Lysosomal Enzymes in Bull and Ram Semen

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Certain lysosomal enzymes in bull and ram semen are sequestered into a membrane-limited cytoplasmic droplet, which is shed from the spermatozoon during maturation but persists in the seminal plasma. Changes in pH or osmoticity may release the sequestered enzymes, thus contributing substantially to the activity of the seminal plasma. Of the enzymes assayed (acid phosphatase, acid protease, β -glucuronidase, ribonuclease and hyaluronidase), only hyaluronidase showed substantial activity in the spermatozoa (Dott & Dingle, 1968). This suggested that lysosomal enzymes involved in carbohydrate metabolism might be sequestered differently from others. To test this, droplets were separated as before and it was found that in the bull the activities of β -N-acetylglucosaminidase, β -galactosidase and hyaluronidase are predominantly in the spermatozoa, but acid phosphatase, β -glucuronidase, acid protease and ribonuclease are concentrated in the droplet; but in the ram the spermatozoa contain appreciable acid phosphatase, acid protease and ribonuclease activities in addition to the other enzymes.

The outer membrane of the egg (zona pellucida) is probably composed of mucoprotein (Austin, 1961), and it is possible that the lysosomal enzymes capable of carbohydrate catabolism are retained within the spermatozoon to assist in its passage through the zona pellucida before fertilization. On the other hand, acid hydrolases that might degrade nucleoprotein are removed from the spermatozoa.

The removal is less complete in ram, which might explain why efforts to store ram spermatozoa *in vitro* have not been successful.

The acrosome of the spermatozoon is formed from granules arising in the medulla (idiosome) of the Golgi apparatus in the early spermatid. The stacked lamellae and spherical vesicles that comprise the rest of the Golgi apparatus at this stage later become detached from the acrosome and migrate towards the other side of the nucleus, where they contribute to the cytoplasmic droplet (Bishop & Walton, 1960). In view of this division of the Golgi apparatus and our findings on the distribution of lysosomal enzymes it seems likely that different enzymes are held in different Golgi vesicles. Thus a study of the distribution of lysosomal enzymes in the developing spermatozoon may be of importance not only in the physiology of reproduction but also in the examination of the control and synthesis of lysosomal enzymes in other cells.

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Lysosomal Nature of the Acrosomes of Ram Spermatozoa

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A study of procedures for detaching acrosomes (head-caps) from washed spermatozoa (Hathaway & Hartree, 1963) led to a method, using the cationic detergent Hyamine 2389, for obtaining suspensions of acrosomes virtually free from spermatozoa (Hartree & Srivastava, 1965). The acrosomes separated from ram spermatozoa disintegrate on storage to yield a lipoglycoprotein solution (LGP) containing hyaluronidase and protease activities (Srivastava, Adams & Hartree, 1965). When freshly ovulated rabbit ova are placed in LGP the cumulus cells and the cells of the corona radiata, which surround the ovum, are dispersed. In addition the zona pellucida, the thick outer membrane of the ovum, begins to disintegrate (Srivastava *et al.* 1965).

These results suggest an analogy between acrosomes and lysosomes. When mammalian spermatozoa are treated with Euchrysene 3R and examined by fluorescence microscopy the acrosomes exhibit the red fluorescence characteristic of lysosomes (Allison & Young, 1964). Histochemical reactions show that the acid phosphatase of spermatozoa, both vertebrate and invertebrate, is concentrated in the acrosome (Ruffili, 1960; Monroy, 1965; Buongiorno-Nardelli & Bertolini, 1967). An esterase of guinea-pig spermatozoa is located exclusively in the acrosomes.

Washing ram spermatozoa with 50mM-tris-HCl buffer removes all seminal plasma and all light-microscopically visible particles except for 1% of the cytoplasmic droplets. LGP from such spermatozoa contained acid phosphatase, β -N-acetylglucosaminidase, arylsulphatase and phospholipase A, and also protease activities against denatured haemoglobin. Enzyme activities in LGP were in all cases considerably lower than activities in the buffer washings. Thus the acid phosphatase of seminal plasma (enzyme in washings not sedimentable at 25 000g) represented 50% of total activity recovered from ram semen. The activities of acid hydrolases

in semen from vasectomized rams were extremely low: acid phosphatase activity was <1% of the total activity of normal semen. Thus the bulk of 'soluble' enzymes recovered in the plasma presumably diffused from organelles (e.g. droplets and acrosomes).

We conclude that the acrosome is a specialized lysosome that has evolved to facilitate fertilization in multi-cellular organisms.

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Distribution and Behaviour of some Glycolytic Enzymes in Spermatozoa and Cytoplasmic Droplets from Mammalian Semen

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Semen from the bull, boar and ram normally contains, in addition to spermatozoa, small spherical particles 2–3 μ in diameter known as cytoplasmic droplets (Dott & Dingle, 1968). It is difficult to obtain a preparation of droplets essentially free of spermatozoa or a preparation of spermatozoa essentially free of droplets. One method for preparing droplets has been published (Dott & Dingle, 1968).

Another method has been developed whereby a single sample of semen can be separated easily into populations of droplets and spermatozoa, each preparation containing less than 5% contamination of the other particle. This method involves slow cooling and storage at 4° in a milk diluent, followed by slow differential centrifugation and washing in a sucrose medium.

Analysis of hexokinase, glucose phosphate isomerase and lactate dehydrogenase activities in our preparations revealed certain intrinsic differences in enzyme distribution between the spermatozoa and droplets: in particular, although both spermatozoon and droplet contain very large amounts of glucose phosphate isomerase (the amount per particle is approximately equal), the droplet contains less than one-tenth the amount of lactate dehydrogenase activity that is found in

the spermatozoon. Hexokinase is partly bound in each type of particle, glucose phosphate isomerase is entirely soluble, but lactate dehydrogenase is partly bound in the spermatozoon and soluble in the droplet.

Because of the great practical importance of storage of deep-frozen semen for artificial insemination, much research has been done on the mortality of spermatozoa when deep-frozen or cold-shocked, and emphasis has been placed on the leakage of enzymes from the spermatozoon during these treatments (Murdoch & White, 1968). We therefore cold-shocked, deep-frozen, and shocked hypo-osmotically our preparations and studied the leakage of enzymes by using glucose phosphate isomerase and lactate dehydrogenase as differential markers. The results show that the droplet preparations are very susceptible to all of the treatments, particularly cold-shock and deep-freezing, and leakage of enzymes occurs readily under these conditions. In all cases, although leakage from spermatozoa occurred, the droplets were more fragile and a much greater percentage of their total enzyme content leaked out.

Thus it seems that when enzyme analyses are carried out on seminal constituents, care must be taken to differentiate between the contribution from the droplets and the contribution from the spermatozoa.

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An Electron-Microscope-Autoradiographic Investigation of the Localization of ²⁴¹Pu in Rat Liver

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The intracellular sites of storage in the liver of various colloidal metals have been investigated as a result of the interest in 'bone-seeking' elements (Langham, 1959; Taylor, 1964). Plutonium has been shown to remain in the liver for several months (Taylor, Sowby & Kember, 1961). The reports from biochemical investigations of cell fractions have been conflicting as regards the association of injected plutonium with lysosomes. Rahman & Lindenbaum (1964) demonstrated a close relationship between lysosomal enzymes and cytoplasmic vacuoles containing segregated polymeric ²³⁴Pu in rat liver. Taylor and associates partially confirmed these findings but proposed in addition that mitochondria may also be sites of