Acta vet. scand. 1960, *1*, 140—160.

From the Department of Obstetrics and Gynaecology, Royal Veterinary College, Stockholm.

THE SPERMIOCYTOGENESIS OF THE BULL

By

O. Knudsen and *N. Bryne.*

Morphological changes in the spermiogenic epithelium have previously been studied mainly by routine histological methods. During the last few years it has become apparent that disturbances in cell division and chromosomal changes in the spermiogenic epithelium can produce a reduction in fertility in cattle (*Knudsen* 1954, 1956). *Krallinger* (1931), *Makino* (1944) and *Melander-Knudsen* (1953) described spermiocytogenesis in the bull from the cytomorphological aspect. Comparative histological and cytogenetical investigation give however a much clearer picture of the course of events in spermiocytogenesis, and in the present work an attempt has been made to illuminate spermiocytogenesis from both cytogenetical and histological aspects.

When a cell divides homologous chromosome complements are placed in opposite halves of the mother cell. From the moment of division of the mother cell's cytoplasm by a cell membrane, two new cells are formed. If these are of another type than the mother cell, the new cell type's name has been used from the moment of formation. On the basis of this, different cell types in the spermiogenic epithelium will be described.

MATERIAL AND METHODS

During a period of five years testicular material was collected from a total of 42 bulls, from 7 months to 8 years of age. Before taking the preparations the bulls stayed in the same environment at least 7 months. The preparations were taken by the methods described by *Knudsen* (1954). The material was fixed in acetic alcohol (1:3), or in Carnoy (*Romeis* 1948). Squash preparations

were stained with Gomory's haematoxylin, according to *Melander-Wingstrand* (1953). 5 μ sections were stained with Gomory's haematoxylin and fast green according to *Knudsen* (1954). In some cases, however, it was necessary to acidify the fast green with 5—10 ml. acetic acid to 100 ml. stain solution. The preparations were examined in a phase-variator. An electron microscope was used in studies of the finer structure of the chromosomes.

RESULTS

The spermiocytogenesis may be divided in three different phases:

I. The divisions of the A-spermiogonia, ensuring a permanent supply of primal cells for spermiogenesis.

II. The divisions of the B-spermiogonia, starting the spermiogenic wave.

III. The divisions of the primary and secondary spermiocytes bringing about the important reduction of the chromosome number (meiosis).

Each cell type includes a resting stage (interphase or interkinesis) and the stages of the cell division prophase, metaphase, anaphase and telophase. With the exception of the spermiogonial interphase stage the chromosomes in the bull are visible during the whole of spermiocytogenesis and their morphology varies from stage to stage. It is thus possible to determine the stage of cell division in a germinal epithelial cell by studying its chromosomes.

In the present work each stage of cell division is demonstrated in three ways 1) by a photograph from a squash, 2) by a photograph from a section and 3) by a schematic picture. In the schematic pictures only two chromosome pairs are shown, one with uninterrupted and the other with dotted lines. The centromere is represented by a small circle and the centrosome by a dot. Note that with the technique used the centromere is not visible in squash and sections. Seven of the squash pictures are taken from *Melander-Knudsen.*

With the technique used here, four different cell types are to be found during spermiocytogenesis (the cell divisions of spermiogenesis) i.e. A-spermiogonia (Fig. 1, A), B-spermiogonia (Fig. 1, B), primary (Fig. 1, I) and secondary spermiocytes (Fig. 1, II).

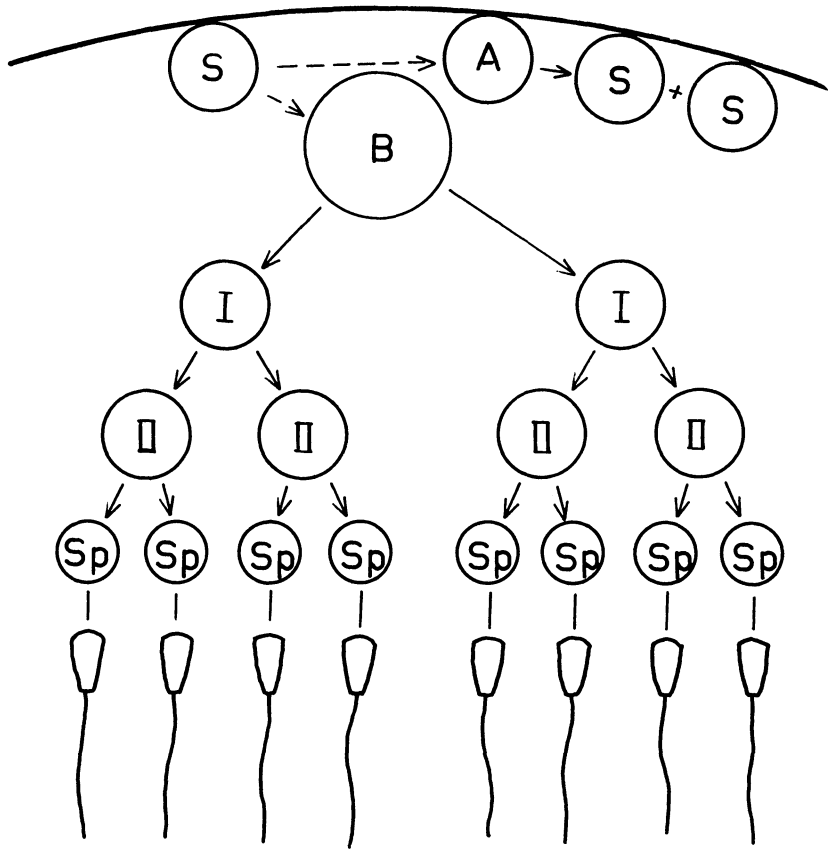

Fig. 1. S, resting spermiogonia cell (A- or B-spermiogonium). A, A-spermiogonium. B, B-spermiogonium. I, Primary spermiocyte. II, Secondary spermiocyte. Sp, Spermid.

Resting A- and B-spermiogonia cells (Figs. 1, S, 2 and 3) have the same morphology until the chromosomes become visible when cell division starts. They are always placed close to the basal membrane of the Tubulus seminiferous.

The A-spermiogonium (or "spermatogonium") divides mitotically like non-germinative cells, and this occurs independently of the spermiogenic wave. The A-spermiogonium by division gives rise to two new resting spermiogonia, either A- or B-spermiogonia.

The B-spermiogonium also undergoes mitotic division, but this differs considerably from that in other cell types. By division it gives rise to two primary spermiocytes.

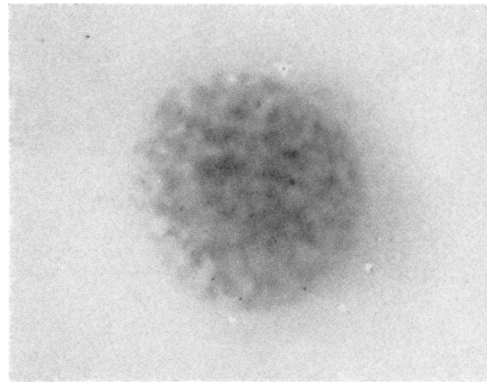

Fig. 2. Resting spermiogonia cell. Squash (approx. 2000 ×).

The primary spermiocyte (or "spermatocyte" or "primary spermatocyte") undergoes the first meiotic division and gives rise to two secondary spermiocytes.

The secondary spermiocyte (or "secondary spermatocyte" or "praespermatid") undergoes the second meiotic division and by division it gives rise to two spermids ("spermatids"). Each spermid (Fig. 1, Sp) transforms to one sperm ("spermatozoa").

The A-spermiogonium is always situated immediately inside the basal membrane in the Tubulus seminiferous. The B-spermiogonium during its first stages is also situated close to the basal membrane, but during cell division it may be displaced somewhat in towards the lumen of the tubulus. More centrally in the tubulus the primary and secondary spermiocytes, spermids and sperms are found lying in this order as near the basal membrane as space permits.

A-spermiogonium.

The diameter of the A-spermiogonium during interphase, prophase and metaphase is about 12 μ, and the nuclear diameter during interphase and prophase is about 8 μ.

Interphase. During interphase no chromosomes are visible in the spermiogonia cells (Figs. 2 and 3). The nucleus is finely granulated and usually entirely spherical. In its centre lies the nucleolus as a sharply contoured sphere.

The division of the A-spermiogonium is demonstrated in Fig. 4.

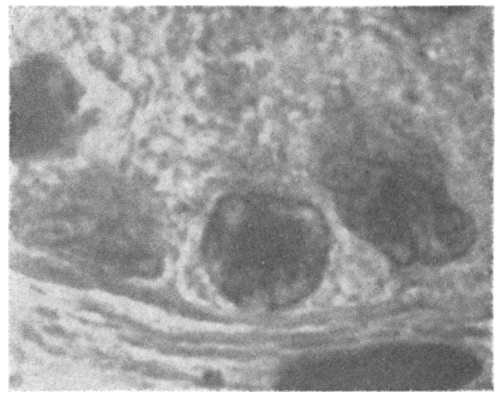

Fig. 3. Resting spermiogonia cell. Section (approx. 2000 ×).

Prophase. The prophase begins when the chromosomes become visible. The chromosomes divide longitudinally very early, each daughter half of the chromosomes being a chromatid (Fig. 4, k and l). The chromatids form two spirals of equal size which are inserted in each other from the side and joined at one point, the centromere, which is concerned with chromosomal movement to the poles. In cattle it is not possible with the technique used to observe this division as the chromatids are so close to each other or in each other (Fig. 4, a and b). In the electron microscope, however, a spiralisation is visible which can be differentiated as a double spiral. In cattle the centromere is situated at the one end of all the chromosomes (telocentric). During the whole prophase the chromosomes become shorter and thicker, which is considered to be due to a continually increasing spiralisation of the chromonemata (the thread-like protein skeleton of the chromosomes), while chromatin is deposited on this skeleton. The chromosomes are rod-shaped during the whole course of A-spermiogonial division, and are practically uniformly thick. In late prophase, however, they become somewhat narrower at the centromeral end (Fig. 4, b).

Metaphase. When the nuclear membrane disappears and the nuclear spindle becomes visible, the cell enters the metaphase stage. The A-spermiogonial spindle forms a double somewhat blunt or right-angled cone about 4 μ diameter in the equatorial plane. The spindle of the A-spermiogonium with the methods used stains less intensely than that of the primary and secondary spermiocytes.

A-Spermiogonium

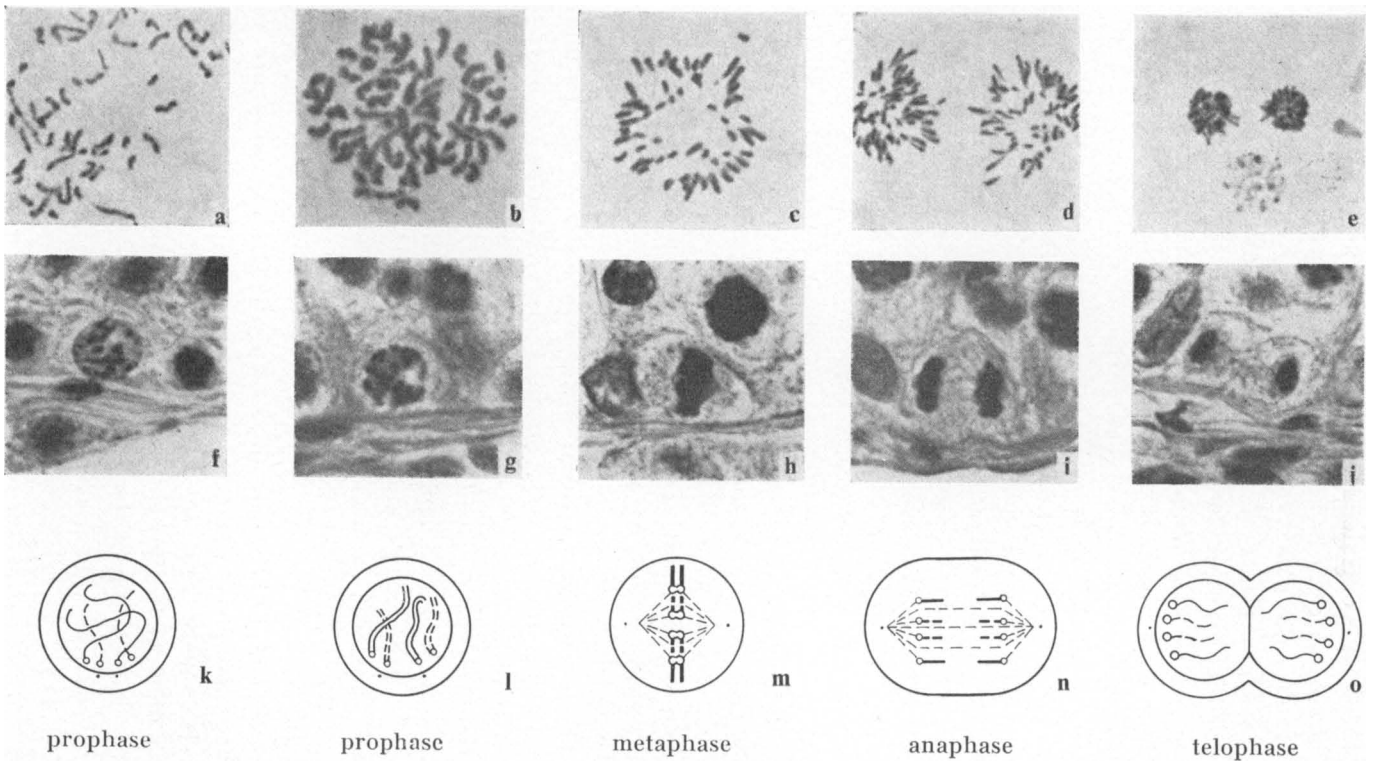

Fig. 4. Squash (upper row), section (middle row) and schematic figures of the different stages in the A-spermiogonium (approx. 2000 ×).

Only occasionally the present authors have been able to observe points outside the ends of the spindle which could be interpreted as centrosomes. Therefore it seems as if the A-spermiogonial centrosomes must be particularly small or be situated very near the ends of the spindle. No astrosphere has been observed in this or any other stage of cell division during spermiogenesis in the bull. When the spindle becomes visible the centromere of each chromosome organizes a spindle fibre which is connected with the poles. The chromosomes become orientated to the spindle's equatorial plane (congression). During congression, before the chromosomes have reached the equatorial plane, these may be placed over much of the spindle which therefore can be difficult to detect. When the chromosomes have reached the equatorial plane in full metaphase, they lie at right angles to the spindle axis with the centromeral ends nearest this. The large chromosomes lie furthest out and the small furthest in and in this manner form a plate.

When the chromosomes are orientated in the equatorial plane (Fig. 4, h), they are maximally contracted (Fig. 4, c). They are rod-shaped and evenly thick or somewhat narrower at the centromeral ends and have a smooth surface.

In metaphase they are easily counted. The chromosome number in cattle is 2n=60 and appears to be very constant in the spermiogenic epithelium of healthy bulls.

The longitudinal division of the chromosomes (see prophase) is completed during the final stage of the metaphase when the centromeres divide. All the chromosomes divide synchronously in these cells.

Anaphase. The anaphase stage covers the period of chromosomal distribution to the poles of the cell. When the chromosomes in late metaphase have divided, the daughter chromosomes are transferred each to its pole with the assistance of the spindle fibres.

The spindle is much more elongated during anaphase than during metaphase. The distribution of the chromosomes occurs synchronously. The chromosomes, still rod-shaped, pass to the poles more or less parallel to the spindle fibres, with the centromeral end leading in the direction of motion.

Telophase. When the two chromosome groups have reached the poles of the cell the telophase stage begins and continues until the cell has been divided by a central constriction. When

the lengthened cell is constricted in the middle the spindle becomes progressively narrower in the constricted region. In the telophase the A-spermiogonial spindle axis of normal bulls is always parallel to the basal membrane. As seen in fig. 4, j this results in both daughter cells being situated close to the basal membrane (*Knudsen* 1954).

The chromosomes become progressively longer and narrower during the telophase and often appear to be disintegrating. This is due to lessening of the spiralisation tension of the chromonemata and to loss of chromatin off the chromonemata.

When the cell has divided, the new-formed cells enter the interphase stage. Whether or not these cells are A- or B-spermiogonia cannot be decided before a new cell-division has commenced and the chromosomes again become visible.

B-spermiogonium.

The B-spermiogonium differs in size, chromosome morphology and spindle morphology from other cell types. Its metaphase and anaphase stages are particularly ephemeral and to obtain these stages in a preparation necessitates very rapid fixation of the testicular material.

The B-spermiogonium in the interphase (Figs. 2 and 3) has a diameter of approx. 12 μ, and the nucleus of approx. 8 μ. During the whole prophase the size of the B-spermiogonium increases to reach a diameter of approx. 23 μ in full metaphase. In the late prophase the diameter of the B-spermiogonial nucleus is about 16 μ.

Interphase. The interphase stage of the B-spermiogonium is morphologically identical with the same stage in the A-spermiogonium (Figs. 1, 2 and 3).

The division of the B-spermiogonium is demonstrated in fig. 5.

Prophase. The chromosomes undergo a very marked contraction during the prophase and remain contracted until the primary spermiocytes are formed. This contraction begins at one end of the chromosomes, presumably the distal end, the opposite end from the centromere (Fig. 5, a, b, k and l). In early prophase the rod-shaped chromosomes thus have a knob at the distal end. The knob becomes progressively larger during the prophase while the rod-shaped part becomes increasingly shorter and thicker. This can be explained by the "ordinary" spiralisation of the chromonemata and the deposition of chromatin on these,

B-Spermiogonium

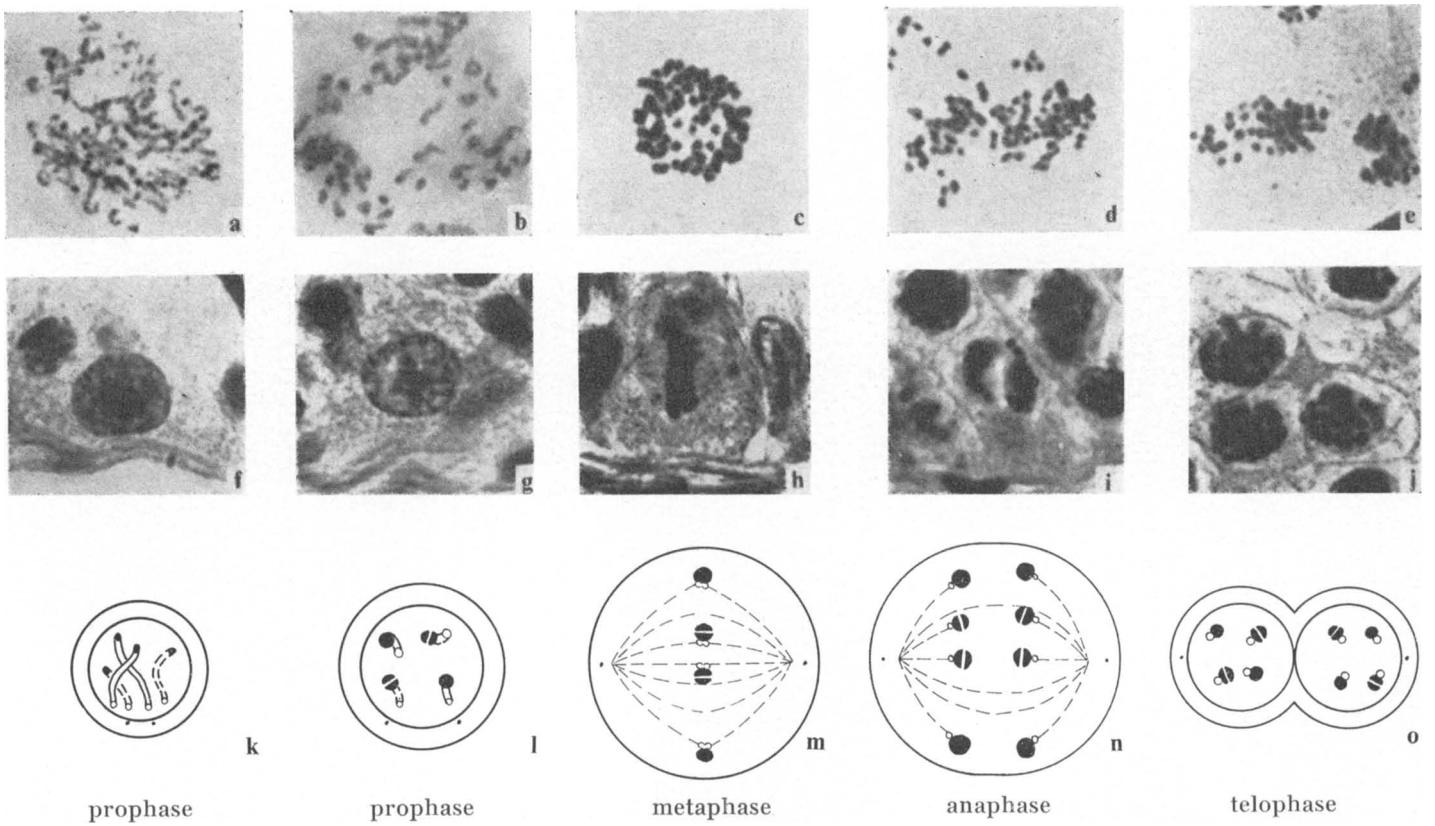

Fig. 5. Squash (upper row), section (middle row) and schematic figures of the different stages in the B-spermiogonium (approx. 2000 ×).

at the same time as more and more of the chromosome is included in the knob. The knob or the "extra" contraction of the chromosomes may be explained as an extra spiralisation (*Knudsen* 1954). Because of the large size of the cell, only relatively infrequently are sections obtained through the sagittal plane. Naturally sections in other planes do not contain the full diameter of the cell. Even in such cases however the cell type is easily identified by the knobbed chromosomes. If the sections is in the sagittal plane, a relatively large and often irregular nucleolus can be seen during the prophase. During the later part of the prophase the chromosomes often lie just inside the cell membrane (Fig. 5, g).

Metaphase. The spindle is fusiform and is approx. 17 μ from pole to pole. Its diameter in the equatorial plane is approx. 11 μ (Fig. 5, h). The spindle stains well with fast green.

The B-spermiogonial centrosomes are visible in an ordinary light microscope as small points situated immediately beyond the spindle apices (Fig. 5, h).

During metaphase the chromosomes reach full contraction and are more or less spherical. The chromosomes are rapidly orientated to the equatorial plane. Attached to the spindle fibres they lie on the outside of these (Fig. 5, h and m) and are thus orientated as in an ordinary mitosis.

Anaphase. The spindle stains poorly with the stains used and is therefore often invisible.

The chromosomes are still maximally contracted. Their distribution to the poles of the cell occur synchronously though this can be difficult to demonstrate in thin sections as the spindle and the chromosomes in this large cell are easily displaced by the microtome.

Telophase. During the telophase only fragments of the spindle can be demonstrated.

The chromosomes are still maximally contracted. In contrast to telophase chromosomes in other cell types, those of the B-spermiogonium do not lie collected at the respective poles, but they are widely spread in their respective cell halves (Fig. 5, j). No despiralisation of the chromosomes has been observed during this telophase stage in the bull.

Primary spermiocyte (Figs. 6 and 7).

The daughter cells of the large B-spermiogonium, the primary spermiocytes, have a diameter of approx. 14 μ, and the nuclear diameter is approx. 8 μ.

The prophase stage is very protracted and complicated and is divided into five sub-stages, i.e. leptotene, zygotene, pachytene, diplotene and diakinesis. The metaphase has a prae-stage which is called praemetaphase.

Interphase. The first stage of the primary spermiocyte, the interphase between mitosis and meiosis, is of variable duration in different cells. As all B-spermiogonia in one area do not divide simultaneously the resulting primary spermiocytes are formed successively. In contrast the change from the interphase to the leptotene stage occurs nearly simultaneously in all primary spermiocytes in the same tubular section. Thus the interphase nuclei formed first await those formed later.

In early interphase the chromosomes are contracted and have the same spherical form as in the final B-spermiogonial stages. Immediately before the leptotene stage this contraction is lost and then it is possible to detect the few short and wide spirals in the chromosomes (Fig. 6, a). The tension in these spirals disappears rapidly and simultaneously in cells situated in the same part of a tubule. In this way the pronounced synchronisation begins, which is characteristic of the spermiogenic wave. When the tension in the chromosome spiral has disappeared the chromosomes are made up of long, thread-like structures and the meiosis starts.

During preparation of sections the contracted spherical chromosomes of the B-spermiogonium and the primary spermiocyte are often displaced outside the cell.

Leptotene. The centrosomes appear to affect the centromeres even during the leptotene stage as the centromeral ends of the chromosomes become orientated towards a definite point at the nuclear membrane outside which it may be assumed that the probably already divided centrosome lies. The long thin leptotene chromosomes thus become arranged in a bouquet. During the leptotene stage the chromosomes are not longitudinally divided as during the early mitotic prophase. At the distal end of each chromosome a small knob can be observed (Fig. 6, b. Note that

the black dots are knobs and not centromeres). The chromosomes contract during the whole prophase and become increasingly shorter and thicker with the progress of the prophase.

Zygotene. During the zygotene stage pairing of homologous chromosomes occurs, i.e. the chromosome of paternal origin comes to lie beside that of maternal origin, and in this manner a bivalent is formed. The zygotene nucleus is thus characterised by paired and unpaired chromosomes, which can easily be observed in squashes with an ordinary microscope (Fig. 6, c. Bivalents are thicker and denser than univalents). The chromosomes are rod-shaped but somewhat shorter and thicker than in the leptotene. They are arranged in a bouquet which becomes increasingly evident as the chromosomes become shorter and thicker (Fig. 6, h, i, j).

Pachytene. During the pachytene pairing has been completed. Now a marked chromomere structure appears. This means that each chromosome in a bivalent is provided with a large number of large or small nodes, chromomeres, arranged in a specific order for each chromosomal pair (Fig. 6, d). It is considered that the chromomeres are of the utmost importance in the pairing of homologous chromosomes. Corresponding chromomeres come to lie in each case exactly beside each other. The chromomeres are concerned with the location of the genes.

During the pachytene a longitudinal division of the chromosomes occurs. The two chromatids from the original chromosome are however still intimately united along their whole length. The chromosomes are held together by chiasmata. A chiasma is understood to be the result of an exchange of chromatid segments between both paired chromosomes in a bivalent. Chiasma formation occurs as follows. One chromatid in each of the two paired chromosomes are broken at exactly the same point. The breakage surfaces formed unite so that the proximal chromatid part in one chromosome unites with the distal part of that in the other chromosome. In this way several chiasmata are usually formed in each bivalent. The chiasmata serve two functions, one is to permit the exchange of genes between homologous chromosomes (crossing-over) and the other is to hold together both chromosomes of the bivalent. Chiasma formation is not visible in pachytene in the bull with the technique used.

The bouquet formation is very obvious in the pachytene with

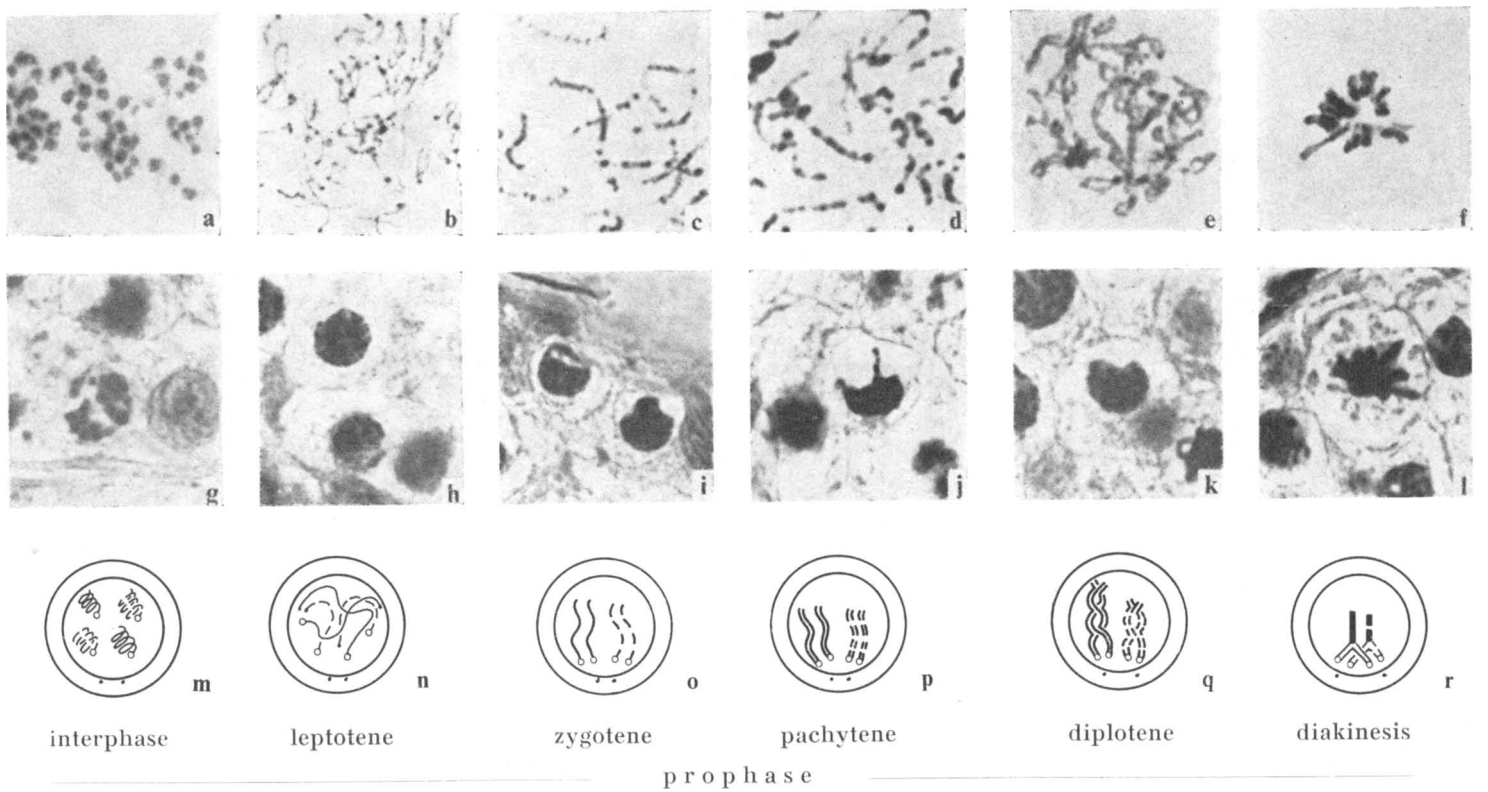

Fig. 6. Squash (upper row), section (middle row) and schematic figures of the different stages in the primary spermiocyte. h and i, two cells in the leptotene and zygotene respectively (approx. 2000 ×).

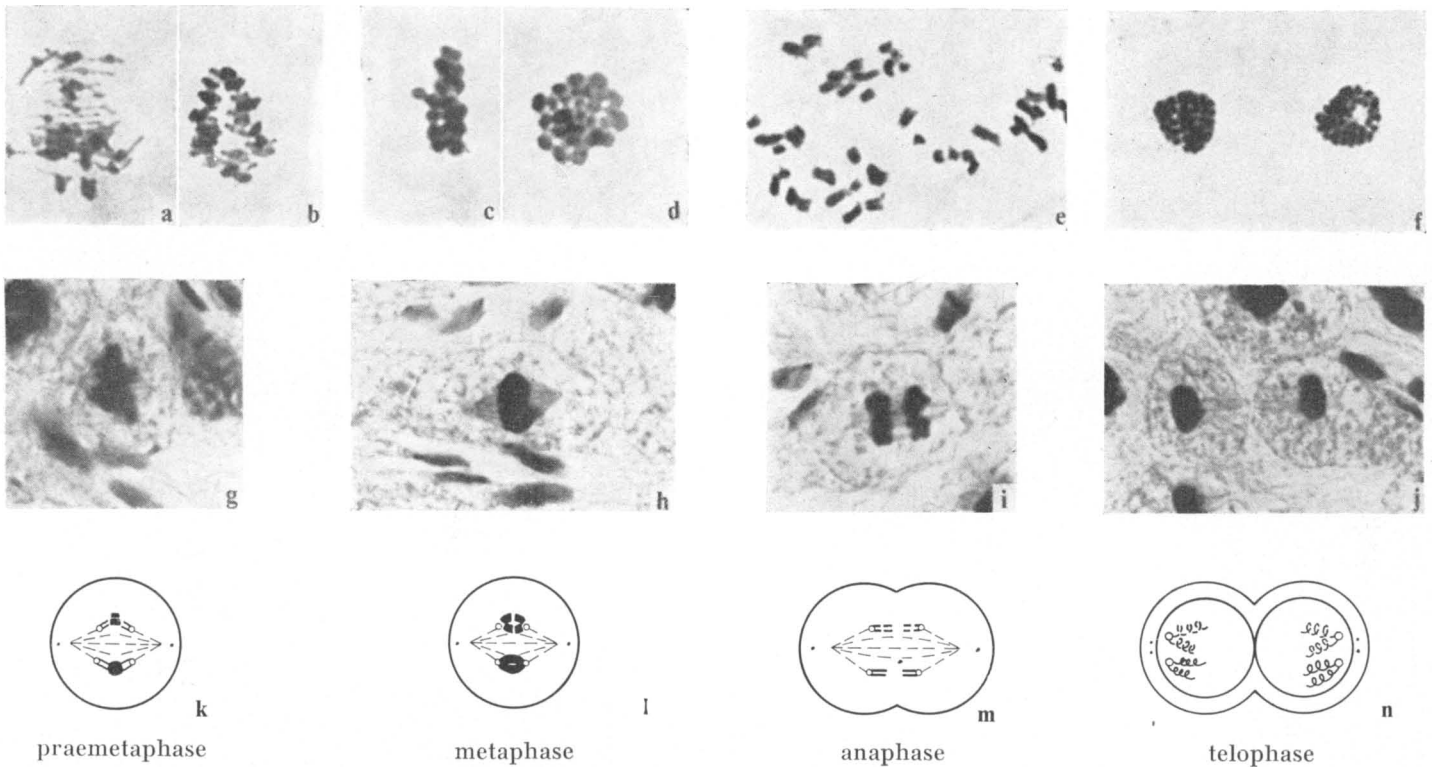

Fig. 7. Squash (upper row), section (middle row) and schematic figures of the different stages in the primary spermiocyte. a, an early stage of congression. b, late stage of congression. c and d, two different projections of a metaphase plate (approx. 2000 ×).

the chromosomes situated in one half of the nucleus. It is also characteristic that the part of the nucleus lacking chromosomes is particularly deficient in structure (Fig. 6, j).

Diplotene. In the diplotene the chromosomes in the bivalents are dragged apart but held together by the chiasmata. This gives the bivalents a chain like appearance (Fig. 6, e).

Diakinesis. During diakinesis, the last stage of the prophase, the centromeral ends of the homologous chromosomes separate and so form two arms, while the distal end of the bivalent is still contracted and appears to be a homogenous body. The length of the centromeral arms is dependant on where the most proximal chiasma is situated. Although the nuclear membrane is still present it appears that the centromeral ends of the chromosome stretch towards the centrosomes which are already migrating to diametrically opposed poles outside the nuclear membrane.

Praemetaphase. When the nuclear membrane disappears and the spindle becomes visible, the cell enters praemetaphase. The spindle is made up of a double cone, slightly acute-angled in both praemetaphase and metaphase, but somewhat more pointed during the latter. The spindle's length is approx. 14 μ and the diameter in the equatorial plane approx. 13 μ (Fig. 7, g).

The centrosomes are well marked, point-like formations lying immediately outside the spindle apices. The centrosomes are apparently very prone to degenerative changes in various diseases in animals (*Knudsen* 1954).

Praemetaphase includes congression of the chromosomes, which differs in the first meiotic division in principle from that in other cell divisions. Each bivalent has two centromeres each of which holds together two chromatids (Fig. 7, k). By co-orientation (*Östergren* 1951) the centromeral arms are stretched each to one pole of the cell. The distal end of the bivalent contracts and becomes a short rod or clump. At the same time the centromeral arms become shorter and seem to be drawn into the contracted bivalent part which is held together by chiasmata (Fig. 7, a and b). This would appear to be due to the general contraction of prophase chromosomes as described previously. During praemetaphase the chromosomes are orientated towards the spindle's peripheral parts and thus form a ring (Fig. 7, b).

Metaphase. When the contraction, which began at the distal ends of the bivalents, reaches its maximum the bivalents appear

to be spherical (Fig. 7, c and d). Occasionally these spherical structures may appear to be hollow.

The chromosomes during the metaphase are arranged in a plate (Fig. 7, c and d) in the equatorial plane (Fig, 7, h). Each bivalent has its diametrically opposed centromeres attached to one spindle fibre and each directed to one pole of the cell (Fig. 7, l). Thus the bivalents lie along the spindle fibres and not outside these, as in the metaphase stage of other cell types. This may be of importance in differentiating the first meiotic division from other types of metaphase in certain cases of pathological spermiogenesis. During the metaphase of the first meiotic division no division of the centromeres occurs. The chiasmata formed which hold both chromosomes in the bivalents together are displaced towards the distal ends of the chromatids (terminalisation). When terminalisation is complete both chromosomes in the bivalents are released from each other and the cell goes into the anaphase.

Anaphase. During anaphase the cell's length and also the spindle's length may vary extensively. The centrosomes may still be seen as pointlike structures.

During the anaphase each chromosome has *one* centromere and *two* chromatid arms, which differentiate it from other anaphase chromosomes. The centromeral end appears cut off while it is possible to distinguish both chromatids at the distal end (Fig. 7, e). Distribution to the poles is particularly rapid. It occurs synchronously except for the x and y chromosomes, which occasionally go before the other chromosomes. The chromosomes are attached to the spindle fibres by their centromeres, and their long axis are parallel to the spindle fibres.

Telophase. During the telophase the cell becomes constricted in the middle between both chromosome groups. During late telophase the spindle is visible running through the small residual canal which connects both daughter halves of the cell (Fig. 7, j).

The chromatid arms of the chromosomes once more undergo a contraction during telophase. When this contraction is complete the chromosomes look like two small touching spheres, which are presumably held together by the centromere (Fig. 7, f). The chromosomes are well collected at the poles of the cell (Fig. 7, j).

The Secondary Spermiocyte (Fig. 8).

The nucleus of the secondary spermiocyte is spherical. It is somewhat larger than the nucleus in the primary spermiocyte and has a diameter of approx. 10 μ. The cytoplasmic body often varies in shape but if it is spherical it has a diameter of approx. 16 μ.

Interkinesis. During interkinesis, the stage between the two meiotic divisions, the contraction of the chromatids, which began in the preceding telophase, reaches its maximum (Fig. 8, k). In certain cases it is possible to observe a spiral structure in the chromatids of the interkinesis chromosomes. This is especially marked towards the end of interkinesis. The tension in this extra spiral appears to be released from the centromeral ends of the chromatids, while it remains in the distal ends of these throughout the prophase.

Prophase. In the prophase the chromosomes have a characteristic knob at the distal end of each chromatid, while the rest of the chromatid is rod-shaped (Fig. 8, b). The knob may be explained as a continuation of the interkinesis contraction of the chromatids. The rod-shaped part becomes shorter and thicker during the prophase. The chromatids are often situated far from each other, especially in squash preparations. No bouquet arrangement of the prophase chromosomes has been observed. They are spread throughout the nucleus (Fig. 8, g).

Metaphase. When the nuclear membrane disappears and the spindle becomes visible, congression occurs rapidly. The spindle is a very pointed, double cone, in which the spindle axis is approx. 15 μ long and the diameter in the equatorial plane approx. 6 μ (Fig. 8, h). The centrosomes may also be observed in the secondary spermiocytes as small points, situated immediately outside the spindle apices.

In early metaphase or at the end of the congression it is occasionally possible to observe some residual knob-formation on the distal chromatid ends of the chromosomes. The chromosomes, which are attached to a spindle fibre by the centromere, lie outside the spindle at right angles to the spindle axis. Both chromatid arms thus lie beside each other but not so close that no space is visible between them (Fig. 8, c). The majority of chromosomes lie outside the spindle and form an obvious ring (Fig. 8, h the left cell). No division of the chromatids occurs

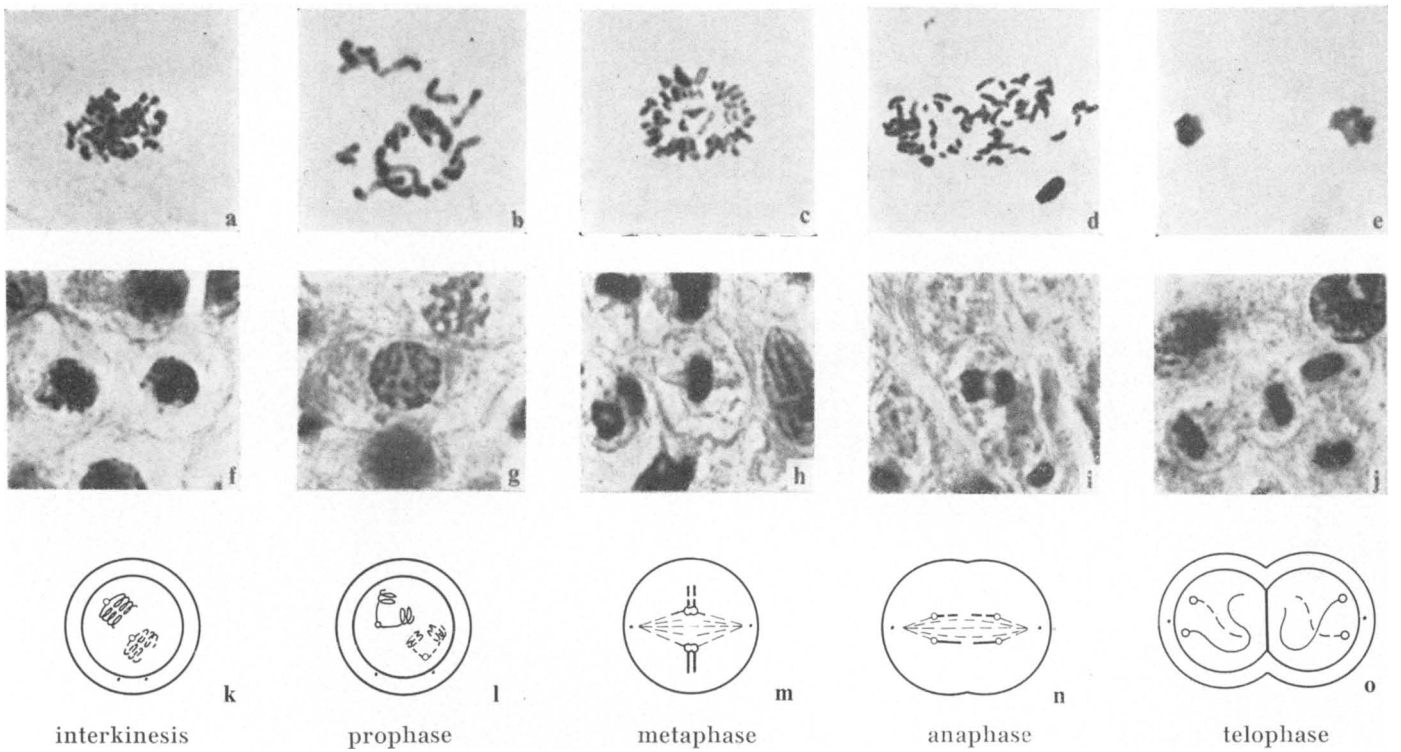

Fig. 8. Squash (upper row), section (middle row) and schematic figures of the different stages in the secondary spermiocyte. f, note how the two interkinesis cells are united by a cytoplasmic bridge. j, two telophase cells just dividing. The right one is older than the left (approx. 2000 ×).

during prophase and metaphase. The metaphase ends when the centromeres divide.

Anaphase. During the anaphase the spindle can be very long but appears to contract and become shorter in the telophase. The chromosomes are now all rod-shaped (Fig. 8, d). Their distribution to the poles occurs synchronously.

Telophase. During telophase the cell is constricted in the middle and the spindle compressed in a small canal between both cell halves (Fig. 8, j).

In the secondary spermiocytes, as in the primary spermiocytes it has not been possible to determine whether complete division of the cell occurs or not.

During telophase the chromosomes become longer and thinner and appear to undergo disintegration. Towards the end of the telophase they are not easily observed (Fig. 8, e).

In the secondary spermiocytes, in contrast to other cell types, no longitudinal division of the chromatids occurs, but division of the centromeres does occur. Reduction of the chromosome number thus occurs during both the meiotic divisions, that is, in both the primary and secondary spermiocytes. In the primary spermiocyte there is no division of the centromeres and in the secondary spermiocyte there is no division of the chromatids. In this way the chromosomes divide once while the cell divides twice and thus the chromosome number is reduced in the spermid.

DISCUSSION

Spermiocytogenesis consists of a series of cell divisions. On the basis of the frequency of the different stages of cell division, it is possible to obtain an idea of the duration of each stage, provided that the tissue has been fixed very rapidly (*Knudsen* 1954). There are considerable variations in duration between the different cell stages. Thus different efficiencies of fixation are required for the study of individual stages of cell division. For the study of e.g. the B-spermiogonial metaphase or any anaphase stage, very small pieces of tissue must be removed quickly from in situ and placed in rapid fixative agent. For certain stages the maximum time for this procedure is said to be 1 minute (*Knudsen* 1954). On the other hand, if one wishes to study the pachytene it appears that tissue which has not been

fixed until 2 days after removal may be used without disadvantage.

A cytogenetic investigation based on the study of squashes is preferable to sections in certain cases of disturbances in fertility, e.g. intrachromosomal aberrations. In other cases, e.g. nuclear spindle disturbances (*Knudsen* 1954) sections are necessary. Electron microscopy would appear to offer great possibilities for the future study of the finer structure of the chromosomes. In the case of a more intensive morphological study of spermiocytogenesis, the problem should be approached with both squashes and sections. Thus the aim in the present work has been to illustrate the various stages of cell division in spermiocytogenesis by a combination of both cytogenetics and histology, and thus facilitate the understanding of the normal events in this part of gamete formation in the bull.

For a discussion of the morphology of the spindle and the chromosomes in the A- and B-spermiogonia and in the primary spermiocytes reference may be made to *Melander-Knudsen* and *Knudsen* (1954).

In the secondary spermiocyte prophase a knob formation is described. This knob becomes apparently smaller with the course of the prophase but may in some cases be discerned in metaphase. This progressive reduction in size of the knob has been interpreted as a despiralisation from the centromeral end of the chromatids.

Burgos and *Fawcett* (1955) in electron microscope studies of spermiohistogenesis found cytoplasmic bridges between several different spermids and they suggested in discussion that complete division of the preceding primary and secondary spermiocytes could not have occurred. The present investigations do not contradict the opinion of these authors (see, for example, Figs. 7, j and 8, f).

After finishing this work a historical account of the studies of chromosomes in bull by *Postiglioni-Grimaldi* (1957) appeared. He also maintains that in order to investigate the spermiocytogenesis in animals it is necessary to use comparative histological and cytogenetical methods.

REFERENCES

Burgos, M., Fawcett, D. W.: J. Biophys. Biochem. Cytology, 1955, I, 287.
Knudsen, O.: Acta pat. microbiol. scand. 1954, Suppl. CI.
Knudsen, O.: Fortpflanzung, Zuchthygiene und Haustierbesamung, 1956, 6, 5.
Krallinger, H. F.: Arch. Tierernähr. Tierz. 1931, Abt. B, 5.
Makino, S.: Cytologia, Tokyo, 1944, 13, 247.
Melander, Y., Knudsen, O.: Hereditas, Lund, 1953, XXXIX, 505.
Melander, Y., Wingstrand, K. G.: Stain Tech. 1953, 28, 217.
Postiglioni-Grimaldi, J.: II Congr. Nacional de Vet., Montevideo, 1957, I, 659.
Romeis, B.: Mikroskopische Technik, München (Leibniz), 1948, § 226.
Östergren, G.: Hereditas, Lund, 1951, XXXVII, 85.

SUMMARY

Each stage of cell division during spermiocytogenesis in the bull may be determined by its characteristic chromosome morphology. In the present work each stage of cell division is demonstrated by a schematic picture, one light microscope photo from a squash preparation and one from a section, and in this way an attempt has been made to illuminate spermiocytogenesis from both cytogenetical and histological aspects.

ZUSAMMENFASSUNG

Die Spermiozytogenese beim Bullen.

Jedes Zellteilungsstadium während der Spermiozytogenese beim Bullen lässt sich auf Grund der charakteristischen Chromosomenmorphologie identifizieren. In der vorliegenden Arbeit wird jedes Zellteilungsstadium mit einer schematischen Zeichnung, einer lichtmikroskopischen Photographie eines Quetschpräparats und einer solchen eines Schnittpräparats dargelegt, um auf diese Weise die Spermiogenese sowohl vom zytogenetischen als auch vom histologischen Gesichtspunkte zu beleuchten.

SAMMANFATTNING

Spermiocytogenesen hos tjur.

Varje celldelningsstadium under spermiocytogenesen hos tjur kan identifieras på grundval av dess karakteristiska kromosommorfologi. I föreliggande arbete är varje celldelningsstadium demonstrerat med en schematisk teckning, ett ljusmikroskopiskt foto från squashpreparat och ett från snittpreparat för att på detta sätt söka belysa spermiocytogenesen från både cytogenetisk och histologisk synpunkt.

(Received October 14. 1958).