Spermiogenesis in the Brush-tailed Possum, *Trichosurus vulpecula* (Marsupialia)

The Development of the Acrosome

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Summary. Acrosome development in the Australian Brush-tailed possum, *Trichosurus vulpecula*, displays a number of extraordinary features. This is particularly evident in the later stages of spermiogenesis, when the area of the nuclear surface bounded by the nuclear ring, and covered by the acrosome, is reduced considerable. As a result, the acrosomal material becomes located over its definitive position on the anterior third of the dorsal nuclear surface; in this process it is thrown into a series of folds, and a wide subacrosomal space is formed.

Further changes around the time of spermiation result in the release of a spermatozoon in which a thin layer of acrosomal material is closely applied to the nucleus over the area of the definitive location of the acrosome, whilst its margins are greatly extended and project freely away from the nucleus. The latter feature does not appear to have been reported for the sperm of other mammals.

**Key words:** Acrosome – *Trichosurus vulpecula* (Marsupialia) – Spermiogenesis – Transmission electron microscopy.

Introduction

The acrosome of the Australian Brush-tailed possum, *Trichosurus vulpecula*, undergoes striking morphological modifications during epididymal transit.
(Harding et al., 1976). In order to provide a basis for understanding these changes, we investigated the development of the possum acrosome during spermiogenesis, and the present paper describes these changes, including the acrosomal form in spermatozoa from the lumen of the seminiferous tubules.

However, acrosome development in *Trichosurus* is of equal interest in its own right. There have been few ultrastructural studies on spermiogenesis in marsupials, and none for *Trichosurus*. Detailed information on the ultrastructure of marsupial acrosome development is available only for the Australian bandicoot, *Perameles nasuta* (Sapsford et al., 1967, 1969), and brief observations for the American opossums, *Caluromys philander* (Phillips, 1970), *Marmosa mitis* and *Didelphis marsupialis virginiana* (Rattner, 1972).

The present study shows that acrosome development in *Trichosurus* displays a number of features which are unique among mammals investigated to date. Differences in this process between marsupials and eutherians are to be expected in view of the disparate nature of the mature acrosome in the two groups (Harding et al., 1976). However, acrosome development in *Trichosurus* also shows distinct differences, particularly in late spermiogenesis, to that in *Perameles* (Sapsford et al., 1967, 1969) the only other marsupial in which acrosome development has been investigated in detail.

**Materials and Methods**

Testicular material was obtained from three adult male brush-tailed possums (*Trichosurus vulpecula*) captured in the Sydney metropolitan area. The tunic of the testis was cut to expose the seminiferous tubules and two types of testis sample were taken. For the first sample, small pieces of the tubules were cut and placed for 30 to 60 minutes in modified Karnovsky's fixative with picric acid added. For the other sample, small pieces of the seminiferous tubules were extensively lacerated with a sharp razor blade under the above fixative and shaken vigorously with fixative in a stoppered centrifuge tube; after which the remaining tubular tissue was removed from the suspension and discarded. After fixation for 30 to 60 min at room temperature, this sample was spun at about 1900 RCF for 10 min. Both samples were then washed overnight in cacodylate/sucrose buffer and post fixation was carried out in the cold for 60 min using osmium tetroxide/cacodylate/glucose, and stained for 30 min in uranyl acetate prior to dehydration. Specimens were examined using a Philips EM 201 or EM 300.

**Results**

In early spermatids of *Trichosurus*, the first evidence of acrosome development is seen when the rounded acrosomal vacuole becomes apposed to the nuclear surface adjacent to the Golgi complex (Fig. 1). Golgi vacuoles are located between the Golgi complex and acrosomal vacuole at this stage (Fig. 1), and presumably add to the latter and bring about its enlargement as in other species (Burgos and Fawcett, 1955; Bedford and Nicander, 1971).

Following this initial apposition the acrosomal vacuole smoothly indents the adjacent nuclear surface (Fig. 2), and its formerly convex outer margin begins to flatten and collapse towards the nucleus (Fig. 3). The Golgi complex commences its movement towards the opposite pole of the nucleus, and is now located to the side of the acrosomal vacuole (Fig. 3).