REPORT OF THE DIRECTOR OF THE REELFOOT LAKE BIOLOGICAL STATION

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The thirty-first session of this research Station, directed by the Tennessee Academy of Science and supported by a grant from the State of Tennessee, was characterized by intensive activities of three research investigators, visits from summer school students and many visitors from the surrounding region.

Dr. Robert J. Schoffman of the Spalding Institute, Peoria, Illinois, spent his twenty-fourth consecutive summer of investigation on the study of age and growth rate of the fishes of the lake.

Dick L. Deonier, graduate student at Iowa State University, Ames, Iowa, used our facilities for his work on the Taxonomy and Ecology of the genus Hydrellia, which is quite abundant on the vegetation of the lake.

A bridge was built across the ditch that has prevented access to the Station for several years by the United States Fish and Wildlife Service, and it is hoped that our facilities may be of greater use and benefit to research workers in the future.

SPERMATOZOA AND SPERMATELEOSIS IN CRYPTOBRANCHUS AND NECTURUS[1]

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INTRODUCTION

The diversity that exists between the seven families of urodeles on the basis of external features is equalled by the differences found in the structure of the spermatozoa from these various groups. It is also expected that phylogenetic relationships can be determined by sperm structure. Although the sperm of all urodeles have some uniformity in general structure such as a prominent flagellum marginal to a conspicuous axial filament, no particular sperm can be said to be "typical."

The sperm from one or more genera of each family of urodeles have been observed and the basic features noted and that of Amphiuma has been reported (Baker, 1962). This is the second of a series dealing with spermateleosis, sperm morphology and motility of urodelean sperm.

Cryptobranchus alleganiensis bishopi, or Ozark Hellbender, has a distinctive sperm in that the neck piece is much reduced, the ring does not elongate down the axial filament, there is no evidence of the cytoplasm and cell membrane passing down the axial filament of the tail, no mitochondria are evident on the tail and the middle piece cannot be distinguished from the principal piece. Mito-

chondria are present about the head in conspicuous protoplasmic beads. The flagellum is quite prominent and has a modified planar motility that is three dimensional. Its wave length and sine amplitude are constant for the species.

The sperm of *Necturus maculosus maculosus,* the Mud-Puppy, by contrast, has an elongate neck piece, the cytoplasm and cell membrane passes down almost the entire length of the tail but there is no evidence of an elongating ring, the mitochondria are present along the entire axial filament and the flagellum is wound about this filament for its entire length. This sperm is longer and of less diameter than any other urodele sperm.

In spermateleosis in both of these urodeles the axial filament and flagellum have an early origin from a prominent granule in the spermatid as a single filament that later separates with the flagellum being considerably longer. Motility is evident quite early and continues throughout spermateleosis. As the sperm matures the parallel flagellum forms into waves alongside or around the axial filament and extends only slightly beyond as a free end piece. Additional observations on Amphiuma indicate clearly a similar origin of flagellum and axial filament which was overlooked in the previous study.

Motility in both urodeles is definitely helical and clockwise.

MATERIAL AND METHODS

The Cryptobranchus were collected below a dam in Spring River two miles south of Mammoth Springs, Arkansas. They are quite abundant here and are picked up at night in shallow water near the shore or from the concrete base of the dam where they are attempting to swim upstream. The river arises from a spring at Mammoth Springs and has a fairly constant temperature of 13 degrees Centigrade. The water at the dam reaches 19 degrees during August. Numerous specimens have been kept in laboratory aquaria for several months.

Necturus were purchased from commercial collectors in Wisconsin. Spermateleosis seems to occur during October or slightly earlier for November through February the testes are enlarged with masses of mature sperm and the sperm ducts are dilated.

All stages of spermateleosis were observed in living state by dark phase contrast microscopy. Permanent preparations showed excellent detail after iron-alum haematoxylin. Tetrazolium chloride salt was used for mitochondrial details and the periodic-acid-Schiff method stains the acroblast and acrosome. These methods are discussed in detail in the paper on Amphiuma (Baker, 1962).

OBSERVATIONS

Cryptobranchus

Testes examined for two years during July and until August 10 had spermatids as the most mature cells while those taken August 12 through September 15 had all stages of sperm development with many bundles of mature sperm, especially from the latter date. By December 1 the testes were considerably enlarged and full of sperm with a few in the sperm ducts, while, by December 15, no sperm were in either testes or sperm duct and the testes were reduced in size. These data are based on animals 25 to 30 cm. long (snout-vent) and it should be noted that the testes of a smaller male, 19 cm. long, had developmental stages on September 24 equivalent to those of August 15 for the larger ones.

The spermatid contains the spherical nucleus, acroblast, ring-granule combination and numerous mitochondria (figs. 1, 2). An orientation of these components occurs as the nucleus elongates. The acroblast is seated on the labile nucleus at its anterior end (fig. 3; Pl. 2a) while the granule and ring are closely appressed to a depression in the posterior end. The nuclear material compresses into a chromophilic mass beginning at its posterior end and proceeding anteriorly (figs. 4, 5), a process that may be the result of fixation as the living cells appear to have nuclei with a homogeneous distribution of chromatin material.

The acroblast appears as a vesicle in the living cell that elongates during spermateleosis. A small dark structure appears at the juncture with the nucleus in stained preparations and it is believed that this is the acrosome (fig. 3-5). The appearance of this vesicle after the periodic-acid-Schiff reaction indicates that the entire acroblast is filled with a homogeneous material (Pl. 1) that is transform(ed) into an acrosome which fits over the anter(ior) extension of the nuclear membrane or perforat(or)ium. A neck piece anlage is not present duri(ng) spermateleosis yet the mature sperm seems to h(ave) a small, slightly spherical, organelle between (the) base of the head and the axial filament (Pl. ?; fig. 8). A ring is prominent in the early stages (of) spermateleosis but disappears (fig 6, 7) with(out) evidence of elongating down the axial filame(nt). There is also no evidence that the cell membra(ne) and cytoplasm extend down the axial filament (or) tail. Associated with this unusual digression fr(om) the pattern of sperm development the mitochond(ria) are never found distal to the nuclear head. Ea(ch) mature sperm, with rare exceptions, has a promi(nent) protoplasmic bead on the head that is fil(led) with mitochondria (fig. 8; Pl. 2d). These bea(ds) are quite tenuous as evidenced by their union in(to) one when several sperm are adjacent to each oth(er.) Despite the lack of mitochondria and protoplasm(ic) beads on the tails, headless sperm have been (ob)served with flagellar movement for a short time.

A filament arises from the granule in the sper(m)atid, passes through the ring and elongates rapid(ly) as the nucleus and cell elongates. This filament (is) vibratile from its early origin, sweeping back a(nd) forth and bending into curves with enough force (to) cause a slight movement of the elongating cell. (It) soon differentiates into an axial filament and (a) flagellum (figs. 4-6; Pl. 2c) and it is apparent fr(om) the motility of a mature sperm that the flagellu(m) alone produces the movement. This axial filame(nt) reaches a maximum length of 185 micra in ea(rly) development (fig. 5) with the flagellum, parrall(el)ing it, extending for twice this length (fig. 7). (It) is quite evident that a sheath, of unknown orig(in) but probably a part of the original filament, hol(ds) these two elongate components together and, as t(he) sperm matures, the flagellum is drawn into t(he) sheath, forming complex waves, with a free e(nd) piece 15-18 micra long (fig. 8). This sheath (is) evident in mutilated sperm where the axial filame(nt) has separated from it (fig. 9; Pl. 2b).

The flagellum has a characteristic wave leng(th) of approximately 6-7 micra and a sine amplitu(de) of 2 micra. Observations on living sperm can (be) quite deceptive in that the field of vision is t(wo) dimensional. Since the flagellum is planar (Pl. ?) from all sides it is obvious that it has an addition(al) curvature, the result of being confined by a shea(th) part of which may be called the undulating me(m)brane. All parts of the flagellum are equidista(nt) from the axial filament which means that betwe(en) each wave there are two arcs with the concave si(de) of the peripheral flagellum facing the axial fil(a)ment.

Motility

The forward movement of the sperm is heli(cal) and clockwise. The axial filament has a sligh(t) three dimensional twist with the flagellum alwa(ys) on the convex side.
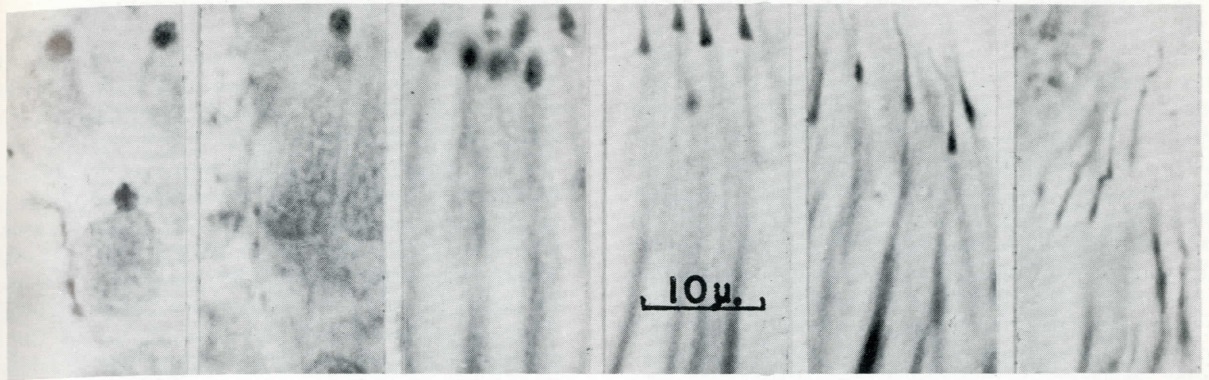
Plate 1. Development of acrosome from spermatid to mature sperm in Cryptobranchus; after reaction with tetrazolium chloride.
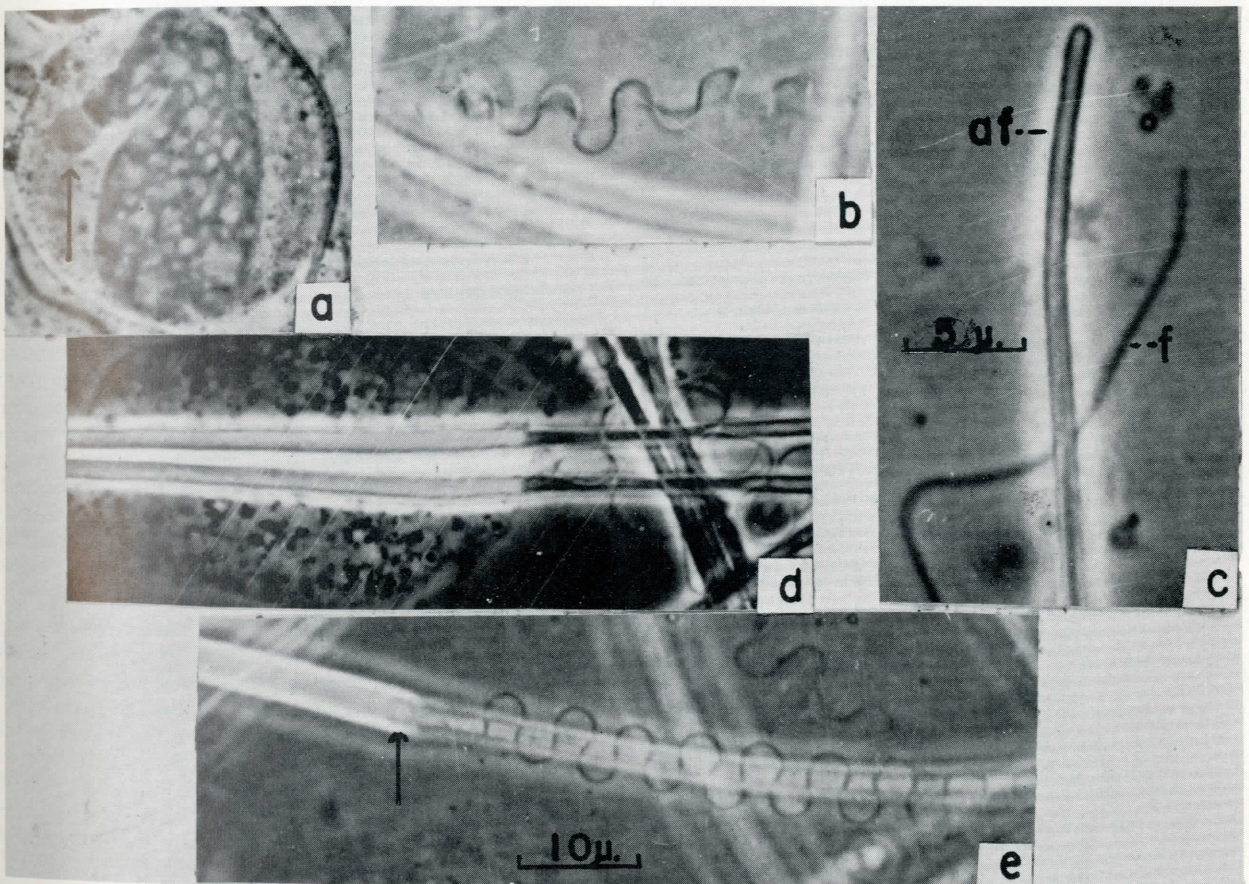

Plate 2. Cryptobranchus sperm; photographs of living cells; a, a spermatid with acroblast (at arrow) at end of the elongating nucleus; b, flagellum and sheath that have slipped from the axial filament; c, axial filament, af, and flagellum, f, after sheath has broken; d, two sperm heads in a single protoplasmic bead; note numerous mitochondria; e, typical head, axial filament and flagellum; possible neck piece at arrow.

A very unusual feature of Cryptobranchus sperm is the lack of motility in those removed from the testes and, examined in istonic salt solution, they have little or no motility, yet when placed in water there is considerable flagellar activity with some forward movement. A sperm suspension kept in isotonic salt solution at 5 degrees Centigrade for ten days had no movement whatever when examined each day, yet when water was added to a drop of the suspension on a slide, there was considerable active motility. By contrast a concentrate of sperm removed from the same testes and placed in pure water at the same temperature showed motility each day for four days when examined under the microscope. It is doubtful that any nutritive material of the medium could be utilized by the sperm during this time.

The flagellar wave normally arises at its attachment to the neck piece and progresses distally. However any impediment as another sperm or small cellular debris may inhibit flagellar movement at the point of origin yet there will be rapid movement distally. Any portion of the flagellum may be motile while other parts are immobile. The Cryptobranchus sperm, like those of other urodeles, exhibit all degrees of speed in wave movement from one wave per second to a speed too rapid to count. Such material is ideal for a study of flagellar movement.

NECTURUS

Spermateleosis seems to occur in September and may extend into October. By November 1 the testes have approximately one-third spermatogonia for the next season and two-thirds bundles of mature sperm. The sperm ducts are distended with sperm from November 1 to March 1 in animals kept in laboratory aquaria.

Spermateleosis is characterized by the presence of a large ring with a densely staining inner portion, a long neck piece, a flagellum winding around the axial filament for its entire length and a very long thread-like sperm.

The acrosome arises from the acroblast as in other urodeles, the chromatin material of the nucleus condenses as a slender filament, and the neck piece arises as a small granule from the posterior end of the nucleus, elongates considerably and remains within the nuclear membrane (figs. 11-15). A definite granule and ring appear in the spermatid. This granule, at first separated from the neck piece (fig. 11), soon contacts it (figs. 12-15) and becomes the base of the axial filament. However an additional basal granule appears beside the posterior end of the neck piece (fig. 15) from which the flagellum apparently extends.

The axial filament and flagellum originate as a single filament from the elongating spermatid, pass through the ring and soon extend for a considerable distance distally. This filament is quite vibratile during these early stages. When maximum elongation is reached (fig. 15) the axial filament is approximately 560 micra in length with the fla-gellum extending an additional 160 micra.

The cell membrane and cytoplasm, with the enclosed mitochondria, pass distally down the fila-ment. Since mitchondria are found the entire length of the axial filament it is safe to assume that the cell membrane extends for its entire length.

The prominent ring is distinctive for Necturus in that it is larger than that of other urodeles and, after iron haematoxylin, has a chromophilic small ring surrounded by a larger chromophobic portion. There is no evidence that this ring elongates down the axial filament and it has not been observed in the mature sperm. In this mature sperm the fla-gellum is wound tightly about the axial filament in characteristic waves or turns (Pl. 3a) and extends for only a few micra as a free end piece.

The mature sperm bears little resemblance to those of other urodeles in its tremendous length, its small diameter and with the encircling flagellum (figs. 16-19). It is difficult to determine the exact length of any sperm for in smears or fixed material there is the possibility of contraction and even living ones may have the ability to modify their length depending on age and the medium. The approximate dimensions, however, in micra are:

perforatorium	8 to 11
nucleus	290 to 310
Neck piece	40 to 45
axial filament	500 to 550
end piece	3 to 5

This means the total length may vary from 8... to 920 micra.

In a living sperm the acrosome is not visible in the perforatorium and this with the nucleus and neck piece, appears as a single structure. A promi-nent helical appearance is evident on the anterior end which diminishes posteriorly through the neck piece (fig. 19; Pl. 3a). The axial filament becomes progressively smaller and this results in flagellar movement becoming increasingly evident distally. The flagellum is closely adherent to the axial fila-ment for its entire length and appears to be held in place by an invisible sheath (Pl. 3).

The mitochondria are distributed as separate granules rather than as masses as in Amphiuma (Baker, 1962) because of the small size of the axial filament. This distribution makes it possible to count them after staining with tetrazolium chloride salts by averaging the number observed in a few of the coils of the flagellum. Sperm removed from the testes in October have 30 to 40 mitochondria per coil (fig. 16), those removed from the sperm duct in December have 12 to 15 per coil (fig. 17; Pl. 3), while those taken from a gelatinous mass on the cloaca in February have only 1 to 3 per coil (fig. 18).

In contrast to the immobility of Cryptobranchus sperm in isotonic salt solution, the Necturus sperm are invariably quite active when observed whether taken from the testes, sperm duct or cloaca. Dense white coils of sperm emerge from a clipped sperm duct and it is therefore possible to obtain a con...
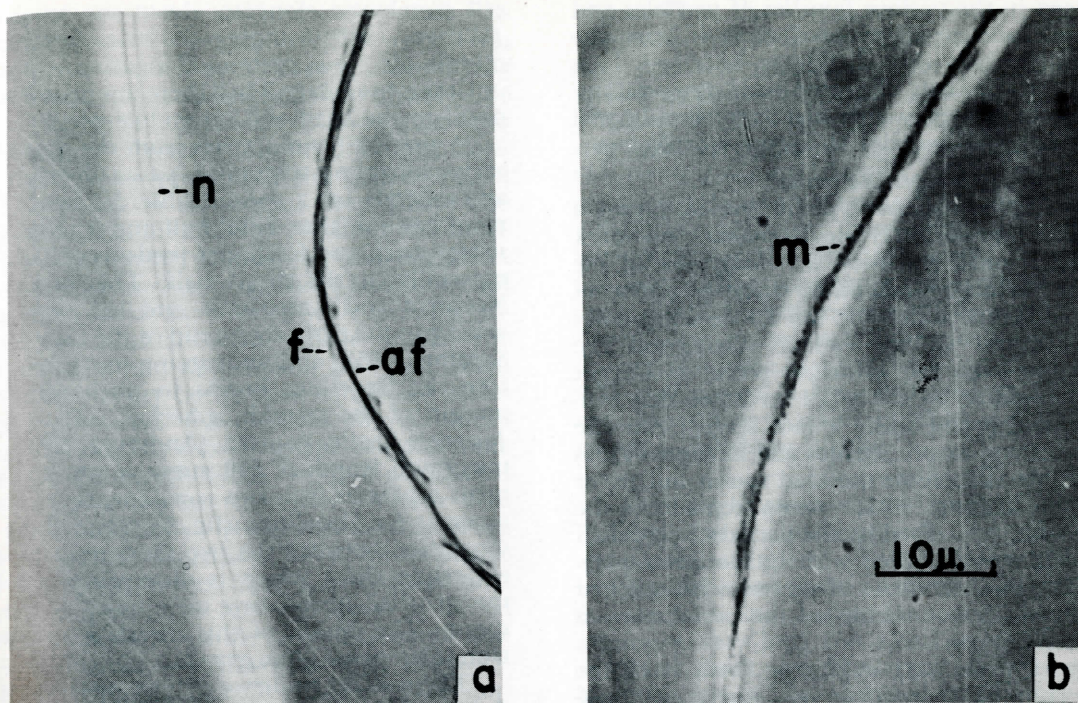
Plate 3. Necturus sperm; photographs of living cells; a, helical curvature of nucleus, n; f, flagellum winding around axial filament, af; b, axial filament after reaction with tetrazolium chloride showing mitochondria, m.

centrate in a substrate of isotonic salt solution with no perceptible nutritive material. Such masses, when placed in a refrigerator at 5 degrees Centigrade, show very rapid motility each day when examined over a period of 16 days. Agitation could possibly induce this motility but it is believed these sperm are moving continuously during this entire time. Changing the substrate to pure water produces no perceptible change in motility nor does there seem to be any noticeable increase in motility when the temperature is raised to 25 degrees Centigrade.

Motility in Necturus sperm is helical and straight with no helical path of motion. The anterior perforatorium, being slightly curved, appears double in rapid rotation because of after image effect. The elongated head, being straight, exhibits little movement other than a slight vibration in the more anterior thinner part due to the centrifugal force of the perforatorium. The axial filament is at first straight but as it decreases in size, the flagellum dominates by producing smaller coils that appear more active than the rest of the sperm. In a dilute suspension on a slide the tail of one sperm may cross the rotating tail of another and the two will wind up and become as one. An individual sperm tail may even contact its own head and result in a double coil. The end of such a coil best illustrates helical motility because the rotation can be clearly observed. Entangled sperm tails are transitory, however, and their continued gyrations soon

separate individuals from each other and from their own parts and normal forward helical motility again ensues.

There is no flagellar wave as observed in Amphiuma and Cryptobranchus due to the close adherence of the flagellum to the axial filament. It is apparent that a sheath holds these components together and it is difficult to envision this sheath as an undulating membrane. Actual movement of the flagellum can be observed by oil immersion magnification and this appears as a slight vibration progressing distally.

DISCUSSION

The original purpose of this study was that of describing the sperm of Cryptobranchus and Necturus and to point out the differences that exist between these and the sperm of Amphiuma. However, to really understand the modification of the organelles, the morphology must be related to spermateleosis, the type of motility, the sexual cycle and the duration of flagellar activity.

Several sperm of the families Ambystomidae, Salamandiae and Plethodontidae have been described (Retzius, 1906) and these all have a neck piece, an axial filament and a flagellum that has conspicuous waves. The process of spermateleosis in Salamandra (Meves, 1897) is in general similar to that of Amphiuma (Baker, 1962) and this has been considered as typical for the urodeles (Wilson, 1928). It is quite apparent, however, that major differences exist in both spermateleosis and sperm

morphology in the various families of urodeles and generalizations must be avoided until at least one representative from each family is described. It is predicted, on the basis of preliminary observations, that different genera within the same family will exhibit diagnostic features in sperm morphology.

Cryptobranchus

Cryptobranchus sperm is distinctive in having a small acrosome in early development, no neck piece evident until maturity, no elongation of the ring down the axial filament, no distal extension of the cell membrane down the tail and consequently no mitochondria on the tail to distinguish the middle piece from the principal piece. McGregor (1897) commented on the small acrosome that cannot be distinguished until a late stage, with the vesicle appearing empty. Burgos and Fawcett (1956) saw a sizeable vacuole in the toad sperm but never an acrosomic granule. This vacuole seems to be the acrosome when visualized by the periodic-acid-Schiff reaction and may be filled with intracellular polysaccharides. The acrosome elongates with nuclear elongation but is not visible on the perforatorium of the mature sperm.

The neck piece may appear to form from the granule (proximal centriole of others) in Salamandra (Meves, 1897; Gatenby, 1931; Bowen, 1922) but in Amphiuma it arises from the nucleus and remains within the nuclear membrane (Baker, 1962). This granule is prominent in Cryptobranchus and is the point of origin of the combined axial filament and flagellum but there is no evidence of a neck piece other than a slightly enlarged end of the axial filament in a mature sperm (fig. 8). This neck piece was observed by Smith (1912), and McGregor (1899) assumed its size was causally connected with the length of life of the sperm. He pointed out that spermateleosis occurs with great rapidity, just prior to the breeding season so that sperm are matured in late August, barely in time to fulfill their function.

Cryptobranchus deposits its eggs in September (Smith, 1912), the female is devoid of spermatheca and fertilization is external; a unique feature for an American urodele. In other urodeles the sperm are deposited in cloacal spermathecae and may remain viable for a year of more (Baker, 1962). Whether the neck piece is related to this specialization is conjecture for its function has not been determined. Although both Smith and McGregor studied the north-east Cryptobranchus sub-species from Pennsylvania the time of spermateleosis seems to be the same. Collections from Spring River, in Arkansas, for two years indicate this uniformity.

The prominent ring (distal centriole of others) appears early in association with the granule. There is no indication that it elongates down the axial filament as described for Salamandra (Meves, 1897) and Amphiuma (McGregor, 1899; Baker, 1962). A resume of this and other problems dealing with the ring-granule (centrioles) is given in detail in the report on Amphiuma. In a majority of sperm the

cell membrane elongates down the axial filament (Wilson, 1928) bringing the mitochondria to the middle piece but such an elongation is not evident in Cryptobranchus. Thus there are no mitochondria distal to the head and no middle piece.

A single filament arises from the granule in the early elongating spermatid, passes through the ring and extends for a considerable distance. This filament has a smaller distal half and is vibratile, i.e. sweeps back and forth and tends to coil and uncoil especially in the distal half. This occurs also in Necturus and Amphiuma and has been observed arising in the early spermatid of the rat (Austin and Sapsford, 1952) and undergoing movement even before the flagellum begins to push out from the margin of the roughly spherical cell, and for the Japanese beetle, Popillia (Anderson, 1950). When a maximum length of 370 micra is attained this filament separates into two components, an axial filament and a flagellum (fig. 5). Since there is little separation between the two there must be a sheath encircling them which is distensible and becomes the so-called undulating membrane of the mature sperm. The details of this sheath must await electron microscope photographs.

There is no evidence of any motility of an undulating membrane or axial filament other than that resulting from flagellar movement. On sperm maturity the elongated flagellum, paralleling the axial filament (fig. 7) shortens by forming into waves the entire length of the axial filament (fig.) with only a short free end piece 12 to 16 micra long. The axial filament increases considerably in diameter, and in the mature sperm it tapers slightly distally.

Mitochondria and Motility

Energy for motility is associated with the mitochondria as Novikoff (1961) concludes: "mitochondria are the chief, if not the exclusive, site of oxidative phosphorylation," and certain tetrazolium chloride salts form formazan, when they react with the substrates, indicating centers of energy transfer. It is assumed that the mitochondria are invariably present in the middle piece of sperm but there is no evidence of such a portion in Cryptobranchus sperm; thus no mitochondria are apparent distal to the head. Large protoplasmic beads are invariably present on the sperm head and two sperm in juxtaposition have a single large bead (Pl. 2d). Smith (1912) observed this mass of protoplasm surrounding the posterior part of the head of Cryptobranchus sperm. These beads contain mitochondria. Even when middle piece mitochondria are present there still is no explanation of how the energy is transferred to the flagellum. Heidenhain (1911) believed the chemical energy must be distributed locally throughout the length of the motile organelle and Nelson (1954) shows ATPase activity in tail fractions of the bull sperm to be three times that of mid-pieces, and in the rat sperm he (1958; 1959) visualizes flagella TAPase succinic dehydrogenase activity in association with the outer

most array of fibrils but not the two central filaments. Bishop (1962), in an excellent review on Sperm Motility, states that ATPase is a contractile protein in the sperm tail, and that muscle like ATPase-active components exist in association with the longitudinal filaments. However he also feels that the mid-piece, containing the mitochondrial system, is essential for movement. Gray (1958) also concludes that chemical energy required for motility must be distributed throughout the flagellum. Headless Cryptobranchus sperm have been observed with flagellar activity for a short while so apparently the energy substrate must reside within the flagellum. This would also explain how ram sperm remain motile twenty hours after exogenous stores have been exhausted (Moore and Mayer, 1941), and how the iceryine coccids sperm can flagellate even though the mitochondria "remain unchanged in the cytoplasm and are discarded in toto with the rest of the cell body" (Hughes-Schrader, 1946).

The prominent flagellum of Cryptobranchus (Pl. 2e; fig. 8) appears to have a two-dimensional motility but regardless of how the sperm is turned the waves are always visible and this could not occur in a two-dimensional system. The flagellum is not helical as every part of it remains equidistant from the axial filament. Confined to the periphery of an enveloping sheath each wave bends in an arc to follow the contour of the sheath. The wave of motility appears to originate at the base of the sperm head and progress distally but short sections can undergo wave formation independent of the remainder, and Bishop (1959) concludes that conversion of chemical energy to mechanical energy occurs in the respective flagellar segments. Perhaps Cryptobranchus sperm flagellum motility approaches that of the sea-urchin (Gray, 1955) where the wave is at first two-dimensional, but as it sweeps distally it is converted into a three-dimensional wave which gives the sperm a helical spin about the axis. Cryptobranchus sperm axial filament has a three-dimensional twist that contributes to the helical motility.

The many problems associated with sperm morphology, physiology, energy transformations, motility and permeability are reviewed in recent publications (Bishop, 1961; 1962a, 1962b) to which the reader is referred for further information. "The nature of the mechanism of sperm motility remains a major problem" (Bishop, 1962b, p. 293).

Hydration and Fertilization

The lack of motility in Cryptobranchus sperm taken from the testes or sperm duct is unusual for urodeles; so also is external fertilization (McGregor, 1899; Smith, 1912). Noble (1931) reports external fertilization characterizes the two most primitive families of urodeles, Hynobiidae and Cryptobranchidae, and possibly the Sirenidae. Reese (1904) stripped Cryptobranchus sperm from males and reported no motility. Other urodeles deposit spermatophores that are taken up or placed in the female cloaca where the sperm are stored in the sperma-

theca. Thus the sperm never contact water. The Cryptobranchus female deposits the unfertilized eggs and the male discharges seminal fluid and secretions from the cloacal glands with the sperm (Bishop, 1943). The sperm must therefore swim in the water to reach the eggs. Sperm removed from the testes have no motility, nor is there motility after eight days in isotonic salt solution at 5 degrees Centigrade, yet when these same sperm are diluted with water active motility results. In pure water for four days at the same temperature the sperm have motility when examined on a slide. It is believed these sperm have been moving continuously during this time. It has been reported that ram and bull sperm become hydrated by permeability changes and as a result capable of full metabolic activity (Salisbury, 1956).

Necturus

This single genus of the family Proteidae, has a sperm that is also diagnostic. It is the longest of any urodelean sperm (approximately 900 micra), has a prominent neck piece that originates from the nucleus and a flagellum that winds tightly around a very long but small axial filament.

Many years ago McGregor (1899) noted that Necturus sperm have matured by October and that maturation probably occurs in early summer. All stages of spermateleosis can be obtained from testes late in September, while mature sperm have been taken from the sperm duct in October until April. Data associated with reproduction in Necturus has been reviewed recently by Harris (1961).

The acroblastic vesicle (fig. 11) has no visible acrosome except when the periodic-acid-Schiff method is used. The granule and ring are typical of other urodeles except that the ring is larger and has an inner chromophilic and outer chromophobic region. This is somewhat comparable to that found by Sotelo and Trujillo-Cenoz (1958) in Ambystoma where the ring does not have a centriolar structure and is called a "distal ring." It is divided into a light distal and dark distal ring on the basis of penetration of fixative for electron microscopy. They further believe the granule (proximal centriole of others) is not a centriole and prefer to call it a "juxtanuclear body". This granule, from which the long axial filament-flagellum arises, is separate and distinct from the neck piece anlage (figs. 11-15). The separation of the axial filament from the flagellum is evident as the cell membrane extends distally. This membrane probably extends down the entire axial filament since mitochondria can be observed for its entire length.

Mitochondria

These minute organelles can be observed individually by light microscopy after reaction with tetrazolium chloride salts (Pl. 3b) due to their distribution on the very slender axial filament. Energy for motility is closely associated with these mitochondria. Sperm removed from the testes or sperm duct always exhibit rapid motility in isotonic solution of salt. An abundance of mitochondria are present

in October, shortly after maturity of the sperm (fig. 16), are considerably less in December (fig. 17) and quite scant in February (fig. 18). It is believed the mitochondria are consumed or eliminated in energy degradation similar to their decrease in maturation of erythocytes (de Robertis et. al., 1954). It has been stated that a decrease in ATP content coincides with impairment and final loss of sperm motility (Mann, 1945).

Motility

The entire sperm is helical in structure beginning with the perforatorium, down the head, the neck piece and through the axial filament (Pl. 3a; fig. 19). The perforatorium spins rapidly as the sperm rotates, with enough force to vibrate the anterior half of the head. The posterior half, being larger, offers more resistance as does the proximal portion of the axial filament. The flagellum winds about this filament and appears to be held tightly by a sheath (Pl. 3a), which is evidently homologous to the broad undulating membrane of other urodele sperm. This axial filament decreases in size distally and this size difference is reflected in movement resulting from flagellar activity. Little motion is noted in the proximal third of the tail as the axial filament resists bending, and little flagellar movement can be detected here other than a rapid vibration passing distally. In the middle third the helical coil is apparent due to the flagellum being able to bend this smaller filament, while in the distal third the flagellum dominates the filament and bends it into a series of small helical coils that rotate with considerable speed. The rotation rate seems to be independent of temperature and when examined on a slide various speeds are apparent in the same suspension.

CONCLUSIONS

The sperm of Cryptobranchus and Necturus seem to be diagnostic for their families. Cryptobranchus sperm has a minute neck piece, the ring does not enlongate, the cell membrane does not extend down the tail, no middle piece can be distinguished and mitochondria are restricted to protoplasmic beads on the head. Motility is helical.

Necturus sperm has a very elongated neck piece, the ring does not elongate as the cell membrane extends down the tail, no middle piece can be distinguished because the mitochondria are distributed along the entire axial filament. The flagellum is wound around the axial filament for its entire length.

Quantitative determinations can be made of mitochondria due to their sparce distribution and they diminish in number from October to February.

Addition of water to a suspension of Cryptobranchus sperm activates flagellar activity and this seems to be correlated with the unusual feature of external fertilization.

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EXPLANATION OF FIGURES

Permanent stained stages of spermateleosis in Crypto-branchus (1-9) and Necturus (10-19). Abbreviations: acb, acroblast; acr, acrosome; af, axial filament; ep, end piece; f, flagellum; m, mitochondria; n, nucleus; np, neck piece; pb, protoplasmic bead; um, undulating membrane or sheath.

1. Spermatid with numerous mitochondria and acroblast.

2. Later spermatid with ring and granule, mitochondria and acroblast.

3. Elongation of the spermatid; acroblast and ring-granule oriented at poles of nucleus; filament that is combination of axial filament and flagellum emerges from granule and passes through the ring. This filament has motility.

4. Later stage. Posterior nuclear material condensing; small acrosome in acroblast; filament is 370 micra long.

5. Axial filament and flagellum are separated with axial filament increasing in size.

6. Elongated acrosome in acroblast outside the nuclear membrane.

7. Perforatorium at anterior end of nucleus; axial filament now of maximum thickness.

8. Mature sperm of Cryptobranchus; protoplasmic bead with mitochondria; flagellum is drawn into waves alongside the axial filament.

9. The end piece and portion of the flagellum and sheath have separated from the axial filament.

10. Spermatid with spherical mitochondria, acroblast, granule and double ring.

11. Elongation of the spermatid nucleus and origin of neck piece inside the nuclear membrane; filament emerges from granule and passes through the ring.

12-14. Further elongation of the spermatid; neck piece is elongating and chromatin is condensing.

15. Maximum elongation; axial filament and flagellum are now separated; cell membrane is elongating down the axial filament.

16. Enlarged portion of the axial filament and flagellum with mitochondria after treatment with tetrazolium chloride; made in October.

17. The same; made in December. The mitochondria are reduced in number.

18. The same; made in February.

19. Mature sperm of Necturus; total length 900 micra. (Note that sketch is one-half size of developmental stages.

BOOK REVIEW

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Exploring the Secrets of the Sea. By William J. Cromie. Prentice-Hall, Inc. Englewood Cliffs, N.J. 315 p. $5.95.

This book is a good introduction for the layman to the science of oceanography. It has been written with a minimum of technicality and enlivened with anecdotes of experiences in the field (or should I say "in the ocean"). The subject matter covers all parts of the oceans from the Arctic to the Antarctic and from the surface of the sea to the bottom of the Marianas Trench. There are one or more chapters on each of the following: the oceans and oceanic circulation, floor of the ocean and its sediments, life in the sea, waves, tides, methods of exploring the ocean. All of these subjects are discussed clearly and some of them very well; the chapter on tides is exceptionally good. The first two chapters, on the origin of the earth and on the origin of life, are less satisfactory since they are based largely on the conjecture and hypotheses of other scientists, and the hypotheses suggested are not directly related to oceanography.

The book is well illustrated with photographs and with maps and drawings executed by George Geygan. There are occasional examples of poor editing, such as a footnote in Chapter 8 saying that a certain process "will be explained in Chapter Two."

The author has succeeded very well in picturing oceanography as a modern and active science with as many clearly recognized problems ahead of it as there are accomplishments behind it. It was prophetic that the pioneering oceanographic vessel which began its first survey in 1872 was named "Challenger", although, on the other hand, oceanographers may consider themselves to be more challenged than vice versa. There are opposing hypotheses for many phenomena observed in the ocean, and these disagreements appear in this book, showing that oceanography is far from a dead science. Although the author does not say so, I am sure that he would agree that many of millions of dollars now being spent on space research could be much more profitably expended for research in the oceans.