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ULTRASTRUCTURE OF THE MATURE SPERMATOZOA AND THE PROCESS
OF SPERMIOGENESIS IN THE COCKROACH, NAUPHOETA CINEREA
(DICTYOPTERA: BLATTARIA: BLABERIDAE)

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

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************

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Ultrastructure of Spermatozoa of the Cockroach, Pycnoscelus
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FIELDS OF STUDY

Major Field: Zoology

Studies in Electron Microscopy: Dr. Wayne B. Parrish

Studies in Entomology: Dr. Frank W. Fisk
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INTRODUCTION

The fine structure of spermatozoa of almost all species of insects has been studied extensively and there is voluminous literature on the subject. Spermiogenesis, the development of spermatids into spermatozoa, has also been the subject of several investigations, more so after the introduction of the electron microscope. In 1959 Gatenby and Tahmisian included one of the first electron microscope observations of an insect spermatozoon. Prior to that most of the investigations were aided by light and phase microscopes, and these revealed mostly the gross structure of the spermatozoa. Recent studies deal with points of finer detail, such as transformation of the various structures within the spermatid cell into more definite complex structures in the sperm. Electron microscopy has made it possible to follow the step by step development from a spermatogonium to spermatids, and the differentiation of the spermatids into mature spermatozoa.

With the help of the electron microscope the studies on spermatogenesis of several species of animals, including insects, have increased tremendously. Considering the existence of such a vast number of cockroach species
(3,500 known species), one would expect numerous fine structural investigations on spermatogenesis in these insects, and a tremendous amount of variation may be expected from species to species. Furthermore, cockroaches are one of the earliest insects known to exist (as early as Carboniferous period) and, since they have evolved through so many generations and have yet retained most of their earlier characteristics, it would be interesting to know if their sperm structure has likewise been conserved. However, few authors have described the sperm of the cockroach, and fewer still have studied and described the process of spermatogenesis in the cockroaches. Nath, Gupta and Sehgal (1957), and Nath (1965), gave a morphological description of the sperm of Periplaneta americana (L.). Richards (1963) described the giant and normal spermatozoa in Periplaneta americana. Frola and Norman (1964) worked on the morphology and the motility of the spermatozoa of Byrsotria fumigata (Guerin); Hughes and Davey (1969) reported on the activity of Periplaneta; Eddelman et al. (1970) described the spatulate acrosome in Periplaneta americana. Lindsey and Biesele (1970) described the development of centriole adjunct in Eublaberus posticus; Liu (1973) described the ultrastructural changes in the spermatid nuclear envelope of Periplaneta americana. Shahaney et al. (1972) described the sperm of Pycnoscelus indicus (Fabricius). Most of these studies stressed the morphology of the sperm, and very
little work was done on spermatogenesis.

Spermatogenesis in the Acridids, which are closely related to the cockroaches, has been more extensively studied. A review of investigations on these species have helped in understanding the development of sperm in cockroaches. Beams et al. (1954) and later Tahmisian, Powers and Devine (1956) described the development of the mitochondrial derivatives in spermatozoa of the grasshopper, *Melanoplus differentialis* Thoma. Yasuzumi et al. (1958) studied the grasshopper *Gelastorhinus bicolor* de Haan. Gatenby and Tahmisian (1959) reported on the centriole adjunct in Orthopteran spermatogenesis. Dass and Ris (1958) studied the organization of the nucleus during spermatogenesis in the grasshoppers, *Chortophaga vividifasciata* (De Geer), *Chorthippus centipennis* (Harris) and *Romalea microptera* (Beauvois). Kaye (1962) described the acrosome formation in the house cricket, *Acheta domesticus* (L.). The development of the microtubular system during spermiogenesis in the grasshopper *Melanoplus differentialis* was followed by Kessel (1967). Shay and Biesele (1968) observed spermiogenesis in the cave cricket *Centhophilus secretus* (Scudder). Szollosi (1975) described the spermiogenesis in *Locusta migratoria*.

Besides the review of investigations on the Acridids, studies of spermatogenesis of various other species of insects have also aided the studies on spermiogenesis in

Besides the above investigations, the study of sperm and their development in species other than insects have also aided in understanding the development of the sperm of the cockroach. Reger (1964; 1966) has described and compared spermatogenesis in isopods, Assellus militaris (Hay), Oniscus asselius and the amphipod Orchistoidea sp. Yasuzumi (1962) worked on the Cipangopaludina malleata. Anderson (1967) studied spermiogenesis in Lumbricus terrestria. Berard (1974) described spermiogenesis in Daphnia magna. Atwood (1975) described the sperm of echinoderms.

Finally, general reviews of development in various species described by Nath (1965), Davey (1965), Franzen (1966), Phillips (1970; 1974), Fawcett (1975), and Baccetti
and Afzelius (1976) have tremendously helped in the description and developmental studies of the cockroach sperm.

As mentioned earlier, since not much has been described in detail on any particular cockroach spermatozoa, it was felt necessary to obtain a better and more detailed account of the development of the cockroach sperm. In 1972, I reported a description of the sperm of the cockroach, *Pycnoscelus indicus* (Shahaney et al., 1972). At that time the development of the spermatozoa was not pursued. For the present study, a closely related species, *Nauphoeta cinerea*, has been selected for further study. This research includes a description of the mature sperm, its development within the testis, and the development of the various organelles. The description of the mature sperm of *Nauphoeta cinerea* is almost identical to that of *Pycnoscelus indicus* (Shahaney et al., 1972), except for the unusual characteristic of dilations in the nucleus which are peculiar to the latter species. The description of mature sperm is included mainly to help in understanding the development of various parts of the sperm. Like most insect sperm, the sperm of the cockroach, *N. cinerea*, consists of a head and a tail—the head being no wider than the tail. The head consists of the acrosome and the nucleus, and the tail consists of the mitochondrial derivatives and the axial filament which extend almost the entire length of the tail. A centriole
adjunct serves to join the head to the tail, and is basically a part of the tail. The centriole adjunct extends from the base of the nucleus posteriorly into the tail for a short distance.

The latter part of this research includes spermiation, during which the spermatid produces a tail and the chromatin or nuclear material greatly condenses; the nucleus elongates and parts of the mature spermatozoon develop from the organelles of the spermatid, while excess cytoplasm is discarded. In this transformation from spermatid to mature sperm there is a very complex and orderly series of events, but it is practically impossible to obtain a complete series of all stages. Hence the present observations are confined mainly to the development of various individual structures, even though it is evident that the events are synchronous and linked. A brief summary of the synchronous development of the organelles, aided by diagrams, follows the development of the following structures: acrosome, nucleus, centriole adjunct, mitochondrial derivatives, and the axial filament. The summary, for convenience is divided into five consecutive stages, each stage showing the synchronous development of the above named structures.

The information provided below on the description and development of the sperm of *Nauphoeta cinerea*, which is a fairly complete study, not hitherto pursued to such an
extent on any other cockroach sperm, should enhance further studies on sperm of other cockroaches and should serve as a basis for comparative studies. Furthermore, insight may be gained by these studies as to how the cockroach has managed to survive through more generations than has any other insect.
METHODS AND MATERIALS

Nauphoeta cinerea specimens were obtained from cultures established in laboratories of The Ohio State University.

Smears of sperm were made for light microscopy and sections of testes and seminal vesicles from nymphs and adult cockroaches were made for both light and electron microscopy. Thick sections (1μ) were used for light microscopy, whereas thin sections (0.05 - 0.1μ) were used for electron microscopy.

Smears. Male cockroaches, both adults and nymphs, were anesthetized with CO₂ and then dissected in cockroach saline (Appendix I). Testes and seminal vesicles were removed and tissues were individually placed on glass slides and squashed with the help of coverslips. The smeared slides were dried over vapors of glacial acetic acid for five minutes, and, without being further air-dried, were stained in Delafield's Hematoxylin. Some smears were counterstained with Eosin. All slides were passed through a graded series of ethanols, cleared in xylene, mounted in Piccolyte and observed under the light microscope. Photomicrographs of whole sperm were taken and diagrams were drawn to depict the full structure of the sperm.
Thick and Thin Sections for Light and Electron Microscopy. Male cockroaches, both nymphs and adults, were anesthetized with CO₂ and dissected. The testes and seminal vesicles were removed and immediately immersed in cold 5% Glutaraldehyde prepared in phosphate buffer, pH 7.2 (Appendix I). While in this fixative, though already fairly small, tissues were cut further into smaller pieces approximately 1mm³ to allow penetration of the fixative into the tissues. After fixation for four hours, tissues were washed in phosphate buffer wash, pH 7.41 (Appendix I) overnight, after which they were post-fixed in cold 1% osmium tetroxide (OsO₄) (according to Millonig) for two hours. After post-fixation, tissues were dehydrated in a graded series of ethanols, then passed through mixtures (ratios 3:1, 1:1, 1:3 absolute ethanol to Spurr's low-viscosity resin, Appendix I), and finally in full strength resin in which they were left overnight to remove any traces of alcohol. The tissues were then embedded in the resin in plastic molds. The molds were first filled with pure resin, then the tissue pieces, one per mold, were carefully placed in the resin and allowed to sink to the bottom of the mold. Care was taken to avoid any air bubbles in the resin. When the tissues had sunk to the bottom, the molds were placed in the oven (70°C) overnight, during which time the resin hardened enough to enable easy sectioning. The resin blocks were then removed from the molds with the aid of pliers or a razor blade. The
faces of the blocks were trimmed to trapezoid shapes of fairly small sizes. This was accomplished by gently trimming the excess resin with sharp new razor blades, with the aid of a cycloptic stereoscopic microscope.

Glass knives were made with an LKB Knifemaker, and thick and thin sections were cut on the Sorvall Porter Blum MT2 Microtome. Thick sections (approximately 1μ) were picked up on glass slides onto a drop of distilled water. The slides were heated gently over a moderately warm hot plate until the water on the slide dried and the sections adhered to the slides. A drop of Toluidine blue stain (Appendix I) was placed over the sections and the slides again warmed. When the drop of stain dried the slides were carefully washed with distilled water and dried again over the hot plate. Sections were then ready to be observed under the light microscope. Photomicrographs were taken of large areas of the testes and the seminal vesicles.

Thin sections of tissues (0.05 - 0.1μ thick) were cut for electron microscopy. The interference colors of thin sections varied from silver (0.05μ) to gold (0.1μ). These thin sections were transferred from the knives onto 200 mesh copper grids. Some copper grids were previously coated with a parlodion film (1.75% parlodion in Amyl Acetate) and with a layer of carbon in a Mikros VE10 Vacuum Evaporator. The sections on the grids were stained with Uranyl Acetate
(Appendix I) for 15 minutes, then rinsed with 50% ethanol followed by a distilled water rinse. Excess water was removed with the help of filter papers. Sections were then counter-stained with Reynold's lead citrate (Appendix I) for five minutes, washed in 0.01 NaOH, and finally rinsed in distilled water. The stained grids were then viewed with an RCA EMU-3G electron microscope. Pictures were taken on Dupont 711 Cronar film. The negatives were developed as per the instructions by the manufacturers of the film and were printed on Kodabromide photographic paper F-3, F-4 or F-5. Pictures were taken at magnifications ranging from 2,000x to 32,000x (Appendix II).
OBSERVATIONS
PART I
ULTRASTRUCTURE OF THE MATURE SPERMATOZOA

The sperm of the cockroach, Nauphoeta cinerea, which is approximately 200µ long and roughly 1µ wide, is divided into two principal parts, the head and the tail (Shahaney et al., 1972). The head is that portion of the sperm which consists of an elongated nucleus capped by a small acrosome (Plate 1). The tail is the portion extending from just behind the nucleus to the posterior tip. The tail consists of a centriole adjunct, axial filament, and two elongated mitochondrial derivatives. The tail may be further divided into three regions: the neck, main-piece and end-piece.

Head. The head of the sperm, measuring approximately 25µ in length and 1µ in width, is elongated and needle-like, consisting of an anteriorly placed small acrosome (Plate 1) followed by an elongate nucleus which occupies the entire head space. The acrosome and the nucleus are enclosed within the plasma membrane which is continuous with the membrane enclosing the structure within the tail.

Acrosome. The acrosome (Plate 1) measures about 3µ in length and is approximately one-eighth the length of the
Plate 1. Diagrammatic representation of a spermatozoon

a - f represent cross sections of the sperm at various levels.

A - F represent longitudinal sections at various levels.
nucleus. It is conical in shape tapering anteriorly. The wider end of the acrosome caps the nucleus. Ultrastructurally the acrosome (Figs. 1-3) is an electron dense mass except for its center which is electron transparent (Fig. 3). Separating the nucleus from the acrosome is a post-acrosomal ring (Fig. 3) which is set into a depression at the anterior end of the nucleus. A fibrous acrosomal rod (Fig. 2) extends through this ring, anteriorly into the electron transparent space in the acrosome, and posteriorly into the anterior invagination in the nucleus.

Nucleus. The nucleus is readily distinguishable due to its intense staining. It appears electron opaque and no detailed structures within it are visible. It occupies almost the entire length of the head, and measures approximately 22μ in length (Plate 1). It is elongate and somewhat cylindrical, and in cross section is bilaterally symmetrical. There are two prominent grooves, one on either side of the long axis (Fig. 4), extending the entire length of the nucleus. It also has an anterior and a posterior medial groove extending to a short distance. The anterior groove accommodates the acrosomal rod (Fig. 2) while the posterior groove houses the centriole or the basal body (Figs. 7, 38) which is continuous with the axial filament in the tail.
**Tail.** The tail occupies about seven-eighths the length of the entire sperm, measuring about 175µ in length and about 1µ in width. It is subdivided into the neck, main piece and end-piece (Plate 1).

The neck region can be distinguished by the presence of a centriole adjunct, an organelle which serves to join the head to the tail. Anteriorly, the centriole adjunct encloses the axial filament and the mitochondrial derivatives; posteriorly it diminishes into a partial sheath (approximately 3µ beyond the base of the tail) and completely disappears after a short distance within the main-piece of the tail (Plate 1). The main-piece, which comprises most of the tail area contains the axial filament and the mitochondrial derivatives. The end-piece, which comprises the extreme tip of the tail, consists of the area beyond the mitochondrial derivatives where only the irregularly arranged fibres of the axial filament remain (Plate 1).

**Centriole Adjunct.** The centriole adjunct is an electron dense mass, which begins at the base of the nucleus and extends posteriorly around the mitochondrial derivatives and the axial filament (Plate 1). Closer to the nucleus, the centriole adjunct completely surrounds the mitochondrial derivatives and the axial filament (Fig. 5) but posteriorly it becomes a partial sheath (Fig. 6) which
gradually diminishes in size (Fig. 6) as it extends posteriorly for some distance until it disappears completely (Fig. 10).

**Centriole.** The centriole, now called the basal body, begins in the posterior invagination of the nucleus and continues into the axial filament in the tail forming a centriole-axial filament complex while confined within the nuclear invagination (Fig. 38). Extended beyond the nuclear invagination it becomes the axial filament.

**Axial Filament.** The axial filament extends posteriorly from the base of the nucleus to the caudal tip of the tail. The centriole-axial filament complex has the basic 9+2 pattern of most cilia and flagella, that is, an inner centrally located pair of fibers and an outer circle of nine doublet tubules (Fig. 8), each doublet consisting of subfiber A and subfiber B, the former bearing a pair of arms. After emerging from the nuclear invagination, it becomes the axial filament and acquires an additional row of nine tubules (Fig. 8), now acquiring a 9+9+2 pattern of tubules. Each of the nine additional tubules is located distal to, but towards subfiber B of the doublet (Diagram below). These tubules referred to as accessory tubules, are electron opaque. Between the accessory tubules, and along the same radius as the doublet tubules, are pairs of fine fibers, each fine fiber connecting with the adjacent subfiber. The fine fiber closer to subfiber B also connects
with the adjacent accessory tubule. From the central pair of tubules extend radial spoke-like projections towards the doublets (Diagram).

The axial filament loses this pattern posteriorly in the end-piece of the tail. The individual tubules thus end abruptly (Fig. 9).

**Mitochondrial Derivatives.** The two elongate mitochondrial derivatives lie on either side of the axial filament. They begin at the base of the nucleus and within the body of the centriole adjunct (Plate 1) and extend posteriorly through the end of the main-piece of the tail.

In cross section (Fig. 10) the mitochondrial derivatives appear eggshaped with the narrow end facing the axial filament. Each mitochondrial derivative in cross section shows: (a) a crystalloid portion; (b) an area occupied by cristae and (c) an electron transparent space. Two-thirds of the total area of the mitochondrial derivatives proximal to the axial filament is occupied by the crystalloid which
appears roughly triangular in cross section. The crystalloid portion is divided into two equal longitudinal halves by a homogeneous dense band and consists of regularly repeating sub-units showing a crystalline array (Fig. 10). The osmophilic subunits that make up the crystal are hexagonally arranged. The cristae (Fig. 11), occupying the remaining one third of the mitochondrial derivatives, distal to the axial filament, lie perpendicular to the elongate structure. Between the crystalloid and the cristae is a transparent zone which is not bound by any limiting membrane, but apparently there are fine filaments located within it.
PART II

THE TESTES

The testes of the cockroach Nauphoeta cinerea are paired organs located laterally between the fifth and sixth abdominal segments. Each testis, embedded in a matrix of fat cells, consists of a number of follicles around a central duct, the vas deferens. The testes are covered by a simple epithelium, and each follicle again is covered by its own simple epithelium. The follicles contain many cysts, each of which consists of a clone of germinal cells embedded in large polyvalent epithelial cells (Baccetti and Bairati, 1964; Cantacuzene, 1968). The gonial and meiotic divisions are synchronous within a given cyst, and the cytoplasmic bridges that result from incomplete cytokinesis in these divisions make each clone a functional syncytium (Baccetti and Bairati, 1964; Hoage and Kessel, 1968).

The testes develop continuously throughout the nymphal stages and reach their maximum size in the sixth instar, becoming smaller in the adult. In the first instar the testes divide into compartments. In the second instar, the testes contain numerous spermatogonia, and in the third
instar the testes increase in size. By the fourth instar the spermatogonia undergo meiotic divisions, resulting in primary and secondary spermatocytes, which further divide into spermatids. In the last nymphal stage, mature sperm are abundant, and various stages of development are still observed, including the presence of some spermatogonial cells. The testes of adult cockroaches contain an abundance of mature sperm, several early and late spermatids, some spermatocytes and few spermatogonia. The overall testis size in adult cockroaches is greatly reduced.

A cross section of a testis from a late nymphal stage or from an adult cockroach shows sections of several follicles. Various developmental stages of the sperm may be observed in a single cross section.

Plate 2 shows:

a. Spermatogonia
b. Primary and secondary spermatocytes
c. Early spermatids showing nebenkern and acroblast formation
d. Late spermatids showing elongation of cell parts and condensation of the nucleus
e. Mature spermatozoa.
Plate 2. Diagrammatic sketch of a cross section of the cockroach testis. Various stages of development of the sperm can be seen within a follicle of the testis.
PART III

DEVELOPMENT OF THE COCKROACH SPERMATOZOA

A very orderly sequence of events takes place during spermiogenesis and at any one stage each organelle is in a definite developmental stage. Development of each structure has been dealt with separately even though it is evident that the synchronous events are linked. A brief description of development of various organelles of the spermatids of the cockroach Nauphoeta cinerea is given below.

Acrosome

There are several dictyosomes scattered in the cytoplasm of early spermatids. These come together towards one end of the nucleus, and form a single Golgi body, now called the acroblast (Figs. 12-14). The acroblast is semi-circular in shape and caps a part of the nucleus. An incomplete double walled membrane encloses the acroblast. This membrane appears to be a continuation of the smooth endoplasmic reticulum from the surrounding cytoplasm. The portion of the acroblast facing the nucleus consists of a row of large electron dense granules (Figs. 13, 14). The nuclear membrane lying adjacent to these granules is thickened, giving an appearance of electron dense granules
The outer margin of the acroblast, which is convex in shape, consists of a row of vesicles immediately enclosed within the membrane (Figs. 13, 14). The center of the acroblast appears to be homogeneous and resembles the ultrastructure of the nucleus. Outside the acroblast several scattered dictyosomes may still be seen (Figs. 12, 14).

The acroblast caps the nucleus in the vicinity of the nebenkern (aggregation of mitochondria in a form of spherical mass) and the axial filament (Fig. 13). The axial filament at this stage can be seen indented in the nucleus (Fig. 40), or it can occasionally be seen projected out of the nuclear indentation (Fig. 13).

The acroblast is first seen when the nebenkern is formed and can still be seen in the same state even after the nebenkern has transformed into two individual mitochondrial derivatives, thus indicating that it persists in that state for a long period. When the mitochondrial derivatives begin to elongate, the dense granules of the acroblast aggregate and with accretion of more dense material a large electron dense granule is formed (Figs. 15, 16). This granule is termed the proacrosome. As the cell elongates the proacrosome rotates towards the anterior end and the axial filament is now on the opposite or the posterior end (Fig. 17). Excess cytoplasm begins to slough
off as the proacrosome appears closer to the plasma membrane and, concomitantly, the surrounding dictyosomes begin to disappear (Figs. 16-17). The proacrosome now acquires a spherical shape and appears to have a double membrane (Fig. 18). The proacrosomal membrane facing the nucleus thickens as does the outer membrane of the facing nucleus. Between these two thick membranes appears a new membrane which becomes the acrosomal plate (Fig. 18).

The proacrosome begins to elongate, and the portion of the proacrosome facing the nucleus invaginates forming a cavity, the main axis of which is parallel to the elongating structure (Figs. 19; 20). This concavity increases in height forming a conical structure with the sides being electron opaque and the center electron transparent (Figs. 20, 21). The acrosomal plate remains dense where the acrosome is in contact with the nucleus. The central concavity and electron transparent area do not show the presence of the acrosomal plate. Therefore the acrosomal plate now appears to be an acrosomal ring (Figs. 3, 22). In the vicinity of the acrosomal invagination the nucleus also invaginates, and within this space, a fairly electron dense acrosomal rod forms (Fig. 20). The acrosomal rod extends through the acrosomal ring into the electron transparent space in the proacrosome to form the acrosome.

As the cell elongates further so does the acrosome until the final length (approximately 3μ) is acquired,
which is approximately one-eighth the length of the nucleus.

**Nucleus**

In the early spermatid the nucleus is spherical with a distinct double membrane (Fig. 25). The nuclear contents are finely fibrillar with several heterochromatic patches, and nucleoli scattered at random (Figs. 23-25). There are several inclusion bodies, some of which are electron dense (Fig. 23), other are granular (Fig. 24), or fibrillar (Fig. 24), while some are coiled ribbonlike structures (Figs. 23 and 25).

During spermiogenesis the spermatid nucleus becomes ovoid in shape and the heterochromatic patches migrate from the periphery towards the center of the nucleus, where the chromatin is more diffuse and homogeneous. The nucleolus disintegrates and its ribonucleoprotein contents are expelled through the nuclear pores into the cytoplasm. The DNA content of the nucleolus is retained within the nucleus. Condensation of the chromatin begins along the periphery of the nucleus (Fig. 26) and progresses inwards until the entire nuclear mass appears to be coarsely granular (Fig. 27). The nucleus now begins to elongate (Fig. 28) and microtubules can be seen encircling the outer surface of the nucleus, and lying parallel to the elongating nucleus (Fig. 27). As the nucleus elongates, the chromatin becomes more organized; the coarse granules fuse with each other
to form a coarse fibrous mass (Figs. 29 and 30). At the periphery the coarse fibers become denser by fusion of adjacent fibers to form dense patches (Figs. 31, and 32). During this condensation and dehydration of the nucleus, lateral grooves (Fig. 31) begin to form, and these are retained in the mature spermatozoa. The fusion of chromatin fibers progresses inwards, and in the center of the nucleus the fibers can be seen fused together to form sheetlike structures (Fig. 32). At the same time some electron transparent spaces may be seen interspersed between the sheetlike structures and the distinct fibers (Fig. 31). As condensation and elongation progresses, the fibers and sheetlike structures fuse together; all vacuole or transparent spaces fill up with condensed chromatin, thus reducing the overall size of the nucleus. The nuclear mass is finally condensed into a compact mass in which no further structures are discernible. The nuclear membrane loses its nuclear pores and there is no more exchange between the nucleus and the cytoplasmic contents. The final nuclear shape being elongate and needle-like, and a length of approximately 22μ is acquired. When condensation and elongation is complete, the microtubules disappear.

**Centriole Adjunct**

The centriole adjunct first appears as a granular organelle surrounding the basal body, or the beginning of the axial filament, which has just begun to elongate and
emerge from the nuclear invagination (Fig. 33). At first the centriole adjunct is cylindrical and contains regularly arranged granules (Fig. 33). These granules appear to be more prominent near the nucleus. A distinct space demarcates the centriole adjunct from the nucleus (Figs. 33 and 34).

As differentiation and development of the sperm proceeds, the microtubules, which were observed around the nucleus, extend posteriorly to encircle the centriole adjunct (Fig. 36) and the regularly arranged granules begin to condense and the organelle becomes more compact (Fig. 34); some irregularly distributed spaces are formed and these are retained for some time (Figs. 34-38). These spaces or vacuoles are still observed when the centriole adjunct begins to elongate and envelopes the axial filament and the mitochondrial derivatives (Fig. 38). The spaces eventually disappear as the centriole adjunct elongates further and completely envelopes the axial filament and the mitochondrial derivatives at the anterior end which lies immediately behind the base of the nucleus (Fig. 37). Caudally the compact centriole adjunct extends for a short distance becoming a partial sheath around the axial filament and the mitochondrial derivatives, and gradually reduces in size further posteriorly until, after a short distance, it disappears completely. With the completion of elongation
and condensation of the centriole adjunct, the microtubules disappear.

**Axial Filament**

In the late spermatocyte two centrioles have been observed lying at right angles to each other (Fig. 39), but in the spermatid only one centriole is retained, the fate of the other is unknown. The single centriole of the early spermatid, now called the basal body, is destined to become the axial filament of the flagellum. At first the basal body is located in the periphery of the cell, but later it moves towards the nucleus and eventually indents into the nuclear mass (Fig. 40). At this stage it shows the basic 9+2 pattern of the tubules, with two central tubules being single and empty and the outer ones being doublets (subfiber A and subfiber B). Subfiber A of the doublet is usually smaller than subfiber B and bears a pair of armlike extensions.

The basal body, indented into the nucleus, is usually seen in the vicinity of the acroblast and the nebenkern (aggregation of mitochondria to form a spherical mass). Later, either the nucleus rotates or the acroblast moves away, and the basal body appears to be on the end opposite the acroblast (Fig. 17). The basal body then extends out from the nuclear indentation (Fig. 41) and is usually seen to be enclosed within a cytoplasmic ampulla (Fig. 42). This
is formed by the projection of the axial filament into the cytoplasm until it reaches the plasma membrane. The plasma membrane envelopes the flageller tip and invaginates into the cell and around the axial filament until it reaches a position close to the base of the flagellum where it evaginates out in close proximity with the projecting flagellum (Fig. 42). The invaginated plasma membrane at its innermost fold is lined by a dense material forming a structure that corresponds to the "ring centriole" (Fig. 42).

In the axial filament the subfiber B of each doublet forms curved arms which eventually join together to form a triplet (Fig. 36). The newly formed third tubule of each triplet later separates from the doublet to form the accessory tubule which is at first empty and later a dense core forms within, giving it an electron dense appearance (Fig. 8). Spoke-like projections arranged radially from the central fibers to the doublets then appear between the central fibers and the doublets (Fig. 8).

By this stage the mitochondrial derivatives have oriented on either side of the axial filament with endoplasmic reticulum extending between them. Cisternae of endoplasmic reticulum encircle the axial filament forming a sheath around it (Fig. 44). Several microtubules lie at random on either side of the axial filament (Fig. 59). The collapsed cisternae around the axial filament lie in contact
with the mitochondrial derivatives and dense areas develop on either side (Fig. 59). The sheath around the axial filament finally disappears but the densities between the axial filament and the mitochondrial derivatives are retained (Figs. 59, 61).

Residual cytoplasm is eventually sloughed off until the tail consists only of the axial filament and the mitochondrial derivatives.

In the posterior tip of the tail the basic 9+2 pattern is lost, doublets disappear first, followed by the remaining tubules. The tubules may be observed within the plasma membrane dispersed at random, without any pattern (Fig. 9).

Mitochondrial Derivatives

Mitochondria of various sizes and shapes appear scattered within the spermatocyte cytoplasm, mostly along or attached to the elongated endoplasmic reticulum. At an early stage of differentiation of the spermatid all mitochondria begin to cluster at one pole of the cell (Figs. 45, 46). They subsequently aggregate at one end, near the nucleus, in a single mass called the nebenkern (Fig. 47). The nebenkern at first has an irregular shape (Fig. 47), but soon becomes spherical or ovoid, with the mitochondria being loosely connected (Fig. 48). Among these loosely connected mitochondria a chromidial body (Fig. 48) may usually be seen, the purpose of which is not known. The mitochondria
begin to elongate, branch, and intertwine to form a compact spherical or ovoid mass. In this compact mass the mitochondria organize concentrically in an onionlike configuration (Fig. 50), being separated from each other by narrow, constant cytoplasmic spaces. A cross section through this nebenkern shows large mitochondrial profiles concentrically arranged (Fig. 50), those in the center being more tightly fused, resulting in a fairly electron dense mass compared to those on the periphery. The cristae in the individual mitochondria are still intact, though they may appear to have reorganized according to the shape of the nebenkern (Fig. 50). The mitochondria then begin to fuse with each other until eventually two large masses appear to adhere to each other with a narrow ridge of cytoplasm separating the two masses (Figs. 51, 53). These two hemispherical masses then separate and orient themselves on either side of the axial filament (Figs. 55, 56). During this separation, islands of cytoplasm seem to be trapped within the nebenkern halves (Figs. 55, 56), but these disappear when the nebenkern masses elongate. The cristae at this stage are still present, giving the nebenkern masses an appearance of large, original type of mitochondria (Fig. 57). The mitochondrial masses now become dense, thin, irregular in shape, and limited by a double membrane (Fig. 57). A row of microtubules encircling the mitochondrial surface, and arranged
parallel to the long axis of the elongating cell, appear
(Figs. 58, 59) and elongation of the mitochondrial masses
begins. As elongation proceeds, the mitochondrial masses
acquire a definite shape, which in cross section, appears
ovoid with the narrow ends extending away from each other,
and the broader ends lying fairly close together (Fig. 58).
The axial filament can be seen oriented centrally between
the two narrow ends of the elongating mitochondrial masses
(Fig. 58). With further elongation, crystallization begins
at the narrow ends of the mitochondrial masses (Fig. 58).
Once the crystallization begins, the mitochondrial masses
are called the mitochondrial derivatives. Crystallization,
beginning at the narrow ends, gradually progresses inwards
towards the wider ends (Fig. 59), until it covers almost
two-thirds of the total mitochondrial derivatives (Figs.
60 and 61). The cristae, which at first were evenly
scattered, resembling those of the original mitochondria,
are now pushed by the growing crystalloid towards the wider
end where they orient themselves perpendicular to the long
axis of the mitochondrial derivatives and can only be
observed in longitudinal sections (Fig. 11). A clear space
forms between the crystalloid and the cristae (Fig. 11), and
this probably is the remaining matrix in which occasionally
fine microtubules may be observed.
The mitochondrial derivatives elongate further until they extend almost to the caudal tip of the tail. They narrow towards the posterior tip and end shortly before the axial filament loses its basic pattern in the end-piece of the tail.

The microtubules surrounding the mitochondrial derivatives disappear when elongation is complete.

**Microtubules**

During spermatid development, microtubules have been seen to encircle the nucleus (Figs. 27-32), mitochondrial derivatives (Figs. 59, 60), centriole adjunct (Figs. 36, 37) and the axial filament (Figs. 60, 61) during the process of nuclear condensation and elongation of various structures. Microtubules are seen to be directed parallel to the long axis of the elongating structures. They first appear at the onset of the elongation process and disappear after the nucleus is fully condensed and the mitochondrial derivatives, centriole adjunct, axial filament and the nucleus are fully elongated.

**Intercellular Bridges**

In insects it is very common to see groups of spermatogonia, spermatocytes, or spermatids connected by intercellular bridges. Early in the meiotic divisions, the cells usually complete cytokinesis, but in some instances, at some point in development, the products of meiotic divisions
do not separate completely (Fig. 62). The cells continue to differentiate and form mature spermatozoa. Figure 63 shows several sperm tails enclosed within a common plasma membrane.

**Cytoplasmic Sloughing**

In spermatozoa of most animal species, the volume of spermatids is reduced several fold during spermiogenesis. Ribosomes, endoplasmic reticulum, vesicles, Golgi complexes and microtubules are lost during the process. This process occurs in absence of a lysosomal system which is generally associated with cytoplasmic degradation in other cell types. In insects, large areas of cytoplasm, apparently containing unwanted structures, are eliminated by pinching off from the spermatid, and these masses may be observed floating free in the cyst lumen (Fig. 64).
PART IV

SYNCHRONOUS DEVELOPMENT OF SPERM ORGANELLES

The development of acrosome, nucleus, centriole adjunct, axial filament and the mitochondria was described individually in the previous section. As we know, this development is synchronous and linked. Looking at a section of a spermatid at any stage will show development of all the above named structures at a particular stage. Not all structures begin and end their development at the same time. The time lapse of all the events has not been pursued, but it is interesting to note how the development of one structure has progressed in relation to that of the other. A series of diagrammatic sketches, for convenience divided into five stages, depicts the development of each structure.
PLATE 3

DIAGRAMMATIC SKETCH OF A DIFFERENTIATING SPERMATID AT STAGE 1

A. Mitochondria beginning to aggregate at one end of the cell.
B. Nuclear chromatin homogenous showing heterochromatic masses and a nucleolus.
C. Two centrioles visible in the cytoplasm.
PLATE 4

DIAGRAMMATIC SKETCH OF A DIFFERENTIATING SPERMATID AT STAGE 2

A. Mitochondria forming a nebenkern.
B. Nuclear condensation begins along the periphery.
C. Acrosome formation begins - acroblast visible.
D. Centriole or basal body comes closer and indents into nucleus.
Plate 4
PLATE 5

DIAGRAMMATIC SKETCH OF A DIFFERENTIATING SPERMATID AT STAGE 3

A. Mitochondrial derivatives as two distinct entities; elongation begins.

B. Nuclear material more homogeneous with no nucleoli; further condensation along the periphery.

C. Proacrosome forming - becomes larger and spherical.

D. Axial filament extending out of the nuclear indentation.

E. Centriole adjunct forms at the base of the nucleus and around the axial filament.
PLATE 6

DIAGRAMMATIC SKETCH OF THE ELONGATING SPERMATID
AT STAGE 4
(Cross Sections at Different Levels Along the Length)

A. Mitochondrial derivatives elongated - crystal formation begins; microtubules visible around the mitochondrial derivatives.

B. Nucleus becomes fibrillar; lateral grooves beginning to form; microtubules surround the nucleus - indicates condensation and elongation.

C. Acrosome elongated; acrosomal rod forming and extending into nuclear indentation.

D. Axial filament shows the regular 9+9+2 pattern - outer row of accessory tubules formed.

E. Centriole adjunct elongates and wraps around mitochondrial derivatives and axial filament for a short distance and then extends posteriorly and gradually decreases.

F. Excess cytoplasm begins to slough off.
Plate 6

- **ACROSOME**
- **ACROSOMAL ROD**
- **CYTOPLASM**

- **NUCLEUS**
- **CYTOPLASM**

- **MICROTUBULES**

- **AXIAL FILAMENT**
- **MICROTUBULES**
- **MITOCHONDRIAL DERIVATIVE**
- **CENTRIOLE ADJUNCT**
PLATE 7

DIAGRAMMATIC SKETCH OF A FULLY DEVELOPED SPERMATOZOOON

AT STAGE 5

(Cross Sections at Different Levels Along the Length)

A. Mitochondrial derivatives fully elongated, extend almost to the posterior tip; crystallization covers two-thirds of the total area, remaining one-third occupied by cristae and matrix.

B. Nucleus fully condensed; elongation complete.

C. Acrosome elongated; acrosomal rod and acrosomal ring fully developed.

D. Axial filament extends to the tip as 9+9+2; at the extreme tip this pattern is lost and fibers end individually and abruptly.

E. Centriole adjunct more condensed - extends for some distance then gradually disappears.

F. Cytoplasm reduced considerably.

G. Microtubules no longer present.
Plate 7
DISCUSSION

The general structure of the spermatozoa of many different species of animals has been studied by various investigators. Many conflicting statements comparing insect and mammalian sperm have been published. The terms applied to parts of the mammalian spermatozoa are probably more standardized than those used for sperm of other animals. The difficulty lies in trying to use the same terms for the insect sperm as those used in mammalian sperm. The parts of a mature mammalian sperm from anterior to posterior are: head and tail, the head consisting of a nucleus capped by an acrosome, and the tail being divided into neck, middle-piece, main-piece and end-piece (Fawcett and Ito, 1965). The neck, which is the base of the tail and lies immediately behind the head, contains a connecting piece (probably derived from the proximal centriole) and the base of the axial filament. The middle-piece, which lies immediately behind the neck, contains mitochondrial derivatives which are arranged in the form of a helical sheath. The main-piece, forming the longest segment of the sperm, lies immediately behind the middle-piece. It consists of the axial filament and is surrounded by a fibrous sheath composed of
anastomozing rib-like structures attached to a thickened region on each side (Fawcett, 1958). The end-piece, consisting of the axial filament enclosed within the cell membrane, forms the distal portion of the sperm tail.

A representative insect sperm consists of a head and a tail, and the tail can be subdivided into neck, main-piece and end-piece. These parts of the tail differ from those similarly named parts of mammalian spermatozoa (Nath, 1956; Davey, 1965). There is no differentiated area free of mitochondrial derivatives that can be called the neck, as that of mammals, but an area of the insect sperm tail, consisting of a centriole adjunct which serves to connect the head to the tail, has been termed the neck. The mitochondrial derivatives in insect sperm are not confined to a relatively short middle-piece as in mammals. Instead, they begin at the base of the head within the body of the centriole adjunct, and extend distally for the greater length of the tail, except for a very small portion of the caudal tip of the tail. This region of mitochondria and axial filament in the insect tail has been termed the main-piece. The fibrous sheath which surrounds the main-piece in mammals is entirely lacking in insects - hence there is no main-piece like that of mammalian sperm, in insect sperm tails. The terminal portion of the insect sperm tail is composed of the posterior extension of the axial filament and this is similar to the end-piece of the mammalian sperm -
hence it can be appropriately termed the end-piece. The insect sperm tail thus has a neck, structurally unlike that in mammals, a main-piece, containing mainly the mitochondrial derivatives and the axial filament, and comprising the major portion of the sperm, again unlike that of mammals. The end-piece is the only structure common to both insect and mammalian sperm tails, and appropriately named.

Thus, it is obvious that except for the end-piece, no other parts of the tail of insect sperm correspond exactly to the neck, middle-piece or the main-piece of the mammalian sperm. This raises the problem of terminology, and it is clear that the use of mammalian terms for the parts of insect sperm tail are misleading, but the terms when applied on their own, and not used for comparison with mammalian sperm, do appear to be appropriate. Hence, it is necessary to state here that the terms used are not homologous to those of mammals.

As stated earlier, little work has been done on the sperm of cockroaches. A few authors have described some parts of the sperm, with most of the work having been done on the various species of Periplaneta. The present research describes more completely the ultrastructure and spermigenesis of a cockroach sperm. The species selected is Nauphoeta cinerea. Such a detailed description and step by step development has not hitherto been shown on any
cockroach sperm. In the following discussion therefore, wherever *Nauphoeta cinerea* is mentioned, it refers only to the present dissertation research. Each sperm structure has been discussed individually and the overall summary at the end stresses some of the features which may be common to other species but are more prominent and clearly shown in the present research.

**Acrosome**

In the sperm of *Nauphoeta cinerea* the acrosome is conical in shape and electron dense with a central electron transparent space, resembling that of the isopod sperm (Reger, 1964). In the transparent space is lodged an acrosomal rod which extends through the acrosomal ring between the acrosome and the nucleus, into the nuclear invagination. The acrosome of *N. cinerea* does not resemble the duckbill structure seen in *Periplaneta americana* (Nath et al., 1957; Richards, 1963; Eddleman et al., 1970). According to Eddleman et al., the duckbill material is actually adhering cytoplasm conforming generally to the outline of the acrosome. This is very common in the early stage sperm but is reduced in size in the later stages by pinching off of excess cytoplasm. Eddleman et al. found similar conditions of retaining excess acrosomal cytoplasm, also termed periacrosomal material, late in spermiogenesis in *P. australasiae*, *P. brunnea*, *P. fuliginosa*, *P. japonica* and
Blatta orientalis. According to Kaye (1962) the acrosome of the house cricket, Acheta domesticus consists of two parts, an inner and outer core. The inner core occurs within the invagination of the base of the outer core. The outer core is derived from the proacrosomal granule, and the inner core from an amorphous material appearing during the early stages of invagination. Kaye thus concludes that the fully developed acrosome is derived from two distinct sources. The axial rod in P. americana is indicated (Eddleman, et al., 1970) to have its origin from a mass of material similar to that which forms the inner core in the house cricket. Eddleman et al. have observed remnants of material exhibiting the same approximate electron density as the axial rod, to be seen adhering to the polar surface of the nucleus. Shay and Biesele (1968) suggest that the axial rod probably arose from a mass of dense material which occurs between the nuclear and the proacrosomal granule membranes when these two bodies are in close apposition. In N. cinerea too, there is a clear thickening of the nuclear membrane between the acroblast and the nucleus. This thickening gives rise to a granular substance which probably is the precursor of the acrosomal plate which later becomes an acrosomal ring through which the acrosomal rod passes, and probably gives rise to the formation of the acrosomal rod itself.
Several authors have described the development of the acrosome and suggest that the Golgi body gives rise to the proacrosomal granule which later transforms into the acrosome, but no one has clearly shown the formation of the granule. Phillips (1970) and Szollosi (1975) have shown the formation of the proacrosome in the concavity of the Golgi body, and this proacrosomal granule is shown to approach the nucleus until its limiting membrane lies parallel and in contact with it. In *N. cinerea*, formation of the proacrosome is very clearly indicated. In the early spermatid a caplike structure approaches the nucleus. It clearly shows vesicles and cisternae indicating the presence of several dictyosomes. The cisternae secrete substances which produce granules that can be seen to be accumulating until a large proacrosomal granule is produced. The Golgi then seems to move away from the nucleus and for some time is clearly seen around the proacrosomal granule. At the time the proacrosomal granule is being formed a clear thickening of the nuclear membrane is observed. This thickening leads to formation of granules and these granules probably later produce the acrosomal plate and the acrosomal rod.

Nucleus

The nucleus of mature sperm of all insects is usually needle-like and consists of a compact mass of chromatin in
which no further substructure is visible. This is the result of chemical changes occurring as the spermatid nucleus elongates, the homogeneous chromatin becomes compact and the nucleus decreases in size. The structural reorganization of the chromatin varies in different species. Generally, the chromatin is very diffuse in early spermatids and very dense in the mature sperm where no substructure is visible, but the intermediate stages vary in different species. Dass and Ris (1958) found the chromatin fibers of the grasshopper *Chorthippus centipennis* to be thicker in the early spermatid, and these fibers underwent divisions which resulted in thinner fibers in the late spermatid. Kaye and Kaye (1966) also found that chromatin fibers became thicker early in spermiogenesis. In *Nauphoeta cinerea* the fibers were noticed to become progressively thicker during spermiogenesis and at any time all the fibers appeared to be of uniform thickness. Phillips (1970) has reported the various conditions in different species. In the treehopper *Ceresa* he noticed that the chromatin at some stages was further advanced in condensation around the periphery of the nucleus than in the center. In the bush katydid *Scudderia*, he noticed that the chromatin at the anterior end of the nucleus was less condensed than in the posterior regions. In such species, Phillips noticed that chromatin strands of several different diameters could be measured in the same nucleus.
In *Nauphoeta cinerea* the condensation of the nucleus was completely contrary to its closely related species, *Pycnoscelus indicus* (Shahaney et al., 1972) where condensation was not only incomplete, but also some nuclei were dilated to almost twice their normal width. The dilations left a space usually filled with some chemical, the nature of which has not yet been ascertained. The chromatin around the space appeared to be arranged in whorls and did not appear as densely condensed as found in nuclei without dilations. Whether the space was formed by incomplete condensation or by disruption of the nucleus due to dilations is not clear.

Elongation of the spermatid nucleus coincides with the appearance of microtubules in most species of insects. In *N. cinerea* microtubules appear in a single row adhering to the nuclear surface, the axis of each microtubule being parallel to the long axis of the cell. The row of microtubules conforms to the general shape of the nucleus, such that when the lateral grooves are formed the microtubules may be found within the grooves also, unlike the condition in the fungus gnat, *Sciara corprophila* (Phillips, 1966) where the microtubules are absent in the lateral grooves. Kessel (1966) has found them in groups of 4 to 13 microtubules, present only in the furrows and depressions of the spermatid nucleus of the dragonfly, *Aeschna grandis*. In
the sperm of the grasshopper, *Melanoplus differentialis*

differentialis Kessel (1967) noticed that the microtubules completely surrounded the nucleus, but from the overlapping appearance of microtubules in oblique sections and appearance of layers of microtubules in approximate transverse sections, he deduced that the microtubules did not extend in a straight line along the surface of the elongating nuclei but rather extended around the nuclei in a helical configuration. Later, during the course of elongation the microtubules appeared to straighten out. Irrespective of the arrangement of microtubules it was noticed that in most cases the microtubules appeared at the onset of elongation and disappeared when elongation was completed.

**Centriole Adjunct**

The origin of centriole adjunct was a controversial point for several years. Its presence was at first doubted by several workers, but the electron microscope has since confirmed that such a structure exists (Gatenby and Tahmisian, 1959). Gatenby and Tahmisian (1959) described the structure as a differentiated area that eventually becomes associated with the flagellar centriole. Since then several workers have observed this structure in several species and designated it with various names until the term centriole adjunct was accepted. Bowen (1920) described a similar structure as a pericentriolar structure and
called it a pseudoblepharoblast. Johnson (1931) called it a post nuclear body; Sotello and Trujillo-Cenoz (1958) termed it the juxtanuclear body; Kaye (1962) called it a basophilic centriole and later Kaye and Kaye (1966) referred to it as a flagellar accessory structure. Kessel (1967) called it a perinuclear aggregate and later (1968) referred to it as a granular material. Phillips (1966) called it a dense fibrous material; Anderson (1967) referred to it as a dense material. The term centriole adjunct was first used by Nunez (1963); and Breland et al. in 1965, 1966, and 1968. Since the use of improved ultrastructural techniques, there is no doubt that such a structure exists. Breland et al. (1966) found a centriole adjunct in the sperm of all species of mosquitoes, and they found the development of the centriole adjunct similar to that described by Gatenby and Tahmisian (1959). Gatenby and Tahmisian (1959) stated that in the ground cricket, *Nemobius*, centriole adjunct arises as spheres and granules which later fuse and form around the centriole. This form of development appears to be true of the sperm of the cockroach, *Nauphoeta cinerea*. Gatenby later (1961) added that it may have originated from the centriolar matrix. Sotello and Trujillo-Cenoz (1958) also believed that the centriole adjunct was in some way related to the centriole, while other workers believe that it originated directly or indirectly from the nucleus.
Clements and Potter (1967) interpreted the centriole adjunct as "the posterior extremity of the nucleus, where the nucleolar contents are almost entirely absent and only the nuclear envelop remains." Barker and Reiss (1966) also believed that this structure originated from the nucleus, if not directly, then possibly some nuclear material organized into a definite mass in the cytoplasm and gave rise to the centriole adjunct. Phillips (1970) described the centriole adjunct as first appearing as a dense material in intimate association with the basal body, and this material increases in amount as spermiogenesis progresses. According to Phillips this organelle is located in a position corresponding to that of columns of the connecting piece in mammalian spermatozoa, and it has been proposed by Breland et al. (1966) and Fawcett and Phillips (1969) that the centriole adjunct may serve to secure the flagellum to the sperm head.

Microtubules surround the centriole adjunct during maturation in much the same manner observed during elongation and condensation of the nucleus and elongation of the mitochondrial derivatives. The centriole adjunct in N. cinerea does not elongate as much as does the nucleus but an equal amount of condensation has been observed. For some time in the late spermatid the centriole adjunct has been seen to retain several vacuoles and has been found to be very granular, but condensation progresses until no
structures are visible within it and with condensation completed the microtubules disappear. Szollosi (1975) has described a similar process of development of the centriole adjunct in Locusta migratoria, except that it eventually transforms into a solid piece constituted of four concentrated lamellae, and that it surrounds the base of the nucleus. The centriole adjunct of N. cinerea appears to serve a mechanical function that is mainly supportive and connects the head to the tail.

Axial Filament

A 9+2 pattern of tubules is characteristic of most cilia and flagella. It holds true for most flagella of insect sperm, though in some insect species variations are known to occur (Phillips, 1969). In addition to the basic 9+2 pattern of tubules, insect sperm flagella usually possess nine outer coarse accessory tubules. According to Gibbons and Grimstone (1960) the doublet tubules appear to be a constant feature of motile sperm, and one member of each doublet, designated as subfiber A, is slightly smaller with a more electron dense center than subfiber B. Subfiber A also bears two arms directed towards the larger subfiber B of the adjacent doublet.

According to Phillips (1970) there are two ways a flagellum can form. In one type a spherical vesicle appears in association with the basal body. As the
flagellum elongates, the vesicle membrane wraps around its tip in its cupshaped depression, and elongates with the flagellum until it comes in contact with the plasma membrane and finally fuses with it. The second type is that seen in *Nauphoeta cinerea* where the flagellum elongates until it reaches the plasma membrane, and then the membrane invaginates into the cell and encloses the flagellum close to the base of the nucleus. In both types a dense material encloses the base of the flagellum and forms the "ring centriole." According to Phillips this "ring centriole", or "annulus", behaves as if it were "anchoring the cell membrane near the basal body during the early growth of the flagellum, but later is released slipping caudally along the flagellum, taking the attached cell membrane with it. As the cell membrane moves caudad the mitochondrial derivatives take up position on either side of the flagellum."

Fawcett and Phillips (1969) have likened this annulus or ring centriole to the annulus in mammalian sperm which similarly moves posteriorly during spermiogenesis.

Some authors (Breland *et al*., 1968) have indicated the presence of two centrioles in the spermatids, whereas others (Kitajima, *et al*., 1972 and Szollosi, 1975) have contradicted this statement. In *Nauphoeta cinerea* two centrioles were observed in the spermatocyte, but only one was seen in spermatids. As the fate of the second centriole is not
known, it is difficult to state positively as to the existence of one or two centrioles in the early spermatids. In either case, one of the two centrioles is destined to become the basal body which is the precursor of the flagellum.

**Mitochondrial Derivatives**

The mitochondrial derivatives in insect sperm were studied even before the use of the electron microscope. Early investigators were particularly interested in the complicated steps by which the small mitochondria of the spermatocyte were transformed into large mitochondrial derivatives of the mature spermatozoa. Bowen (1920) described how the previously spherical mitochondria became elongate structures by telophase of second meiotic division, and were pinched in half by the cleavage furrow, thus resulting in equal distribution of mitochondria to daughter cells at the end of the second meiotic division. After meiosis, the mitochondria again became spherical and clustered together in one area of the cell. There was then a complex series of rearrangements and fusion that lead to their integration into a large spherical mass - the nebenkern. The fusion of mitochondria continued until the nebenkern consisted of two large interlocking mitochondria masses. These underwent a series of convolutions and the nebenkern eventually separated into its two constituent
mitochondrial derivatives. Each large mitochondrial derivative then assumed the form of an ellipse, and the two took position directly behind the nucleus on either side of the basal body of the spermatid flagellum. The mitochondrial derivatives remained closely associated with the flagellum and subsequently elongated parallel to its long axis. During the elongation process they became narrow and long and extended from the base of the nucleus to very nearly the end of the tail.

Careful electron microscopic analyses of spermiogenesis have exposed complexities in the development of mitochondrial derivatives. Mitochondrial development in various species has been reported by several investigators. Pratt (1968) has given a detailed description of nebenkern formation in Murgantia histrionica. A very similar process occurs in Nauphoeta cinerea. Here one can see clearly the aggregation of several mitochondria towards one pole of the cell, to form a spherical mass, the nebenkern. The mitochondria are at first loosely clustered. They then begin to branch and intertwine with each other still retaining their double membrane and the cristae. The walls of individual mitochondria fuse with walls of the adjacent mitochondria until two distinct hemispherical masses remain. These two closely adhering masses retain their shape while the cristae re-orient themselves to conform to their newly acquired shape.
The two hemispherical masses then pull apart and orient themselves on either side of the elongating axial filament.

In *Nauphoeta cinerea* elongation begins immediately after the two constituents of the nebenkern separate, even before they position themselves on either side of the flagellum, and continues during the period of sperm maturation. During elongation the cristae gradually realign to one side and the folds become perpendicular to the long axis - hence not visible in cross sections. This feature appears to be common to mitochondrial derivatives of most insect species. After the alignment of cristae, beginning of crystallization is noticed at the end opposite to the cristae. This gradually progresses towards the cristae. In cross sections the crystalloid appears to be in a hexagonal array, and in longitudinal sections it shows a herring bone pattern. The paracrystalline material progresses until it obliterates most of the matrix leaving behind only a small area of electron transparency between the cristae and the crystalloid. The paracrystalline portion was first observed by Andre (1962) and confirmed by Meyer (1964), and has since been observed by various investigators.

In most species of insects mitochondrial development is identical until the two nebenkern derivatives have taken up position on either side of the base of the flagellum,
after which they begin a process of internal organizations which may differ from species to species. The result in most species is two mitochondrial derivatives, identical in size and shape, as is the case in *N. cinerea*. In a few species the mitochondrial derivatives are of unequal size (Yasuzumi and Oura, 1965), or they may be similar in size but assume a different shape (Herold and Munz, 1967). In some species only one mitochondrial derivative persists in the mature sperm as seen in the Caddis fly (Phillips, 1970). In cross sections the shape of the mitochondrial derivatives, in most cases, is ovoid as seen in *N. cinerea*, but it can assume bizarre shapes which may be characteristic of some species, as seen in the plant hopper, *Acanolonia* (Phillips, 1970).

The complex process of mitochondrial aggregation and fusion to form a nebenkern, followed by a separation into two mitochondrial derivatives, and their elongation and internal reorganization, has not been hitherto observed in any somatic cells; its functional significance is not yet understood. Until the two mitochondrial derivatives separate, a typical mitochondrial profile is retained, with a double limiting membrane and internal cristae. It is only during the elongation process that reorganization occurs; the general (overall) electron density increases, the cristae orient themselves to one end, while progressive crystallization begins at the opposite end. It is during
this process that the microtubules are observed encircling the long axis of mitochondrial derivatives and the axial filaments. The microtubules disappear once elongation is complete.

**Microtubules**

Microtubules have been observed in elongating spermatids of several species of animals. Several pieces of evidence point to the microtubules as playing an important role in the condensation and elongation of the nucleus. The fact that the microtubules are so closely applied to the entire length of the nucleus suggests an important functional association. Also, the fact that they first appear before elongation begins and disappear from the fully matured spermatozoa indicates that their functional role in shaping of the nucleus has ended.

The microtubules, also present in the tail region, encircling mitochondrial derivatives and the axial filament, probably play the same role of shaping and elongating structures, but they are also recognized to provide a mechanism for motility (Kessel, 1967).

In *Nauphoeta cinerea*, as in most insect species, microtubules have been observed around the nucleus, axial filament, centriole adjunct, and the mitochondrial derivatives. They are in almost all cases arranged parallel to the long axis, though the number and arrangement may vary.
In *Locusta murgantia* (Szollosi, 1975) more than two rows of microtubules were observed around the nucleus; in *Aeschna grandis* (Kessel, 1967) in groups of 5-9 microtubules in nuclear depressions; whereas in the cockroach, *N. cinerea*, there is clearly one row of microtubules around the nucleus, centriole adjunct, and the mitochondrial derivatives, and random clusters around the axial filament. Irrespective of the arrangement, the microtubules appeared at the onset of elongation and disappeared when elongation was completed. The microtubules in *N. cinerea* clearly appear to aid in the process of elongation and condensation of the structures they encircle. McIntosh and Porter (1967) have proposed that the microtubules play a very important role in cell elongation, whereas Fawcett et al. (1971) show arguments that these organelles do not play a determinant role in shaping the sperm nucleus. Such controversies still exist and the exact purpose of microtubules has yet to be determined.
SUMMARY AND CONCLUSIONS

Summing up the description and development of the sperm of cockroaches, it may be seen that the sperm of *Nauphoeta cinerea* is a "typical flagellate sperm" according to Nath (1956). It consists of a needlelike head followed by a thread like tail. The head consists of a nucleus capped by an acrosome. The tail is further subdivided into a neck, main piece, and end piece. The neck consists of a centriole adjunct which encloses the base of the axial filament, and the mitochondrial derivatives. The main piece consists of the axial filament and the mitochondrial derivatives, while the end piece consists of the caudal tip of the axial filament which now no longer retains its basic 9+2 pattern of tubules.

**Acrosome**

The acrosome, situated anterior to the nucleus, is a capshaped structure originating from the golgi complex. It consists of an outer core surrounding the inner acrosomal rod. The acrosome is formed from the proacrosomal granule which is the result of accretion of a granular material secreted by the dictyosomes. The dictyosomes in the spermatids cap around the nucleus to form an acroblast. Facing the nucleus, the proacrosomal granule forms a
concavity, and as it continues to elongate anteriorly it forms a dense cone-like structure. The facing nucleus indents, and along with the concavity of the acrosome an electron transparent space is produced in which appears the acrosomal rod. The acrosome of *N. cinerea* differs from that found in the various species of the genus *Periplaneta*. The "duckbill" feature of *Periplaneta sp.* is not observed. The development of the acrosome has not hitherto been described in detail before. It is a well known fact that the acrosome arises from the golgi body. Most authors merely indicate that a proacrosomal granule is produced by the golgi body and the granule moves towards the nucleus where it continues to develop into an acrosome. In *N. cinerea* a distinct acroblast is seen capped around the nucleus. Small granules produced by the acroblast accumulate until a large proacrosomal granule is formed. The golgi body then can be seen to move away and the proacrosomal granule becomes spherical, acquires a double membrane, initiates the production of the acrosomal plate, and then indents forming a cavity facing the nucleus. In the cavity the acrosomal rod begins to form from the dense material produced between the proacrosomal granule and the nucleus.
Nucleus

The nucleus of the spermatid is spherical consisting of a homogeneous chromatin material with a few heterochromatic patches. During spermiogenesis the heterochromatic bodies move towards the center of the nucleus and condensation begins at the periphery and progresses inwards. The chromatin then alters from a homogeneously granular material to a fibrous content. The fibers begin to fuse with each other and condense into a compact mass. During the process of condensation the nucleus also elongates. At the onset of elongation and further condensation, microtubules appear. The microtubules are arranged parallel to the long axis of the elongating nucleus, in a single row of tubules adhering close to the surface of the nuclear membrane. When elongation and condensation of the nucleus is complete the microtubules disappear, indicating that they are responsible for the process of elongation and condensation. The nucleus is similar in almost all species of cockroaches, except Pycnoscelus indicus, in which nuclear dilations were observed (Shahaney et al., 1972). N. cinerea, being a very closely related species, and having almost an identically structured sperm, did not show any nuclear dilations. In P. indicus the dilations are very prominent and they appear during condensation of the nucleus. In other species vacuoles or electron transparent
spaces are initially present but when chromatin condensation is complete they disappear. Whether these dilations, which rupture the nuclei, serve a useful purpose, or, are accidents of nuclear condensation, is not known.

**Centriole Adjunct**

The centriole adjunct begins at the base of the nucleus and around the basal body. It begins as a granular structure which condenses into a compact mass and ensheathes the elongating axial filament and the mitochondrial derivatives. The centriole adjunct serves to join the head to the tail. At the base of the nucleus it completely encloses the axial filament and the mitochondrial derivatives, and as it extends posteriorly it reduces in size, forming a partial sheath, later becoming crescent shaped, and finally disappearing in the main piece of the tail. The centriole adjunct mainly serves the purpose of support. It serves to join the head to the tail and acts as a means of support during motility when the axial filament undulates at a very rapid speed.

**Axial Filament**

The axial filament begins in the posterior indentation of the nucleus where it has the typical 9+2 pattern of tubules of all cilia and flagella. As it emerges out of the nuclear indentation and passes through the centriole adjunct it acquires another row of tubules, the coarse outer
tubules, now having a 9+9+2 pattern of tubules. This pattern continues in the main piece of the tail but is lost in the end piece. In the end piece of the tail, doublets of the initial 9+2 pattern of tubules are lost first, followed by the remaining tubules. The tubules in the end piece are arranged randomly and they end individually and abruptly before reaching the extreme tip of the tail. The axial filament of _N. cinerea_ appears to be the structure that most resembles that of other species of insects. The accessory tubules, which are formed after the initial 9+2 pattern of tubules has been established, appear after the axial filament has begun to elongate and has extended beyond the nuclear indentation. According to Phillips (1970) the accessory tubules, in some species of insects, extend more anteriorly than the 9+2 tubules.

**Mitochondrial Derivatives**

The mitochondrial derivatives extend almost the entire length of the tail. They begin in the neck region, where they are ensheathed by the centriole adjunct, and they end just before reaching the end piece of the tail. The mitochondrial derivatives result as a product of fusion of the spermatid mitochondria. The spermatid mitochondria aggregate to form a nebenkern where they fuse with one another resulting in a bipartite structure. The constituents of the bipartite structure separate and position on
either side of the elongating axial filament where they conform to their newly acquired shape which is ovoid in cross section. Crystallization begins at the narrow end of the nebenkern constituents, and progresses, thus pushing all the cristae to the wider end. Once crystallization begins the products of the bipartite structure are termed the mitochondrial derivatives. Most authors have observed a similar type of mitochondrial derivative development, but apart from Pratt (1968), none have shown a clear reduction of the nebenkern into a bipartite structure. In *N. cinerea* the bipartite structure is distinctly depicted.

Since the events during spermiogenesis are synchronous and linked, it is not sufficient to give a detailed description of individual structures. It is necessary to include a brief description of the synchronous events. For a matter of convenience the events are grouped into five distinct stages where each stage shows the major changes occurring to produce the following structures of the mature sperm: acrosome, nucleus, centriole adjunct, axial filament and the mitochondrial derivatives.

Stage 1. The mitochondria aggregate to one pole of the cell. The nucleus is homogeneous with a few heterochromatic patches, and there are two centrioles visible, one of which later indents into the nucleus and becomes the basal body, while the fate of the other is unknown.
Stage 2. The mitochondria join together to form a nebenkern where they begin to fuse with one another. In the nucleus the heterochromatic patches move towards the center and condensation of the chromatin begins at the periphery of the nucleus. The centriole, now termed the basal body, indents into the nucleus. The dictyosomes gather at one pole of the cell and cap around the nucleus to form an acroblast.

Stage 3. The nebenkern becomes bipartite and the two constituents separate, elongate and position on either side of the elongating axial filament. Chromatin condensation in the nucleus progresses further. The basal body, which has the basic 9+2 pattern of tubules, projects out of the nuclear indentation and acquires an additional row of tubules, the accessory tubules. The acroblast secretes a granular substance, which by accretion results in a proacrosomal granule. The remaining Golgi body eventually disappears. The centriole adjunct begins as granules around the elongating axial filament. The granules condense and an almost compact centriole adjunct is formed. At this stage it still contains several vacuoles and condensation is not complete.

Stage 4. The whole cell is in the process of elongation. The condensation in the nucleus progresses further, and fibrous structure is visible. Microtubules appear around the elongating organelles. The nucleus conforms to its final shape by producing lateral grooves. The acrosome is
formed from the proacrosomal granule by elongating and producing a concavity in which grows the acrosomal rod. The centriole adjunct extends posteriorly and wraps around the mitochondrial derivatives and the axial filament. It still retains some vacuoles and condensation is not complete. The axial filament elongates, retaining its typical pattern until it reaches the end-piece where the pattern is lost. The mitochondrial derivatives elongate, crystallization begins at one end and progresses towards the other pushing the cristae to the opposite end where they are oriented perpendicular to the long axis of the cell. Microtubules appear around the mitochondrial derivatives in a single row adhering close to the longitudinal cell surface.

Stage 5. Elongation is complete and condensation of the nucleus and centriole adjunct is also complete. Microtubules disappear, while mitochondrial derivatives are two-thirds crystallized. Mitochondria end before reaching the end-piece of the tail. The tubules of the axial filament end abruptly in the end-piece, and the pattern of 9+2 tubules is lost. Excess cytoplasm is sloughed off, and the newly formed and completed organelles are enclosed within a plasma membrane and a minimum of retained cytoplasm.
Figure 1. Cross section of an acrosome (a) showing the acrosomal rod (a.r) x35,700

Figure 2. Longitudinal section of the acrosome (a) showing the acrosomal rod (a.r) passing through the acrosomal ring into the anterior invagination of the nucleus (n). x29,800
Figure 3. Longitudinal section of an acrosome (a) showing the acrosomal ring (ac.r).
\( \times 29,800 \)

Figure 4. Cross sections of sperm heads showing nuclei. \( \times 17,000 \).
Figure 5. Cross sections of sperm tails showing the centriole adjunct (c.a) completely enclosing the axial filament (a.f) and the mitochondrial derivatives (m.d). x22,700.

Figure 6. Cross sections of sperm tails showing the centriole adjunct (c.a) reduced in size further posteriorly along the length of the tail. x17,000.
Figure 7. Longitudinal section showing centriole adjunct (c.a) completely enclosing the mitochondrial derivatives (m.d) at the base of the nucleus (n).  x55,300.

Figure 8. Cross section of the axial filament (a.f) in the main-piece of the tail.  x145,450.
Figure 9. Cross section of the axial filament (a.f) in the end-piece of the tail. The basic 9+2 pattern of tubules (t.) is lost. x55,800.

Figure 10. Cross section of mitochondrial derivatives (m.d) showing crystalloid (c), cristae (cr), electron transparent space (ets) and the dense band (db). x76,550.
Figure 11. Longitudinal section of the mitochondrial derivatives (m.d) showing crystalloid (c), cristae (cr) and electron transparent space (ets). x22,700.

Figure 12. Acroblast (ac) capping the nucleus (n). Golgi body (gb) in the vicinity. x12,600.
Figure 13. Acroblast (ac) in the vicinity of the nebenkern (ne). x10,700.

Figure 14. Acroblast (ac) showing vesicles (v), and granule (gr) secretion. x5,150.
Figure 15. Proacrosomal granule (pr) with Golgi body (gb) in the vicinity. x12,600.

Figure 16. Proacrosomal granule (pr) getting larger and closer to the nucleus (n). x5,750.
Figure 17. Proacrosomal granule (pr) becoming spherical, and golgi body moved away. Axial filament (a.f) and mitochondrial derivatives (md) now on side opposite the proacrosome. x11,340.

Figure 18. Proacrosomal granule (pr) showing double membrane (mb) and formation of acrosomal plate (ap). x29,800.
Figure 19. Beginning of concavity in the proacrosomal granule and formation of the acrosome (a) x19,900.

Figure 20. Acrosomal rod (a.r) beginning in the acrosomal (a) concavity, and extending into the nuclear (n) invagination. x18,900.
Figure 21. Cross section of the developing acrosome (a). x8,500.

Figure 22. Longitudinal section of the developing acrosome (a) showing the acrosomal space (as) and the acrosomal plate (a.p). x42,500.
Figure 23. Cross sections of spermatid nuclei showing heterochromatic inclusions. x4,300.

Figure 24. Spermatid nucleus showing fibrous (f) and granular (gr) inclusions. x11,350.
Figure 25. Spermatid nucleus with a double nuclear membrane (n.m), showing a ribbonlike inclusion. x55,800

Figure 26. Nuclei showing peripheral condensation of the chromatin. Arrows indicate condensed areas. x5,750.
Figure 27. Cross section of nuclei (n) showing fibrous chromatin. Beginning of microtubules (mt) along the nuclear surface. x12,600.

Figure 28. Longitudinal section of nucleus (n) showing chromatin condensation. x18,900.
Figure 29. Cross sections of nuclei showing chromatin condensed in the form of fibers. x13,900.

Figure 30. Cross section of a nucleus (higher modification) showing fibrous contents and surrounded by a ring of longitudinally oriented microtubules (mt). x51,000.
Figure 31. Cross sections of nuclei showing fibers fusing and forming sheetlike structures (str). x15,100.

Figure 32. Cross section of a nucleus showing fibers clumping along the periphery and forming sheetlike structures towards the center. x53,600.
Figure 33. Beginning of centriole adjunct (c.a) in the form of granules, encircling the axial filament (a.f). x41,700.

Figure 34. Condensation of the centriole adjunct (c.a). x16,400.
Figure 35. Centriole adjunct (c.a) condensed further and beginning to encircle the mitochondrial derivatives. x11,400.

Figure 36. Cross section of a condensing centriole adjunct (c.a) with microtubules (mt) along its surface. x17,650.
Figure 37. Cross section of a condensing and elongating centriole adjunct (c.a) wrapped around the axial filament (a.f) and the mitochondrial derivatives (md). Microtubules (mt) can be seen along the surface of the centriole adjunct. x18,300.

Figure 38. Longitudinal section of a developing centriole adjunct (c.a) showing vacuoles (vac), axial filament (a.f) and mitochondrial derivatives (md) with their cristae (cr). Microtubules (mt) can be seen running parallel to the elongating structure. x29,800.
Figure 39. Late spermatocyte showing two centrioles (ce) perpendicular to each other. x12,300.

Figure 40. Basal body (b.b) indented into the nucleus (n). x25,200.
Figure 41. Basal body projecting out of the nuclear indentation to form the axial filament (a.f). x10,000.

Figure 42. Elongation of axial filament (a.f). 'Ring centriole' (r.c) formed. Cross sections at levels A, B and C, showing axial filament are indicated in Figure 43. x12,600.
Figure 43. Cross sections of axial filament at different levels along the length of the elongating structure.
a. shows cross section closer to the nucleus before the ring centriole level - A in Fig. 42.
b. shows cross section immediately beyond the ring centriole hence showing a double membrane-B in Fig. 42.
c. shows cross section where the axial filament has extended beyond the cell membrane - C in Fig. 42. x17,650.

Figure 44. Cross sections of the tail showing mitochondrial derivatives positioned on either side of the axial filament, and endoplasmic reticulum (e.r) forming a sheath around the axial filament. x6,950.
Figure 45. Mitochondria (m) beginning to aggregate at one end of the cell. x2,950.

Figure 46. Mitochondria (m) aggregating near the nucleus (n). x5,750.
Figure 47. Loosely clustered mitochondria (m). x14,500.

Figure 48. Mitochondria clustered in a spherical mass forming a nebenkern (ne). A chromidial body (c.b) can be seen within the body of the nebenkern. x15,120.
Figure 49. Mitochondria fusing together within the nebenkern (ne). A chromidial body (c.b) present in the vicinity. x14,500.

Figure 50. Fusion of mitochondria within the nebenkern progressing. Cristae (cr). within fused mitochondria orienting along the walls. x14,500.
Figure 51. Nebenkern fused into a compact mass forming a bipartite structure. Cristae (cr) orienting along remaining walls. x13,230.

Figure 52. Fusion of Nebenkern mitochondria forming a bipartite structure. Cristae (cr) still visible. x13,230.
Figure 53. Nebenkern fused into a bipartite structure. xl3,700.

Figure 54. Cross section of one of the segments of the bipartite nebenkern. xl7,650.
Figure 55. Nebenkern segments separated and positioned on either side of the axial filament. xl1,850.

Figure 56. Cytoplasmic islands (c.i) trapped in the nebenkern halves. xl3,230.
Figure 57. Nebenkern halves showing cytoplasmic islands (c.i) and cristae (cr). x16,730.

Figure 58. Mitochondrial derivatives (m.d) assumed their definitive shape. Crystallization (cs) beginning at one end. Microtubules (mt) present. x15,760.
Figure 59. Crystallization (cs) progressing towards wider end of the mitochondrial derivatives (m.d).  x25,500.

Figure 60. Microtubules (mt) forming a single row around the mitochondrial derivatives (m.d) and arranged randomly around the axial filament (a.f).  x39,700.
Figure 61. Mitochondrial derivatives showing the crystalloid (c).  x59,540.

Figure 62. Intercellular bridge between two cells.  x12,600.
Figure 63. Due to incomplete cytokinesis during development, several sperm tails can be seen enclosed within a common plasma membrane. 
a.f - axial filament.  x17,000.

Figure 64. Large areas of cytoplasm (indicated by arrows) apparently containing unwanted structures are pinched off and can be seen floating free in the cyst lumen.  x13,870.
APPENDIX I
PREPARATION OF MATERIALS

COCKROACH SALINE (O.S.U. Formula)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>10.93 grams/liter</td>
</tr>
<tr>
<td>KCL</td>
<td>1.57 grams/liter</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>.85 grams/liter</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>.215 grams/liter</td>
</tr>
<tr>
<td>H₂O</td>
<td>Remainder of liter</td>
</tr>
</tbody>
</table>

Final pH of saline should be between 7.0 and 7.5. If not to this range, adjust with NaHCO₃.

PHOSPHATE BUFFER AND PHOSPHATE BUFFER WASH

A. 0.2M solution monobasic sodium phosphate (1.38gm in 50 ml distilled H₂O).

B. 0.2M solution dibasic sodium phosphate (5.68gm Na₂HPO₄ in 200ml distilled H₂O) or (14.34gm Na₂HPO₄·12 H₂O in 200 ml distilled water).

<table>
<thead>
<tr>
<th>BUFFER:</th>
<th>19ml A</th>
<th>81ml B</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUFFER WASH:</td>
<td>19 ml A</td>
<td>81 ml B</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>60 ml dist. H₂O</td>
<td>100 ml dist. H₂O</td>
</tr>
<tr>
<td></td>
<td>160 ml BUFFER</td>
<td>200ml BUFFER</td>
</tr>
</tbody>
</table>
SPURR'S LOW-VISCOSITY EMBEDDING MEDIUM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>wt. in grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCD</td>
<td>10.0</td>
</tr>
<tr>
<td>DER 736</td>
<td>6.0</td>
</tr>
<tr>
<td>NSA</td>
<td>26.0</td>
</tr>
<tr>
<td>DMAE</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Cured at 70°C for 8 hours.

TOLUIDINE BLUE STAIN (For Thick Sections)

2% Stock Toluidine Blue
2% Stock Sodium Borate
1% Pyronin B

When mixing the stain the proportions of the three solutions are identical to the percentage - 2 : 2 : 1

URANYL ACETATE

A saturated solution of uranyl acetate in 50% ethanol.

LEAD CITRATE STAIN (REYNOLD'S)

1. Place 1.33 gm Pb(NO₃)₂, 1.76 gm Na₃(C₆H₅O₇).2H₂O and 30 ml H₂O in a 50 ml volumetric flask.
2. Shake vigorously for one minute and allow to stand with intermittent shaking for 30 minutes.
3. Add 8.0 ml of freshly prepared IN NaOH and dilute suspension to 50 ml and mix by inversion.
APPENDIX II

MAGNIFICATIONS OF ELECTRON MICROSCOPE EMU-3G AT 50KV AS CALIBRATED ON APRIL 19, 1973

<table>
<thead>
<tr>
<th>Magnification</th>
<th>Magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,075x</td>
</tr>
<tr>
<td>2</td>
<td>2,200x</td>
</tr>
<tr>
<td>3</td>
<td>2,869x</td>
</tr>
<tr>
<td>4</td>
<td>4,184x</td>
</tr>
<tr>
<td>5</td>
<td>5,670x</td>
</tr>
<tr>
<td>6</td>
<td>7,564x</td>
</tr>
<tr>
<td>7</td>
<td>11,907x</td>
</tr>
<tr>
<td>8</td>
<td>17,010x</td>
</tr>
<tr>
<td>9</td>
<td>22,306x</td>
</tr>
<tr>
<td>10</td>
<td>30,618x</td>
</tr>
</tbody>
</table>
APPENDIX III

CLASSIFICATION OF SOME OF THE COMMON COCKROACHES
ACCORDING TO MCKITTRICK

PHYLUM: ARTHROPODA
CLASS: INSECTA
ORDER: DICTYOPTERA
SUBORDER: BLATTARIA
SUPERFAMILY: BLATTOIDEA
FAMILY: CRYPTOCERCIDAE
SUBFAMILY: CRYPTOCERCINAE
TYPICAL SPECIES
Cryptocercus punctulatus
FAMILY: BLATTIDAE
SUBFAMILY: BLATTINAE
TYPICAL SPECIES
Blatta orientalis
Periplaneta americana
Periplaneta australasiae
Periplaneta brunnea
Periplaneta fuliginosa
SUPERFAMILY: BLABEROIDEA

FAMILY: BLATTERLLIDAE

SUBFAMILY: PLECTOPTERINAE

Supella supellectilium

SUBFAMILY: BLATTELLINAE

Blattella germanica

Parcoblatta pensylvanica

FAMILY: BLABERIDAE

SUBFAMILY: BLABERINAE

Blaberus giganteus

Byrsotria fumigata

SUBFAMILY: PYCNOSCELINAE

Pycnoscelus surinamensis

Pycnoscelus indicus

SUBFAMILY: DIPLOPTERINAE

Diploptera punctata

SUBFAMILY: OXYHALOINAE

Leucophaea maderae

Nauphoeta cinerea
LITERATURE CITED


