A Lectin-Histochemical Study on the Seminiferous Epithelium of the Northern Smooth-Tailed Tree Shrew (*Dendrogale murina*) and the Java Tree Shrew (*Tupaia javanica*)

By

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Summary: Lectin-binding patterns in the testes of the northern smooth-tailed tree shrew, *Dendrogale murina* and Java tree shrew, *Tupaia javanica* were studied by light microscopy and compared the data with those of the common tree shrew. Four lectins (PNA, SBA, BPA and GS-II) were used in this study. Peanut (*Arachis hypogaea*) agglutinin (PNA), soybean (*Glycine max*) agglutinin (SBA) and *Bauhinia purpurea* agglutinin (BPA) showed a strong reaction in the acrosomal region from Golgi to acrosome-phase spermatids in three species of tree shrews. These lectins also showed a granular positive reaction in the cytoplasm from acrosome to maturation-phase spermatids in three species, except that BPA revealed no granular reaction (though it was positive) in the spermatid cytoplasm of the northern smooth-tailed tree shrew and that PNA revealed no reacion in the spermatid cytoplasm of the common tree shrew. While, *Griffonia simplicifolia*-II agglutinin (GS-II) showed a positive reaction in the acrosomal region of Golgi-phase spermatids in three species of tree shrews. Although GS-II was positive in the spermatocyte cytoplasm of three species, it showed granular in the northern smooth-tailed tree shrew and common tree shrew but not granular in the Java tree shrew. Thus, the lectin-binding patterns in testes were similar among three species belonging to the Order Scandentia. However, slight differences were also detected even among these phylogenetically-close species.

A number of lectins have been used as histochemical reagents to detect the distribution of glycoconjugates in various tissues. Lectin-histochemistry has been carried out in the testes of many mammalian species (Yamamoto, 1982; Arya and Vanha-Perttula, 1984, 1985, 1986; Lee and Damjanov, 1984, 1985; Malmi *et al.*, 1987, 1990; Malmi and Söderström, 1987; Wollina *et al.*, 1989; Kurohmaru *et al.*, 1991, 1995, 1996; Kurohmaru and Hayashi, 1998; Arenas *et al.*, 1998), indicating that lectin-binding patterns reveal the differences among each species. In our previous study (Kurohmaru *et al.*, 1996), we examined the seminiferous epithelium of the common tree shrew (*Tupaia glis*) by lectin-histochemistry and compared the data with those of insectivores (phylogenetically close to tree shrews), especially with the musk shrew (Kurohmaru *et al.*, 1995). As a result, the lectin-bindings of the common tree shrew were somewhat different from those of the musk shrew. However, it is still uncertain whether these lectin-binding patterns are common among tree shrews (Order Scandentia). In order to solve this problem, the present study was proposed to examine the seminiferous epithelium of other species belonging to the Order Scandentia, such as the northern smooth-

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tailed tree shrew (*Dendrogale murina*) and Java tree shrew (*Tupaia javanica*), and to compare the data with those of the common tree shrew (*Tupaia glis*) and other mammalian species.

Materials and Methods

Three adult male northern smooth-tailed tree shrews (body weight; 38–41 g), captured in Thailand, and three adult male Java tree shrews (body weight; 57–75 g), captured in Indonesia, were used in this study.

Light Microscopy

Under pentobarbital anesthesia, the animals were perfused with Ringer's solution followed by Bouin's fixative through the left ventricle. The testes were surgically excised, sliced into slabs and immersed in the same fixative for 2–3 h. They were then dehydrated in a graded series of ethanol, infiltrated in xylene, and embedded in paraffin wax. The sections (5 μm) were deparaffinized, stained with periodic acid-Schiff (PAS)-hematoxylin and examined by light microscopy to judge whether spermatogenesis was active or not.

Lectin Histochemistry

Four lectins were used in this study:peanut agglutinin (PNA, *Arachis hypogaea*), soybean agglutinin (SBA, *Glycine max*), *Bauhinia purpurea* agglutinin (BPA) and *Griffonia simplicifolia* agglutinin II (GS-II). It has been demonstrated that these lectins react with spermatogenic cells in mammals.

Sections (5 μm) of testes previously embedded in paraffin wax were deparaffinized and rehydrated, treated with 1% bovine serum albumin (BSA) in 10 mM phosphate-buffered saline (PBS), pH 7.2, and incubated with biotinylated lectins (Vector Laboratory, Burlingame, CA, USA, 25 μg/ml) in 1% BSA-PBS for 30 min. After washing with PBS, sections were incubated with avidin-biotin peroxidase complex (ABC, Vector Laboratory) for 30 min. Samples were washed again with PBS, immersed in 3,3′-diaminobenzidine (DAB. 0.2 mg/ml)-H_2O_2 (0.005%) for 10 min and rinsed in distilled water. They were stained with hematoxylin and observed by light microscopy.

Negative controls (incubated without lectins) were processed in parallel.

Results

The testes of all animals used in this study revealed active spermatogenesis. In the northern smooth-tailed tree shrew and the Java tree shrew, all lectins used here were positive in spermatogenic cells, but not in Sertoli cells. Additionally, the spermatids in these species could be easily subdivided into four phases (Golgi-, cap-, acrosome- and maturation-phases). SBA, BPA and PNA, indicative of N-acetyl-D-galactosamine and/or D-galactose residues, showed a strong reaction in the acrosomal region from Golgi to acrosome-phase spermatids of both species. These lectins also revealed a granular positive reaction in the cytoplasm from acrosome to maturation-phase spermatids, except that BPA showed no granular reaction in the spermatid cytoplasm of the northern smooth-tailed tree shrew though it was positive. In both species, GS-II, indicative of N-acetyl-D-glucosamine residues, gave a positive reaction in the acrosomal region of Golgi-phase spermatids. GS-II was also positive in the spermatocyte cytoplasm. It showed granular in the northern smooth-tailed tree shrew, but not granular in the Java tree shrew. Thus, the lectin-binding patterns of two species of tree shrews were similar with each other, except for some slight differences.

No reaction was observed in control sections.

Discussion

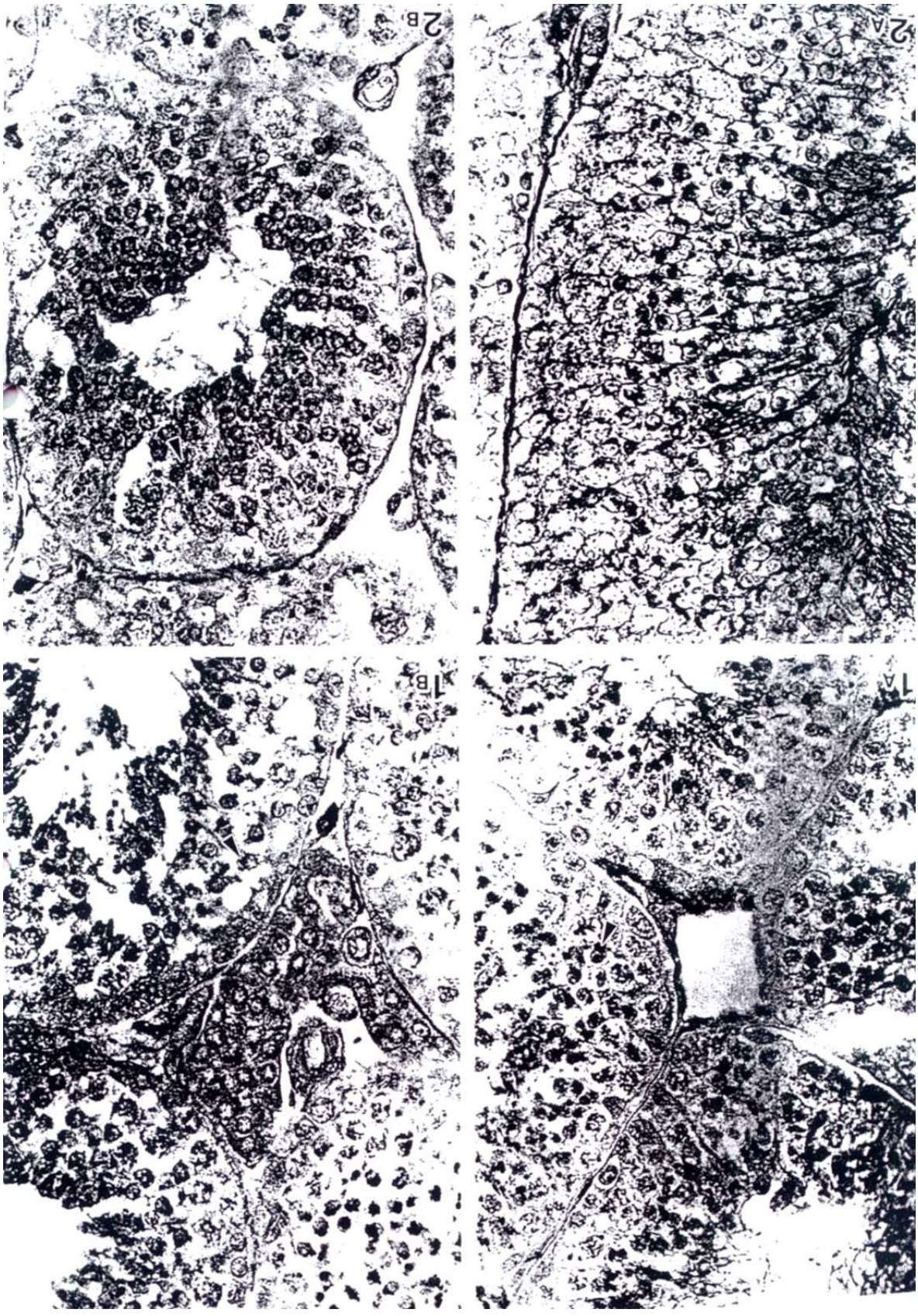
Although it has been reported in many mammalian species that SBA, PNA, BPA and GS-II show a positive reaction in the acrosomal region of spermatids, the period of the appearance/disappearance

Explanation of Figures

Plate I

Fig. 1. SBA binding sites (arrowheads) in the tree shrew seminiferous epithelium. BPA shows a reaction in the acrosomal region of round spermatids in the northern smooth-tailed tree shrew (A) and Java tree shrew (B). ×360 each.

Fig. 2. PNA binding sites (arrowheads) in the tree shrew seminiferous epithelium. PNA shows a reaction in the acrosomal region of round spermatids in the northern smooth-tailed tree shrew (A) and Java tree shrew (B). ×360 each.


2B
2A
1B
1A

of the reaction with each lectin differs for each species. For example, the PNA reaction in the acrosomal region appeared in the acrosome-phase spermatids of the guinea pig (Yamamoto, 1982) and human (Malmi *et al.*, 1987), from Golgi to cap-phase spermatids of the bull (Arya and Vanha-Perttula, 1985), from Golgi to acrosome-phase spermatids of the musk shrew (Kurohmaru *et al.*, 1995) and common tree shrew (Kurohmaru *et al.*, 1996), and from Golgi to maturation-phase spermatids of the goat (Kurohmaru *et al.*, 1991). Similar to the common tree shrew (Kurohmaru *et al.*, 1996), SBA, PNA and BPA reacted with the acrosome from Golgi to acrosome-phase spermatids of the northern smooth-tailed tree shrew and Java tree shrew. This finding indicates that glycoconjugates containing N-acetyl-D-galactosamine and/or D-galactose residues are formed in the acrosome from Golgi to acrosome-phase spermatids and disappear in maturation-phase spermatids.

Similar to the common tree shrew (Kurohmaru *et al.*, 1996), GS-II reacted with the acrosome of Golgi-phase spermatids in both species, indicating that glycoconjugates containing N-acetyl-D-glucosamine residues are formed in the acrosome of Golgi-phase spermatids and immediately disappear.

The positive reaction of BPA showed granular in the spermatid cytoplasm of the common tree shrew and Java tree shrew, but not granular in that of the northern smooth-tailed tree shrew. PNA reacted with the spermatid cytoplasm of the Java tree shrew and northern smooth-tailed tree shrew, but did not react with that of the common tree shrew. Additionally, the positive reaction of GS-II showed granular in the spermatocyte cytoplasm of the common tree shrew and northern smooth-tailed tree shrew, but not granular in that of the Java tree shrew.

Thus, the lectin-binding patterns in testes were similar among three species of tree shrews; common tree shrew, northern smooth-tailed tree shrew and Java tree shrew. However, even among these phylogenetically-close species, some slight variations of lectin-bindings in testes were recognized.

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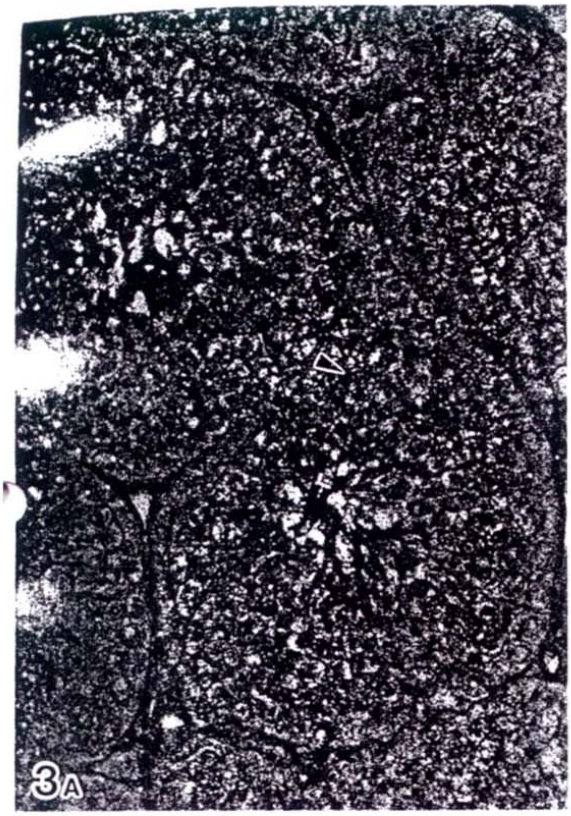
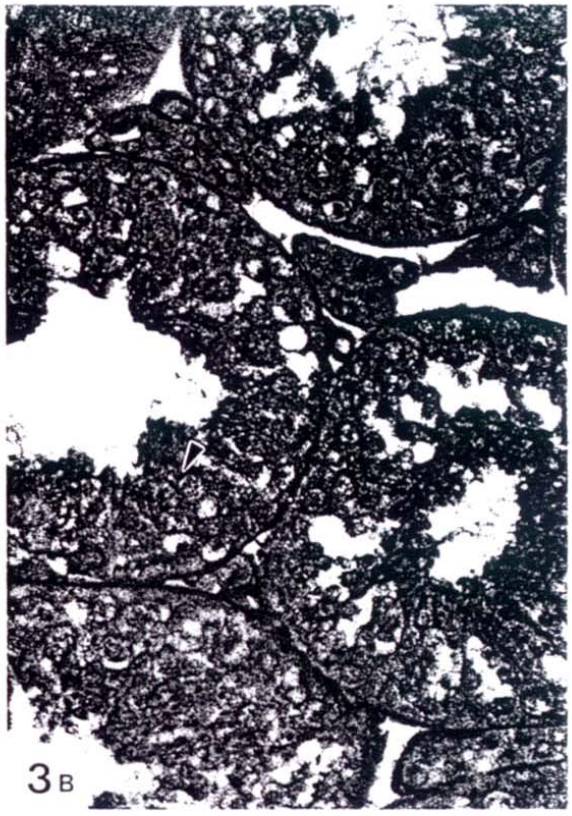
Plate II

Fig. 3. BPA binding sites (arrowheads) in the tree shrew seminiferous epithelium. SBA shows a reaction in the acrosomal region of round spermatids in the northern smooth-tailed tree shrew (A) and Java tree shrew (B). ×360 each.

Fig. 4. GS-II binding sites in the tree shrew seminiferous epithelium. GS-II shows a reaction in the acrosomal region of Golgi-phase spermatids (arrowheads) and the spermatocyte cytoplasm (arrows) of the northern smooth-tailed tree shrew (A) and Java tree shrew (B). ×360 each.


3A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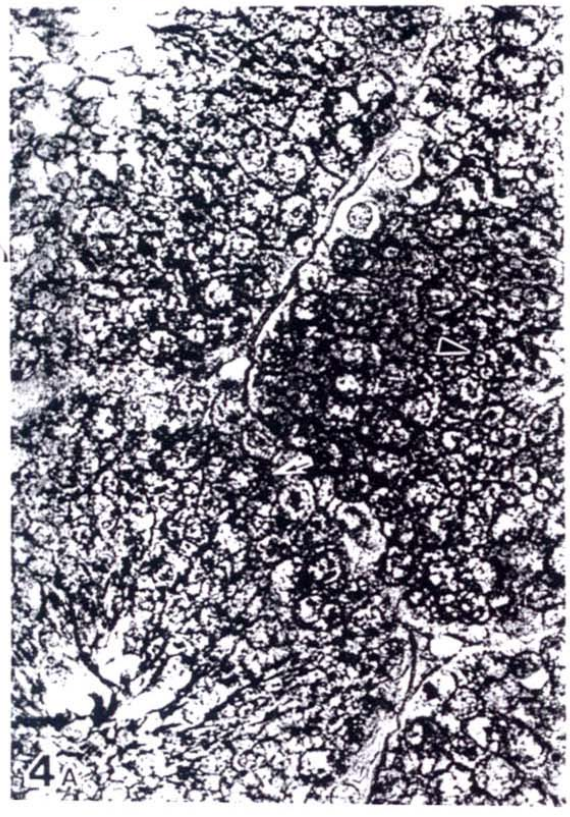
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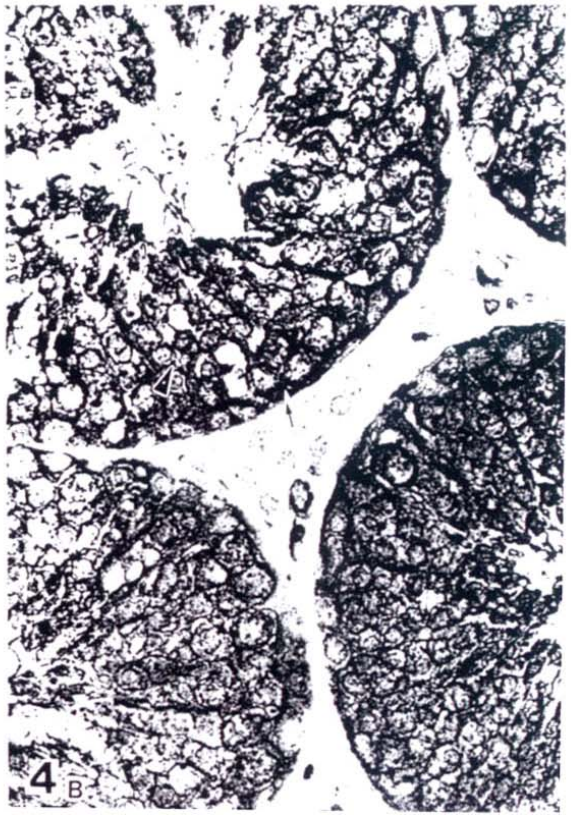
4B

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