ULTRASTRUCTURAL STUDIES OF CYTOPLASMIC DROPLETS OF SOME PLETHODONTID SALAMANDER SPERMATOZOA

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Ultrastructural studies of cytoplasmic droplets of the spermatozoa of bulls, rams, rabbits, rats (Bloom & Nicander, 1961) and bats (Fawcett & Ito, 1965) revealed only numerous membranous vesicles and tubules in the matrix of the droplets. These authors agreed that the membranous elements were probably of endoplasmic reticulum and/or Golgi complex origin. The simplicity of the ultrastructure and lack of other organelles and inclusions in the droplet matrix led Fawcett & Ito (1965) to doubt suggestions that cytoplasmic droplets contain endogenous substrates for epididymal sperm survival.

Recent biochemical and cytochemical analyses of cytoplasmic droplets in spermatozoa of different domestic animals (Dott & Dingle, 1968; Harrison & White, 1972; Moniem & Glover, 1972) showed a rather high activity of hydrolytic and glycolytic enzymes. Harrison & White (1972) suggested that at least some of the enzymes found in the seminal plasma may have their origin in disrupted cytoplasmic droplets.

In our study (Martan, Brandon & Wortham, 1973) of interspecific variation in sperm morphology within the salamander family, Plethodontidae, we have observed that the spermatozoa have a characteristic cytoplasmic droplet. The seven genera studied were Desmognathus, Eurycea, Typhlotriton, Aneides, Ensatina, Plethodon and Bolitoglossa. Cytoplasmic droplets of sixteen species were examined with the light microscope, and those of Plethodon glutinosus were also examined with the electron microscope.

For light microscopy, seminal fluid was removed from the ductus deferens of freshly killed or anaesthetized males, spot-smeared on slides lightly coated with albumin, and fixed for 30 min in 95% ethanol. Slides for cytological details were stained in Heidenhein iron haematoxylin, while slides for localization of RNA were stained by the azure B technique (Swift, 1955). Control slides were exposed for 2 hr to RNAsase before staining (Barka & Anderson, 1963). Living spermatozoa were supravitally stained with Janus green.

For electron microscopy, spermatozoa were obtained by the same procedure and immediately fixed at 4° C in paraformaldehyde (Lynn, Martin & Race, 1966) and post-fixed in 1% osmium tetroxide buffered with s-collidine buffer (Bennett & Luft, 1959) at pH 7-4. The fixed spermatozoa were resuspended in 2% agar after centrifugation and cut into 0-5-mm blocks and stained with 1%
methylene blue for ease of locating the spermatozoa in the agar. The agar blocks were dehydrated through a graded ethyl alcohol series (to 100%) and then through two changes in propylene oxide and finally embedded in Epon 812 by the method of Luft (1961). Capsules were hardened overnight at room temperature, followed by 3 days’ incubation at 60°C. Polymerized blocks were sectioned with a diamond knife on a Reichert Om U2 ultramicrotome. Sections were heated at 60°C for 20 min and then double-stained with 5% aqueous uranyl acetate for 1 hr at room temperature, followed by 0.25% lead citrate for 5 min. Specimens were examined with an Hitachi HU11AB electron microscope operating at an accelerating voltage of 50 kV.

The characteristic cytoplasmic droplet observed on spermatozoa of all species examined is an elongated bulbous structure attached laterally to the spermatozoon anywhere from the head to the end of the mid-piece (Pl. 1, Fig. 1). These cytoplasmic droplets measure from 11 µm to 30 µm in length and many contained a dense body (bodies) located close to that surface of the droplet which is not attached to the spermatozoon (Pl. 1, Fig. 1). The whole cytoplasmic droplet showed a diffuse but strong reaction for RNA which was abolished by pretreatment for 2 hr with RNase. The Janus green reaction in living spermatozoa indicated the presence of mitochondria in the cytoplasmic droplets.

The cytoplasmic droplet of Plethodon glutinosus was surrounded by a single membrane, somewhat porous in appearance. A characteristic structure observed in many cytoplasmic droplets was a complex of several concentric membranes forming a closed system shown in Pl. 1, Figs 2 and 4. Sometimes, the portion of the membrane complex appeared to be unwinding or reorganizing into simpler membranous units indicated by arrows in Pl. 1, Fig. 2. Tubular components were often found near this membranous complex (Pl. 1, Figs 4 and 5). The amorphous granular contents within the membranous structure were similar to cytoplasmic matrix outside the structure. The membranous complexes were always located at the outer (unattached) surface of the cytoplasmic droplet corresponding to the dense bodies observed with the light microscope (Pl. 1, Fig. 1).

The presence of mitochondria was confirmed in thin sections, the organelles being randomly located in the cytoplasmic droplet. This contrasted with the

EXPLANATION OF PLATE 1

Fig. 1. Photomicrograph showing a characteristic cytoplasmic droplet attached laterally to the spermatozoon and containing a dense body (DB).

Figs 2 to 5. Transmission electron micrographs of cytoplasmic droplets of plethodontid salamander spermatozoa.

Fig. 2. Cytoplasmic droplet illustrating concentric membranes (CM) comparable to the dense body in Fig. 1. Arrows indicate portions of the membrane complex which appear to be unwinding or reorganizing into simpler membranous units. Note the regularly placed mitochondria (M1) surrounding the axial filament, compared to the random locations of mitochondria (M2) in the cytoplasmic droplet. Vesicles (V) surrounded by a single membrane and cytoplasmic structures (CS) are also present. The latter are located predominantly near the outer membrane.

Fig. 3. Cytoplasmic droplet containing an osmiophilic body (OB).

Fig. 4. Cytoplasmic droplet illustrating concentric membranes (CM) and tubular components (TC) in juxtaposition.

Fig. 5. Tubular components (TC) from Fig. 4 shown at a higher magnification.
regular placing of the mitochondria in the cytoplasm surrounding the axial fibre (Pl. 1, Fig. 2).

The cytoplasmic droplet contained many variably sized vesicles limited by a single membrane containing granular material (Pl. 1, Fig. 2). Cisternal structures in the cytoplasmic droplet were located predominantly near the outer membrane (Pl. 1, Fig. 2). Osmiophilic bodies were occasionally observed in the cytoplasmic droplet (Pl. 1, Fig. 3). These could be lipid droplets or accumulated breakdown material.

The localization of mitochondria, cytoplasmic RNA and a highly complex membrane system in cytoplasmic droplets suggests that the droplets are functional and may play an important rôle in the maturation of spermatozoa of plethodontid salamanders.

Wilder (1913), who called the cytoplasmic droplet 'a corpuscle', observed it on spermatozoa taken from the cloaca of female Desmognathus fuscus. Her report indicates the longevity of the cytoplasmic droplet and supports our contention that this structure has a functional rôle. As early as 1963, Baker noticed mitochondria in cytoplasmic droplets of salamander spermatozoa. Recently, Hruban, Martan & Aschenbrenner (1971) reported on the complexity of the membrane system found in the cytoplasmic droplet of spermatozoa in the flying squirrel.

The highly organized structure of the cytoplasmic droplet in the flying squirrel and plethodontid salamander makes it seem unlikely that the droplets in these groups are only cytoplasmic remnants without only functional significance.

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