CHAPTER 4

DISCUSSION

The glycoconjugates of ventral prostate

The rat ventral prostate has been the preferred organ for studies on androgen action, morphology, biochemistry, and molecular biology. The major protein secreted by the ventral prostate is spermine or prostatein (Gerhardt *et al.*, 1983). Mannose (Man) is the common sugar in the core region of oligosaccharides of glycoprotein, and is the first sugar added to glycoproteins in the rER, these Man-specific lectins could also stain the prostatein in the epithelial cells. This protein is a major steroid-binding glycoprotein secreted by the ventral prostate. It is known that the extension of N-linked oligosaccharides occurs in the Golgi cisternae, which contain many glycosyltransferases reponsible for the final steps of oligosaccharide synthesis. (Chan and Ho, 1999)

In this study, the rER-Golgi region in all age groups of rat prostates contain α-D-Man, α-D-Glc, GlcNAc, α-D-GalNAc, α-D-Gal, α-L-Fuc, as shown by Con A, DBA, MPA, PSA, and UEA-I. These lectins were moderately reacted within the rER-Golgi region of 2 week-old rats, but their reactions declined as they grew older (Table 4). Especially for Con A and DBA, the intensity staining was clearly different among groups (Figure 30, 31). In contrast, β-D-Gal (1-3)-D-Gal Nac, shown by staining with PNA were weakly stained in 2 week, 1 month, and 3 month-old rats, but moderately stained in the 14 months old (Figure 30, 32). The intensity of WGA staining in the

rER-Golgi region of all groups was slightly different. This suggests that (β-(1-4)-D-GlcNAc)$_2$ NeuAc which is specific to WGA is slightly changed in 2 week, 1 month, 3 months, and 14 month-old rats.

A previous study in adult rats by Chan and Ho (1999) showed that the Golgi area of the ventral prostatic epithelial cells was well developed, and contained abundant GlcNAc, α/β-GalNAc, and NeuAc(2,6)GalNAc residues. The binding sites for Con A, WGA, PNA and DBA were found from day 10 to 13 post partum onwards (Hauke *et al.*, 1989). Tsukise and Yamada (1981) showed that the luminal surface and secretory glanules of the ventral prostatic epithelial cells were rich in glycoconjugates with 1,2-glycol groups (including Glc/Man, Gal, and Fuc).

The glycoconjugates of the dorsal prostate

The dorsal prostate is known to secrete spermine or a 60000-dalton protein (Gerhardt *et al.*, 1983; Chan and Ho, 1999). In all three age groups of dorsal prostates, the secretory epithelial rER-Golgi region contains α-Man,α-Glc,α-GlcNAc, α-L-Fuc, (β-(1-4)-D-GlcNAc)$_2$ NeuAc, as shown by its positive staining with PSA, UEA-I, and WGA. Their intensity staining was slightly different among age groups. The reactivity of DBA, Con A, specific to α-D-GalNAc, α-D-Man, α-D-Glc, GlcNAc were very weak in 14 month-old rats when compared with the younger groups (Figure 30, 33). In contrast, PNA, which specific to β-D-Gal (1-3)-D-GalNAc, showed clearly different staining among age groups; a strong reaction in the 14 months, a moderate reaction in the 3 months, and very weak reaction in the 1 month-old rat respectively.

According to the study of Chan and Ho (1999), the apical cytoplasmic blebs were rich in Man, GlcNAc, Gal/GalNAc, Fuc, NeuAc(α 2,6)

Gal/GalNAc, and oligosaccharide residues. Two major androgen-dependent secretory glycoproteins, dorsal protein I (DP I), and II (DP II), are secreted by the dorsal prostate (also coaglulating gland) DP II follows a slower pathway through the Golgi, while secretion of DP I is rapid and dose not go through the Golgi (Seitz, 1990; Chan and Ho, 1999).

The glycoconjugates of lateral prostate

The lateral lobe is always described together with the dorsal lobe as the dorsolateral prostate, but their epithelial cells are different in their ultrastructure. The lateral prostate is made up of dense secretory granules, microvilli, and rough endoplasmic reticulum with flattened cisternae, but these features are not found in the dorsal lobe (Schrodt, 1981). The lateral prostate is also rich in zinc. (Gunn and Gould, 1957; Gerhardt *et al.*, 1983)

In secretory epithelial rER-Golgi region of all age groups there was moderate to strong reaction to PSA, UEA-I, and WGA, which are specific to α-Man,α-Glc,α-GlcNAc, α-L-Fuc, $(\beta\text{-}(1\text{-}4)\text{-D-GlcNAc})_2$ NeuAc, and their intensity are slightly different among age groups (Table. 10). DBA, specific to α-D-GalNAc was moderately stained in the rER-Golgi region of the 1 month-old rats and increased to strongly stained in 3 month and 14 month-old rats (Figure 30, 34). Also resembled in PNA, specific to β-D-Gal (1-3)-D-GalNAc, there was a weak reaction in 1 month-old rats and an increase to moderate to strong reaction in the 3 month and 14 month-old rats.

Previous studies in adult rats by Chan and Ho showed that the Golgi region of the epithelial cells was rich in GalNAc residues as shown by its moderate staining by SBA (Glycine max), HPA (Helix pomatia), HAA (Helix asperasa), BPA (Bauhinia

purpurea), and SJA (Sophora japonica). Those authors also described, the lateral prostate as able to elaborate fewer glycoconjugates as compared to the ventral and dorsal lobes, and they reported that it is unclear whether the weaker lectin binding is correlated to its high concentration of zinc, as compared to the other two lobes. On the contrary, in the present study, the secretory rER-Golgi region of 3 months-old rat (adult rat) showed moderate to strong reaction with Con A, DBA, MPA, PSA, UEA-I, and WGA (except PNA). However, the secretory functional significance in this lobe is unclear (Hayashi *et al.*, 1991; Chan and Ho, 1999).

The glycoconjugates of the anterior prostate

The coaglulating gland is rich in fructose and glucose (Gunn and Gould, 1957; Gerhardt *et al.*, 1983). The coaglulating gland is known to secrete tranglutaminase, a family of extra and intracellular enzyme that catalyzes the cross-link between proteins and/or polyamines. In rodents, prostatic transglutaminase has been shown to be responsible for the formation of the copulatory plug (Seitz *et al.*, 1990).

In the secretory epithelial rER-Golgi region of all age group, their staining property were intermediating between weak to very weak reaction by DBA, MPA, PNA, and PSA, which are specific to α-D-GalNAc, α-D-GalNAc, α-D-Gal, β-D-Gal (1-3)-D-GalNAc, α-Man,α-Glc,α-GlcNAc, and their intensities are slightly different among age groups. Con A, specific to α-D-Man, α-D-Glc, GlcNAc showed strong reactions in the 1 month-old rats, very weak reactions in 3 month-old rats, and weak reactions in 14 month-old rats (Figure 35). UEA-I, specific to α-L-Fuc, showed weak to moderate reactions in all age groups. WGA, specific to $(\beta\text{-}(1\text{-}4)\text{-D-GlcNAc})_2$ NeuAc, showed very weak reaction in 3 month-old rats, but showed moderate reactions in both 1 month and 14 month-old rats.

The secretion of the anterior prostate can coagulate the monomeric proteins and basic clotting proteins from the seminal vesicles (Cunha *et al.*, 1987). As described by Hayashi et *al.* (1991), the rat-coagulating glands arise as one main duct per side, grown into and arborized within the mesenchyme of the seminal vesicle. This explained the direct apposition of the coagulating gland to the seminal vesicle in adulthood. Therefore, the close relation among the anterior lobe and seminal vesicle may be influenced and associated with lectin reactivity.

Patterns of glycoconjugates synthesis in various age groups

Glycoconjugates were separately synthesized in different cells of different lobes of the prostate gland at different times (Figure 13, 18, 22, and 30). However, each of them did not synchronously synthesize, some were produced in the early stage of life (see Con A and DBA of ventral lobe, Figure 13, 30) whereas some were produced later (see PNA of every lobe and DBA of lateral lobe, Figure 18, 30). This reflected the different timing of expression of glycoconjugates of the rat system, as well as the non-synchronous stoppage of glycoconjugates. The secretory epithelial cells in the rat's prostate glands may stop synthesizing some sugar residues (α-D-Man, α-D-Glc, GlcNAc, and α-D-GalNAc) when they are getting older (see Con A and DBA of ventral lobe, Figure. 13, 30), while the other glycoconjugates still appeared.

Works on correlation between the morphofunction features and age of the prostate glands are scanty. Harkin (1961) in his ultrastructural study of prostatic epithelial cells found that there was more ergastroplasmic region in the cells of sexually mature rats compared with the younger and older groups. Avtsyn *et al.* (1981) in their study on the histological structure of the human prostate and its zinc

content at various ages found a positive correlation. In the prepubertal group whose histological appearance was inactive, there was lesser zinc content in the dry tissue of the gland (48.10 ± 9.10 vs 64.10 ± 6.10 mg/kg) and this index tended to be less in the older group. Battaglia *et al.* (1994) in their study on age-related distribution of endocrine cells (chromogranin A) in the human prostate found the same pattern i.e.: there were more endocrine cells in the 25-54 year group compared with the younger and the older groups. But this present study cannot find out such a positive correlation between any glycoconjugates and the peak of their activity at the adult group. Some of them showed a declining pattern (e.g. α-D-Man, α-D-Glc, GlcNAc which is specific to Con A and α-D-GalNAc which is specific to DBA in ventral lobe), some showed an up-rising pattern (e.g. β-D-Gal (1-3)-D-GalNAc which is specific to PNA) whereas some exhibited a bimodal pattern (e.g. α-Man,α-Glc,α-GlcNAc which is specific to PSA in ventral and dorsal lobe)

Application of the knowledge to the human understandings

1. Application to the human pathology

Data on the human prostate glands are very limited. Most studies of them were concentrated only on certain aspects. Any supplemented studies on human specimen for their glycoconjugates will be beneficial understanding on the biology and pathology of the human prostate. Perhaps, one can answer the question of which lobe of glands is taking more risk on getting to adenocarcinoma and at which age group.

2. Application to the forensic medicine

After obtaining a small piece of prostatic tissue, and after its glycoconjugates were examined, one can come to the conclusion of the age of the specimen

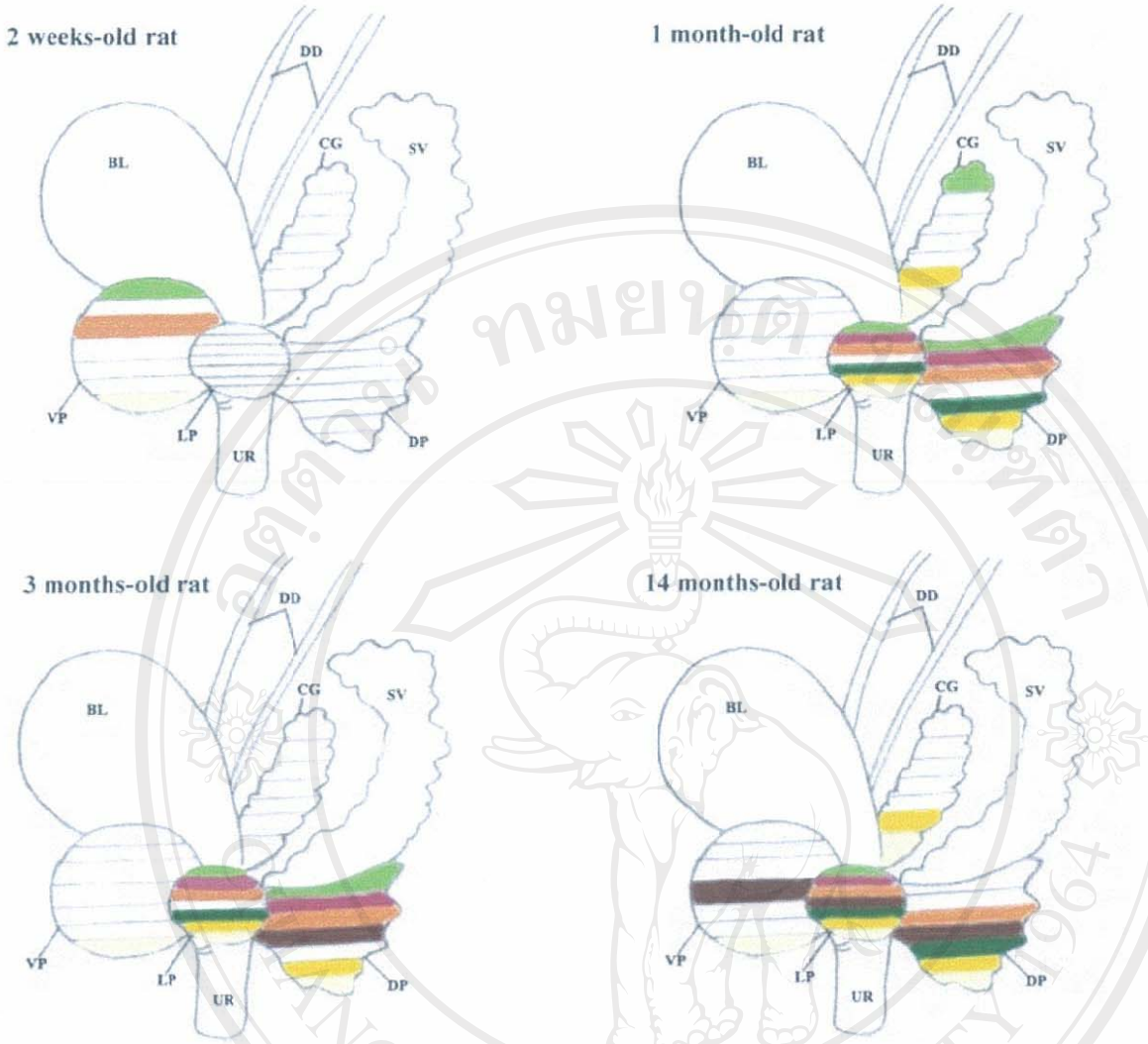

Figure 30 Diagram of the distribution of lectin binding reaction (moderate to strong) in different lobes of the prostate gland of the rat at various ages.

CG, coagulating gland; VP, ventral prostate; LP, lateral prostate; DP, dorsal prostate; BL, urinary bladder; SV, seminal vesicle; DD, ductus deferens; UR, urethra.

- Con A
- DBA
- MPA
- PNA
- PSA
- UEA-I
- WGA

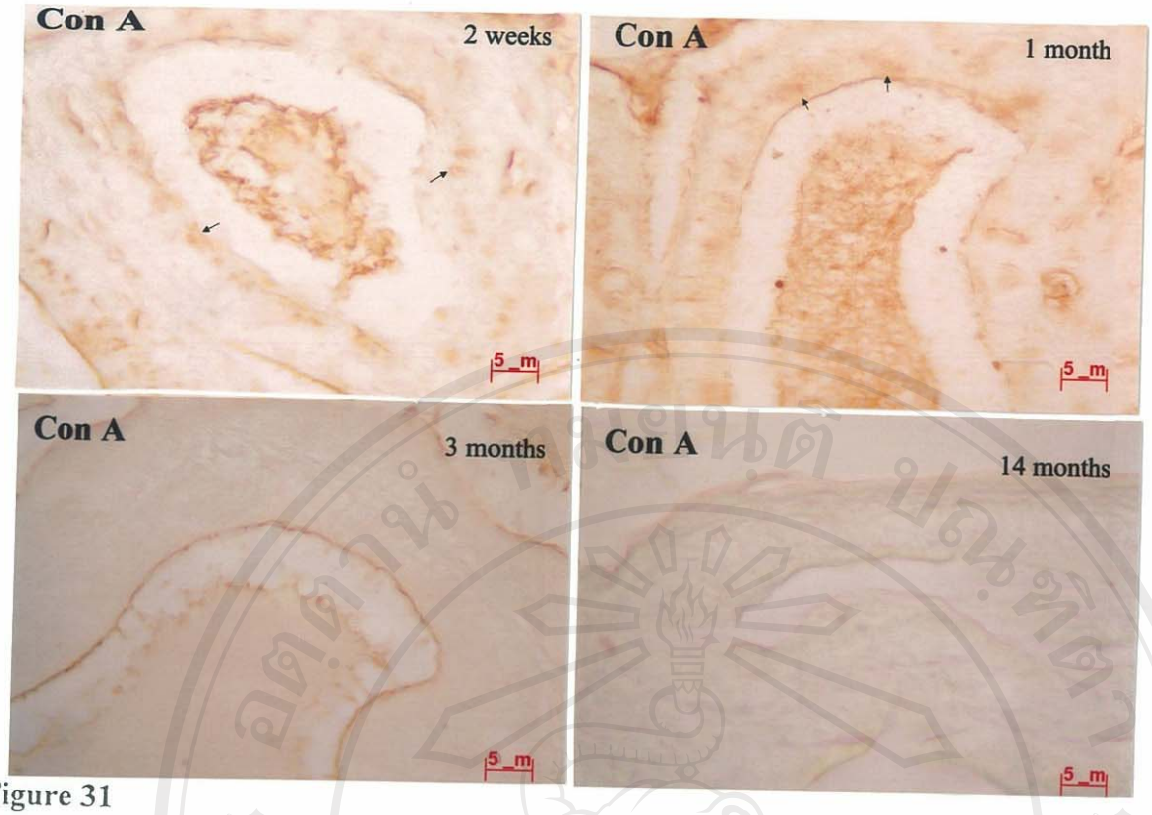

Figure 31

Comparison of Con A staining in the ventral prostate of 2 week (++), 1 month (+), 3 month (±), and 14 month-old rats (±). *Abbreviation: _m; micron.*

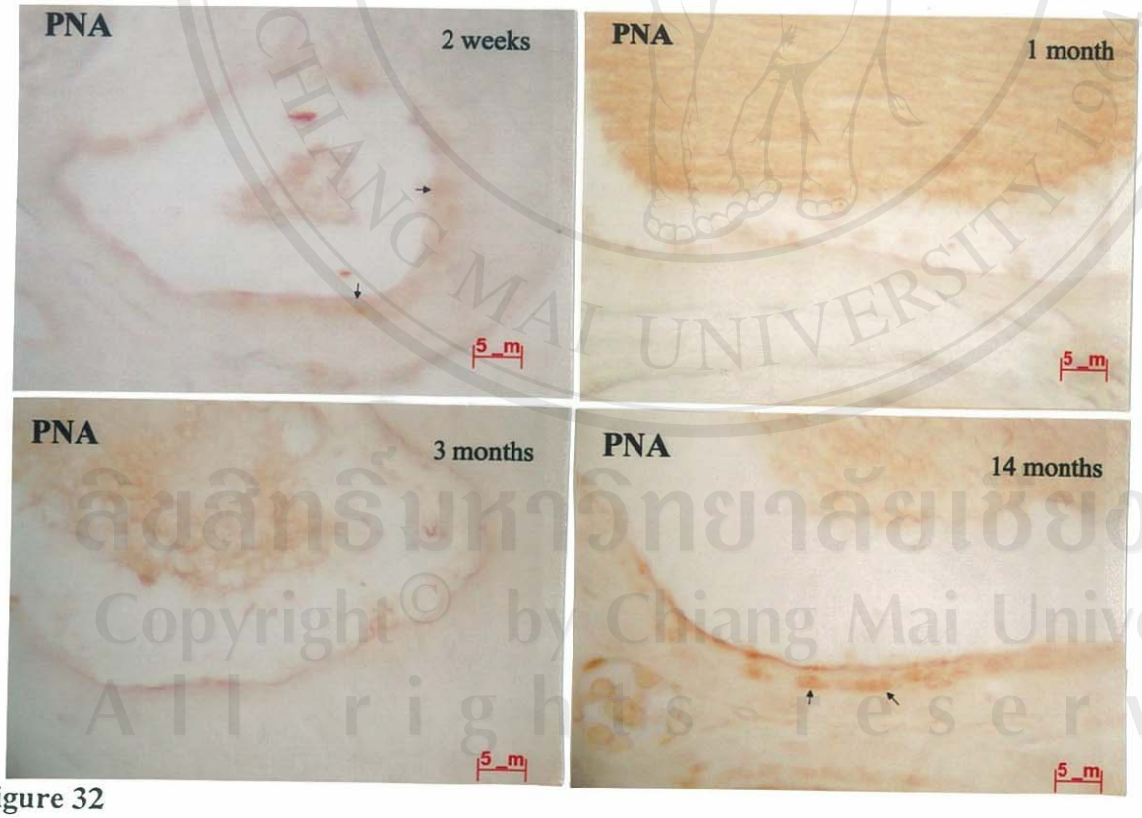

Figure 32

Comparison of PNA staining in the ventral prostate of 2 week (+), 1 month (±), 3month (±), and 14 month-old rats (++). *Abbreviation: _m; micron.*

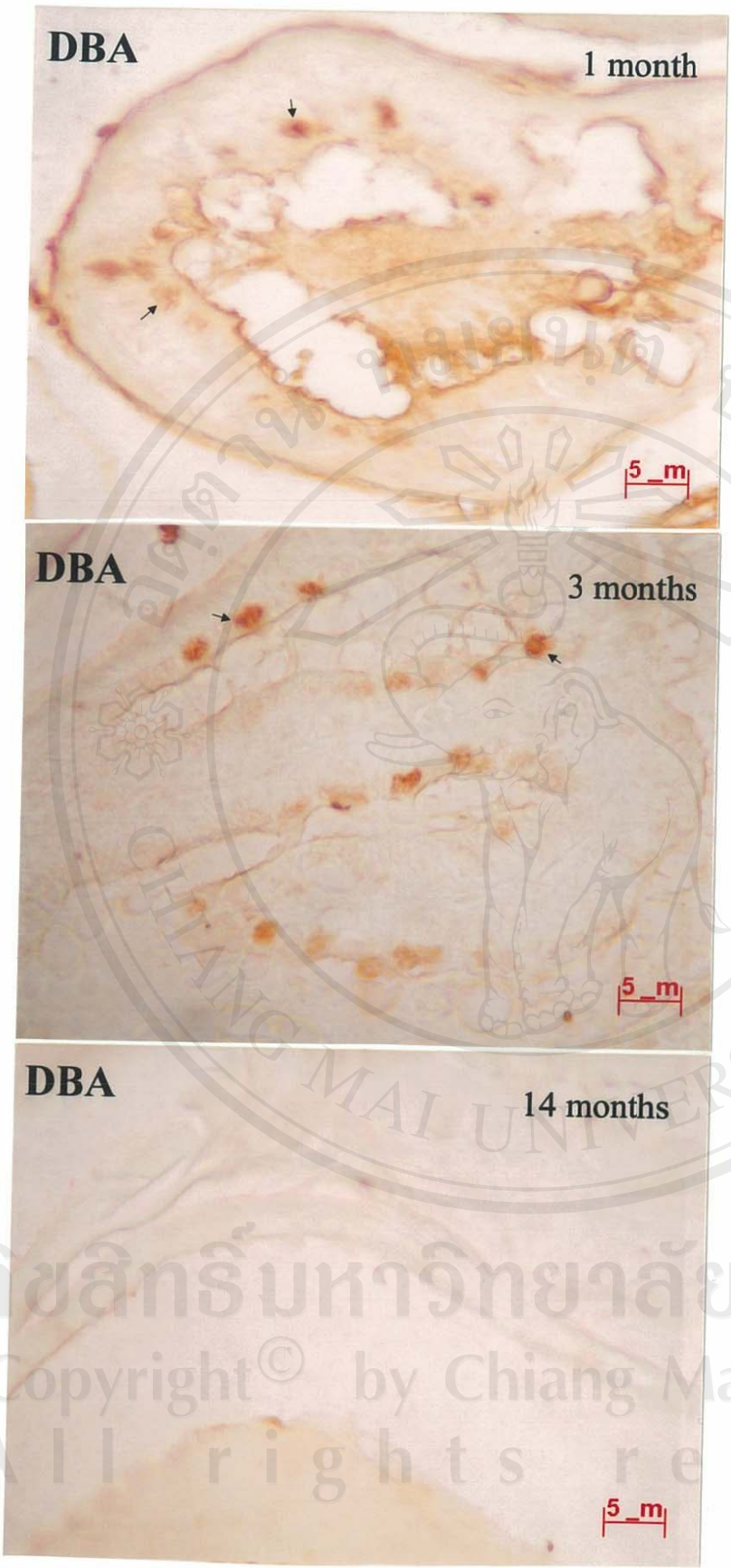

Figure 33

Comparison of DBA staining in the dorsal prostate of 1 month (++), 3 month (++), and 14 month-old rats (±). *Abbreviation: _m; micron.*

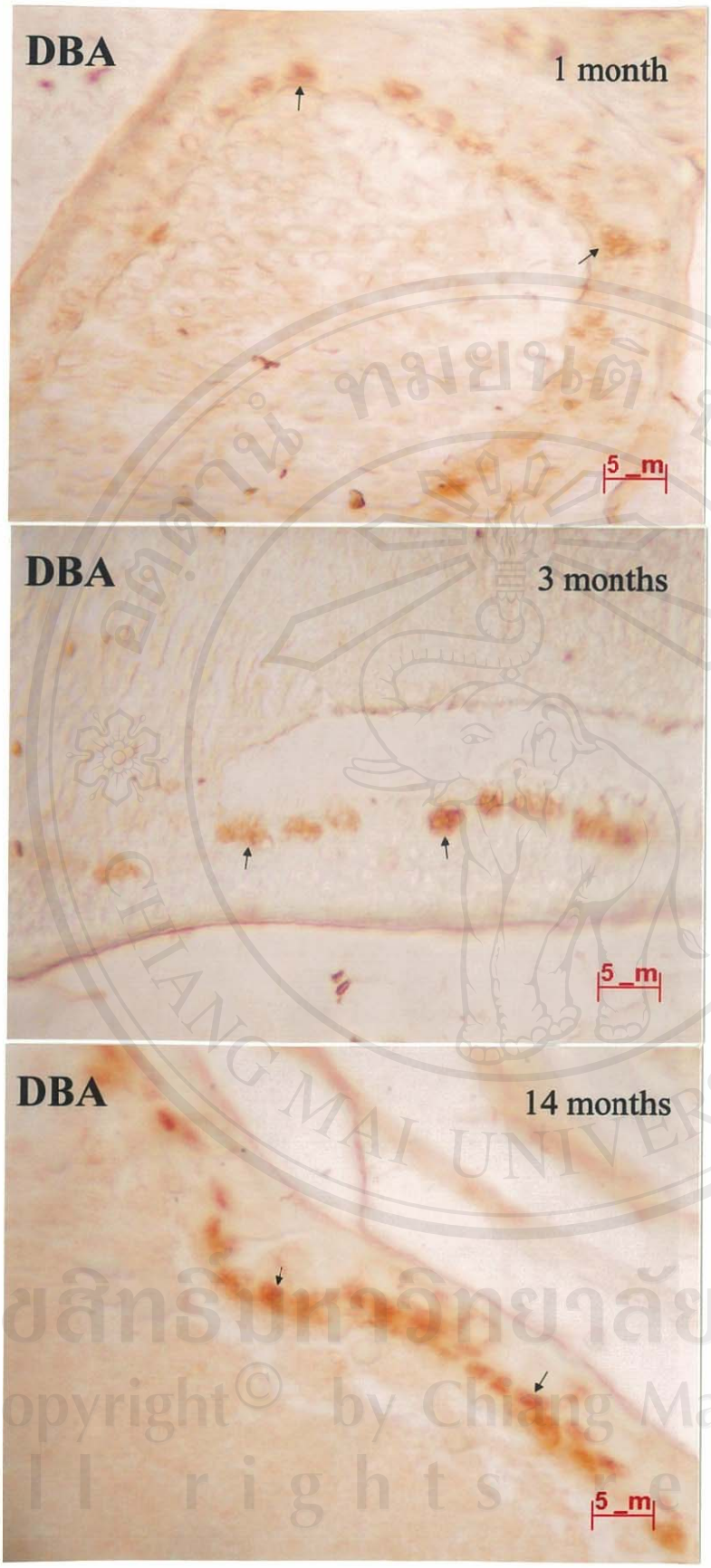

Figure 34

Comparison of DBA staining in the lateral prostate of 1 month (++), 3 month (+++), and 14 month-old rats (+++). *Abbreviation: _m; micron.*

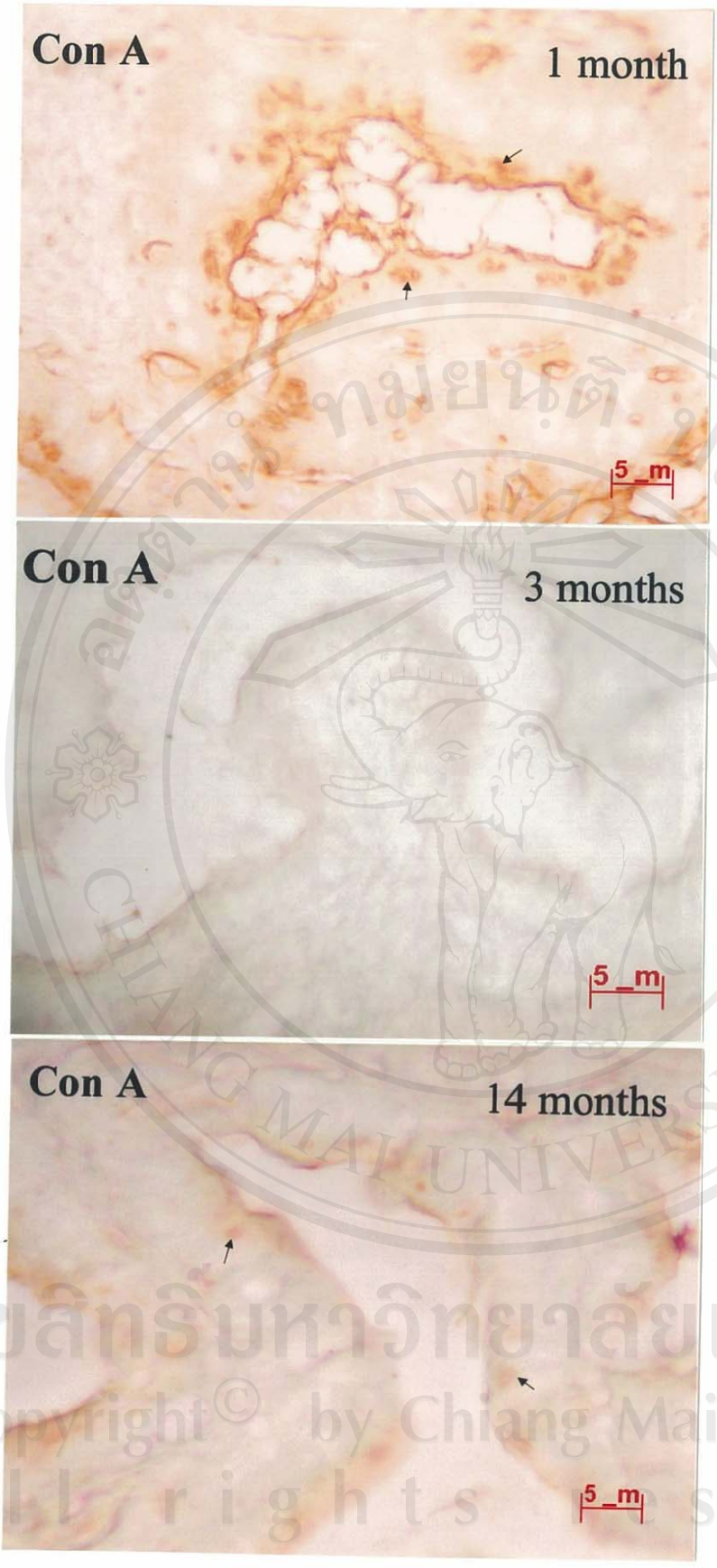

Figure 35

Comparison of Con A staining in the anterior prostate of 1 month (++), 3 month (±), and 14 month-old rats (+). *Abbreviation: _m; micron.*