banach space $\mathfrak{B}$, with $\|T_\alpha\| \leq C$, $T_{\alpha+\beta} = T_\alpha T_\beta$, $(\alpha, \beta \in G)$. We suppose that for each x in $\mathfrak{B}$ the set of points $T_\alpha x (\alpha \in G)$ is a separable subset of $\mathfrak{B}$ and that for each $\bar{x} \in \bar{\mathfrak{B}}$ (the space conjugate to $\mathfrak{B}$) and x in $\mathfrak{B}$ the numerical