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MORPHOLOGICAL STUDY ON THE PENILE SKIN OF BUFFALO AND DOG WITH SPECIAL EFERENCE TO THE CELLS OF THE IMMUNE SYSTEM AND THEIR RELATION TO KERATINOCYTES

(With 16 Figures)

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دراسة جلد القضيب في الجاموس والكلاب مع إلماع خاص على خلايا الجهاز المناعي وعلاقتها بخلايا الأدمة

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تم الحصول على عينات هذه الدراسة من ٨ ذكور من الجاموس و ٨ ذكور من الكلاب. جلد القضيب في الجاموس والكلاب عبارة عن تركيب بسيط أو بمعنى آخر جلد متحور لايحتوي على بصيلات شعر أو غدد عرقية أو غدد زهمية أو حبيبات صبغية. أكثر خلايا الجهاز المناعي شيوعاً في جلد قضيب الجاموس هي الخلايا البلازمية والخلايا البلعمية والخلايا الليمفاوية والخلايا البدنية بينما في الكلاب كانت الخلايا الليمفاوية والخلايا البدنية هما السائدتان. أظهرت الدراسة وجود خلايا بلازمية وخلايا بلعمية داخل فجوات خاصة بين خلايا الأدمة لجلد قضيب الجاموس وترجع الدراسة هذا التواجد إلى وجود علاقة وظيفية بين كل من الخلايا البلازمية والخلايا البلعمية من ناحية وخلايا الأدمه من ناحية أخرى وتفسر الدراسة تفاوت ذلك بين الجاموس والكلاب أنه راجع إلى إختلاف النوع.

SUMMARY

Materials were collected from 8 males of both buffaloes and dogs. There were no, hair follicles, sweat glands, sebaceous glands and pigment granules in the penile skin of buffalo and dog. The most prominent cells of the immune system in the penile skin of buffalo were plasma cells,

macrophages, lymphocytes and mast cells while in dog these cells were lymphocytes and mast cells. Aggregations of plasma cells appeared in the narrow long dermal papillae of the penile skin of buffalo which extending toward the epidermis and sometimes become near the epidermal surface. Plasma cells could be demonstrated singly or in groups in small lacunae between the keratinocytes. The plasma cells inside the lacunae developed from cell proliferation and maturation of migranting B-lymphocytes. Macrophages associated the aggregations of plasma cells. Macrophages sometimes partially embedded toward the keratinocytes; this may indicate their active movement. By this movement and the action of wear and tear forces on the epithelium, the macrophages are deviated with compensatory keratinocytes to be seen even in lacunae between the keratinocytes. Lymphoid follicles were few in the penile skin of buffalo but were frequent in dog. The lymphoid follicles form dome – shape with the epidermis of the penile skin of dog. The epidermis was so thin at the central free part of the dome and its innermost keratinocytes were interrupted due to the infiltration by many lymphocytes; moreover the keratinocytes of the central free point of the dome sometimes appeared so loose. All these features enable lymphocytes to face down the foreign antigen on the external surface of epidermis. The germinal center of lymphoid follicles in dog was not in the center. The distribution of mast cells was similar in both buffalo and dog. The present study suggested that there is a functional relationship for both plasma cells and macrophages with keratinocytes and this relationship is species dependent.

Key Words: *Skin, Penis, Immune System*

INTRODUCTION

Several studies were applied on the skin particularly its glands and hair follicles (Hifny et al., 1984; Kamel et al., 1986). Certain physiological aspects were studied also in the penis of cat, dog and rat (Johnson et al., 1986; Johnson and Kitchell, 1987; Johnson and Halata, 1991). The lymphocytes and its relation to the different types of epithelium were studied by different methods in mammals (Bundo et al., 1996; Krakauer, 1996). M cells as a component cells of the epithelium over the Peyer's patches (Dellmann, 1993) are able to harbour variable amounts of immunocompetent cells inside peculiar invaginations of their

basolateral cytoplasmic membrane, currently referred to as pockets (Regoli et al., 1995). Immunocompentent cells and Langerhans cells location and locomotion within the oral epithelium were studied (Burkhardt, 1992). The same author noticed that about 40 percent of the lymphocytes and 70 percent of the Langerhans cells show signs of locomotion, predominantly in a lateral and basal direction. Keratinocytes as a major cells of the skin epithelium (Banks, 1993) showing an interaction with leucocyte in vitro (Boyera et al., 1993). However to our knowledge, no histological studies demonstrating the relation of the cells of immune system with keratinocytes. Therefore, the aim of the present investigation is to give more information on the penile skin of buffalo and dog and to clarify the of the cells of the immune system with the kertinocytes of the penile skin in these animals of two different species.

MATERIALS and METHODS

Materials were collected from 8 males of buffaloes (Bos bubalis L.) obtained from Kafr – El – Sheikh and El – Mansoura slaughter houses, and 8 males of dogs obtained from Kafr – El – Sheikh and El-Mansoura. All the sampled animals appeared healthy. The ages of the animals ranged from 6 months to 36 months. Dentition was applied after bleeding according to Miller and Robertson (1959) in buffalo and Scott and Fuller (1965) in dog. Sections of 5 mm thickness including the whole contour of the penis with its covering skin (penile skin or lamina penis preputii) were taken as samples. Two samples were taken from the glans and another two samples were taken from the free part of the penis. The bulbus glandis and the pars longa glandis of the dog glans were included in sampling. The os penis was removed carefully from the penis of dog. Fixation was carried out in 10% neutral buffered formalin and Bouin's fluid. Then processing was applied and paraffin serial sections of 4 micrometers (μm) thick were prepared and stained by Harris hematoxylin and Eosin (H& E), Van Gieson technique for collagen fibers, Periodic Acid – Schiff technique for carbohydrates and Acidified Toluidine blue method for mast cells. These methods were reported (Woods and Ellis, 1994; Bancroft and Stevens, 1996).

RESULTS

Buffalo

The penile skin of buffalo exhibited so simple structure that it could be termed a modified skin. The epidermis was thick but only consisted of stratum basale and stratum spinosum; the cells of the stratum basale were less crowded but widely arranged over the tip of dermal papilla (Fig. 1&8). The stratum corneum was not identifiable. The dermis was characterized by well-developed dermal papillae. The dermal papillae were mostly branched (compound) and greatly extended leading to a considerable variation of thickness of the epidermis which appeared very thin at the tip of dermal papillae (Fig.1&2). The hair follicles, sweat glands, sebaceous glands and pigment granules could not be observed in the penile skin of buffalo.

The most prominent cells of the immune system in the penile skin of buffalo were plasma cells, lymphocytes, macrophages and mast cells. The number of these cells sometimes varied from one animal to another and from part of the penis to another except the mast cells. There was no age dependent in this respect.

Aggregations of plasma cells appeared in the narrow long dermal papillae and their branches which, were extending toward the epidermis and sometimes become near the epidermal surface (Fig. 1). The dermal papillae were sometimes crossly cut (Fig. 3&4) and filled with plasma cells, some macrophages and lymphocytes, thin strands of collagen fibers and few fibroblasts (Fig. 3 & 4). The dermal papillae also contained small blood vessels (Fig.1). The plasma cells were typical in structure and could be demonstrated singly or in groups in small lacunae between the keratinocytes near the surface of penile skin (Fig. 5, 8, 6). The lacunae were spaces between the keratinocytes because they were free from collagen fibers and blood vessels. The lacunae sometimes progressed by the spaces of usually degenerated keratinocytes where degraded nuclei of these cells (karyorrhexis) appeared in close association or inside the lacunae (Fig.5). The plasma cells inside the lacunae were formed by the division and maturaof migrant B-lymphocytes between the keratinocytes. Suggestion is that the keratinocytes may have a role in the differentiation of plasma cells from B- lymphocytes (functional relationship).

The macrophages were associating the aggregations of plasma cells and lymphocytes in the dermal papillae; they were located between

the plasma cells or bordering the keratinocytes (Fig 1 & 4). Macrophages sometimes partially embedded toward the keratinocytes and this may indicate the active movement of macrophages. By this movement and the action of wear and tear forces on the epithelium, the macrophages are deviated with compensatory keratinocytes to be seen even in lacunae between the keratinocytes (Fig. 7). This striking behavior of the macrophages not only to meet foreign antigen but also refers to a possible cooperation or functional relationship between them and keratinocytes. Dense heterogeneous granules of variable size showing PAS- positive reaction, the lysosomes, filled the cytoplasm of macrophages. Few vacuoles also may be seen (Fig. 1& 4 & 7).

Lymphocytes were seen dispersed or in the form of few lymphoid follicles (Fig. 8). Migrant lymphocytes between the keratinocytes were frequent and located in lacunae (Fig. 5 &6).

Mast cells were often seen in association to the connective tissue of the dermis and deep to it (Fig. 9) and around the blood vessels. Sometimes they appeared between the plasma cells (Fig. 10). There was no close location of mast cells toward the epidermal keratinocytes although very few or singular mast cell may be located in the dermal papillae; these mast cells were poorly granulated. The number of mast cells did not accompany the variation of the number of other studied cells of the immune system.

Dog

The configuration of the layers of penile skin of dog was simple (modified) like that of buffalo however some variations were noticed. The epidermis was thinner (Fig. 16). The stratum basale was well demonstrated (Fig.11) because its cells were more crowded and with more basophilic cytoplasm (Fig.16). The dermal papillae were simple structure but more developed in the glans and sometimes slightly branched (Fig. 15&16).

The most important cells of the immune system in the penile skin of dog were the lymphocytes and the mast cells. The most prominent cells of the immune system in the penile skin may be controlled by their functional relationship with keratinocytes; species difference occurred in this aspect. The lymphocytes were arranged in follicles and sometimes dispersed in the dermis (Fig. 11). The lymphoid follicles were frequent and their number may be varied from one part of the penis to another and from one animal to another without age dependent. The lymphoid follicles form dome – shape with the epidermis. The epidermis was so

thin at the central free part of the dome (Fig. 11) and its innermost keratinocytes were interrupted and appeared isolated due to the infiltration by many lymphocytes (Fig. 12 & 13). Sometimes the keratinocytes of the central free point of the dome appeared so loose (Fig.13). All these features enable the lymphocytes to face down the foreign antigens on the external epidermal surface. The germinal center was seen in lymphoid follicles but it was not in the center; it was present occupying the internal side of the follicles (Fig. 11). Therefore the lymphoid follicles appeared to be formed of an internal pale – stained area, erroneously the germinal center that covered only toward the epidermal aspect by a crescent cap of lymphocytes (Fig. 11). Few migrant lymphocytes through the epidermis could be encountered. Lacunae containing only a singular plasma cell were rare (Fig. 13).

Mast cells could be seen in larger number in association with blood vessels deep to the dermis (Fig. 14). There was no close relation between the number of mast cells and the number of other cells of the immune system because the number of mast cells did not change. Also there was no close association between mast cells and keratinocytes although poorly granulated mast cells rarely occurred in the dermal papillae (Fig. 15). Therefore the distribution of mast cells in the penile skin of dog was similar to that of buffalo.

DISCUSSION

Unlike the skin (Dellmann, 1993), the penile skin of buffalo and dog not containing hair follicles, sweat glands, sebaceous glands and pigment granules. The epidermis consisted only of stratum basale and stratum spinosum. The stratum corneum was not clear. Therefore the penile skin of buffalo and dog was simple or modified. However the stratum corneum was marked over the glans penis of Surti buffalo (Das and Vyas, 1989). The degree of cornification depends upon the pressure and abrasion to which the epithelium is subjected (Banks, 1993). The stratum basale was well demonstrated in the penile skin of dog because its cells were more crowded and with more basophilic cytoplasm. The proliferation of the cells of stratum basale is responsible for the continual renewal of the epithelium (Fawcett, 1994). The dermal papillae of the penile skin of buffalo were mostly branched and greatly extended leading to a considerable variation of the thickness of the epidermis, but those of dog were simple and sometimes slightly branched in the glans.

Dermal papillae increase surface contact with the epidermis (Banks, 1993). This to increase the supply of nutrient materials from blood vessels of the dermis to the epidermis and to support the epidermis.

Species differences could be recognized between the buffalo and dog in the study of most prominent cells of the immune system of the penile skin. In buffalo these cells were plasma cells, lymphocytes, macrophages and mast cells, while in dog these cells were lymphocytes and mast cells. Similar cells were reported in the skin of the body (Banks, 1993; Dellmann, 1993). The variation in the number of studied cells of the immune system in the penile skin of buffalo and dog was not age dependent. Immunologically there were no significant correlations between intrapreputial immunoglobulin concentration and age (Flower et al., 1983).

Aggregations of plasma cells appeared in the narrow long dermal papillae of the penile skin of buffalo which extending toward the epidermis and sometimes become near the epidermal surface. Numerous plasma cells were found in the canine nasal mucosa that surfaced by stratified squamous epithelium (Adams and Hotchkiss, 1983). Singular and groups of two or three plasma cells sometimes could be demonstrated in small lacunae between the keratinocytes near the surface of penile skin of buffalo. The plasma cells inside the lacunae developed from cell proliferation and maturation of B-lymphocytes. Occasional lymphocytes were seen crossing the basal lamina of oral epithelium of normal murine and human (Burkhardt, 1992), who added that close apposition, or rarely a denticulate surface of lysis of desmosomes were present, both probably indications of cellular interactions. Moreover Nickoloff (1988) suggested that the balance of cutaneous immunohomeostasis may involve several different keratinocyte – derived factors which, may be either lymphocyte activating or lymphocyte inhibitory. In another respect the lymphocytes themselves were found to have an effect upon the keratinocytes (Barker et al., 1988; Stoof et al., 1992). The latter authors explained the direct adhesion-mediated activation of keratinocytes by T- lymphocytes, that it may be important in the genesis of cutaneous inflammation by amplifying the original stimulus, as well as contributing to the trafficking pattern of inflammatory cells as they leave the general circulation and enter the skin. Also human T- lymphocytes have a proliferative response on the intestinal crypt epithelial cells and resting porcine endothelial cells respectively (Evans et al., 1992, Vallee et al.,

1998). Other types of epithelium such as human biliarepithelial cells were found to have an immunogenic and activation role toward T-lymphocytes (Leon et al., 1997; Scholz et al., 1997; Cruickshank et al., 1998). The recent study of Watanabe et al. (1995) suggesting that human intestinal epithelial cells and epithelial goblet cells produce interleukin-7 which serve as a potent regulatory factor for intestinal mucosal lymphocytes through their activation or indirectly their inhibition. Therefore the present study suggested the presence of a role of keratinocytes in the differentiation of plasma cells from B- lymphocytes (functional relationship).

Macrophages were associating the aggregations of plasma cells and lymphocytes in the dermal papillae of penile skin of buffalo. Sometimes macrophages are found close to the epithelial cells (Paul, 1999) and become motile in response to stimulation (Dellmann, 1993). Also in the present study macrophages sometimes partially embedded toward the keratinocytes and this may indicate their active movement. By this movement and the action of wear and tear forces on the epithelium, the macrophages are deviated with compensatory keratinocytes to be seen even in lacunae between the keratinocytes. This striking behavior of the macrophages not only to meet foreign antigen but also refers to a possible cooperation or functional relationship between them and keratinocytes. Macrophages epithelium relationship was studied, where monocyte macrophage differentiation induced by coculture of retinal pigment epithelium cells with monocytes (Osusky et al., 1997). Moreover human monocytes activate porcine endothelial cells, resulting in increasing of some immunological factors such as monocyte chemotactic protein-1 (Millan et al., 1997).

Lymphocytes were seen dispersed or in the form of few follicles in the penile skin of buffalo. Solitary lymph nodules were found in the skin over the penis inside the preputial cavity of Surti buffalo (Das and Vyas, 1989). The lymphoid follicles were frequent in the penile skin of dog. The lymphoid follicles form dome – shape with the epidermis. The epidermis was so thin at the central free part of the dome and its innermost keratinocytes were interrupted due to the infiltration by many lymphocytes; moreover the keratinocytes of the central free point of the dome sometimes appeared so loose. All these features enable the lymphocytes to face down the foreign antigens on the external surface of epidermis. The germinal center of lymphoid follicles was not in the center but it was occupying the internal side of the follicle and covered

only toward the epidermal aspect by a crescent cap of lymphocytes. Similar structure of lymphoid follicle was reported by Fawcett (1994). It is probable that the nomenclature and the role of the germinal center are still questionable.

Mast cells were found in the dermis and deep to it and around the blood vessels in the penile skin of buffalo and dog. The number of mast cells did not accompany the variation of the number of other studied cells of the immune system. Mast cells were present in all samples of oral mucosa of aging mice in the more vascularized areas (Raffaniello and Roy, 1990). Also mast cells of intestinal mucosa of normal rat showing no changes with that of globular leukocytes during the life (Ikeda and Yamashina, 1993). The present study referred to that there was no close association between the mast cells and the keratinocytes although poorly granulated mast cells rarely may occurred in the dermal papillae. This may indicating that the mast cells receive their antigens from blood vessels rather than external epithelium.

The present study revealed a variety of the most predominant cells of the immune system in the penile skin of buffalo and dog. This may be attributed to the difference in the functional correlation or immune response between cells of the immune system and the keratinocytes of penile skin of these two species. The finding of Matsueda et al. (1991) exhibited that the intraepithelial lymphocytes of the duodenum were more prominent than in the large intestine. They suggested that there is a variation of local immune response and the difference of assigned immunological functions among the various sites of the intestine; this variation may support the present study howbeit it is in the same animal. The functional correlation between the cells of the immune system and the different types of epithelium is still very much a matter of search.

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LEGEND

Fig.1: Penile skin of buffalo showing dermal papilla near the epidermal surface containing, (1) Aggregation of plasma cells; (2) Small blood vessel; (3) Macrophage bordering the keratinocytes and filled by lysosomes. (4) Very thin epidermis at the tip of dermal papilla and showing widely arranged stratum basale cells (arrows). (H & E; X1000).

Fig.2: Penile skin of buffalo showing branched dermal papilla. (Van Gieson; X100).

Fig.3: Penile skin of buffalo showing crossly cut dermal papilla. (1) Plasma cells. (2) Fibroblasts. (3) Collagen fibers. (Van Gieson; X1000).

Fig.4: Penile skin of buffalo showing crossly cut dermal papilla. (1) Plasma cells. (2) Lymphocytes. (3) Macrophages filled by lysosomes and the left middle one showing vacuole; they bordering the keratinocytes (arrows). (PAS; X1000).

Fig.5: Penile skin of buffalo. (1) Singular plasma cells in lacunae between the keratinocytes. (2) Degraded nuclei of keratinocytes. (3) Lymphocyte in lacuna. (H&E;X1000).

Fig.6: Penile skin of buffalo. (1) Two plasma cells in lacuna. (2) Lymphocytes in lacunae. (H&E;X1000).

Fig.7: Penile skin of buffalo. (1) Macrophage in lacuna. (2) Macrophage partially embedded toward the keratinocytes. Note the lysosomes. (H&E;X1000).

Fig.8: Penile skin of buffalo showing thick epidermis; stratum basale (arrows heads); stratum spinosum (asterisk); lymphoid follicle (arrow). (H&E;X100).

Fig.9: Penile skin of buffalo showing mast cells in the dermis (arrows). (Toluidine blue; X1000).

Fig.10: Penile skin of buffalo showing mast cell (arrow) between plasma cells (arrows heads). (Toluidine blue; X1000).

Fig.11: Penile skin of dog showing lymphoid follicles (arrows), (1) Thin epidermis at the central free part of the dome. (2) Well identifiable stratum basale. (3) Erroneously the germinal centers. (PAS, X40).

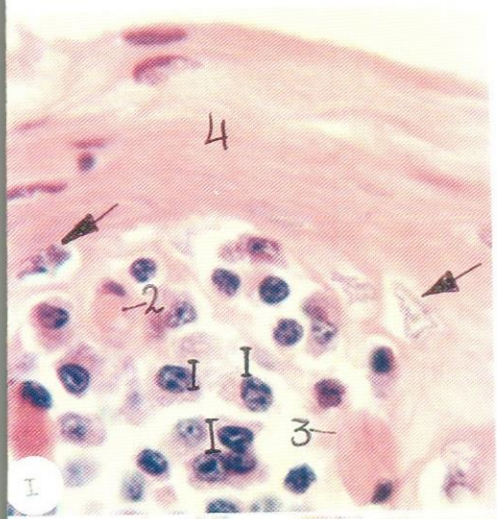
Fig.12: Penile skin of dog showing the central free part of the dome of lymphoid follicle with epidermis infiltrated by lymphocytes (arrows) and interrupted keratinocytes (arrows heads). (PAS; X1000).

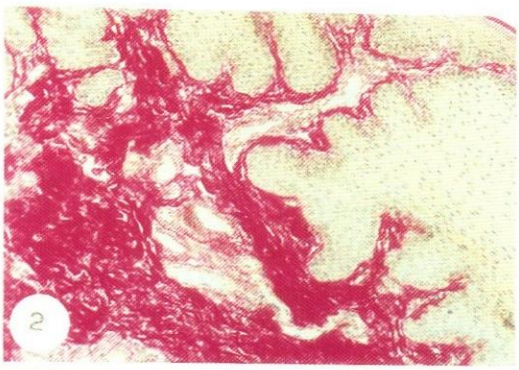
Fig.13: Penile skin of dog showing the central free part of the dome of lymphoid follicle with epidermis infiltrated by lymphocytes (small arrows), interrupted keratinocytes (large arrows), plasma cells in lacuna (open arrow) and so loose keratinocytes at the central free point of the dome (asterisk). (PAS; X1000).

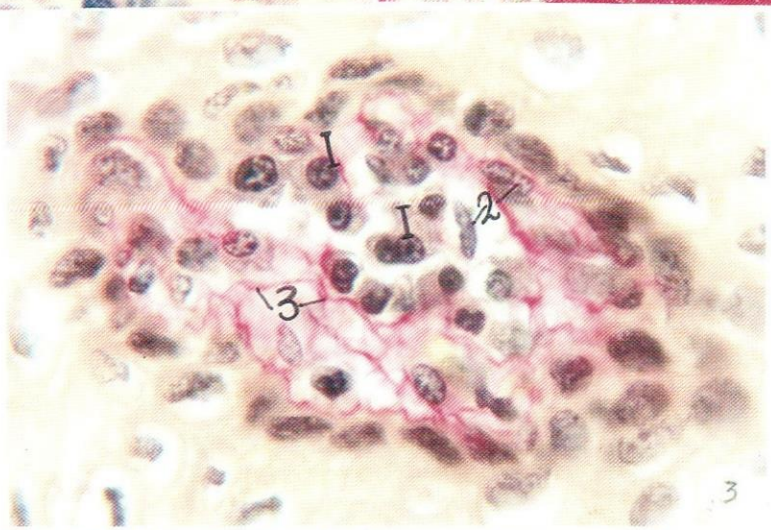
Fig.14: Penile skin of the dog showing mast cells in larger amount (arrows) associating blood vessel (asterisk). (Toluidine blue; X400).

Fig.15: Penile skin of the dog showing poorly granulated mast cell (arrow) in the simple dermal papilla. (Toluidine blue; X1000).

Fig. 16: Penile skin of dog. (1) Thin epidermis. (2) More crowded cells of stratum basale. (3) Slightly branched dermal papilla. (PAS;X100).







g.1: Penile skin of buffalo showing dermal papilla near the epidermal surface containing, (1) Aggregation of plasma cells; (2) Small blood vessel; (3) Macrophage bordering the keratinocytes and filled by lysosomes. (4) Very thin epidermis at the tip of dermal papilla and showing widely arranged stratum basale cells (arrows). (H & E; X1000).

g.2: Penile skin of buffalo showing branched dermal papilla. (Van Gieson; X100).

g.3: Penile skin of buffalo showing crossly cut dermal papilla. (1) Plasma cells. (2) Fibroblasts. (3) Collagen fibers. (Van Gieson; X1000) .

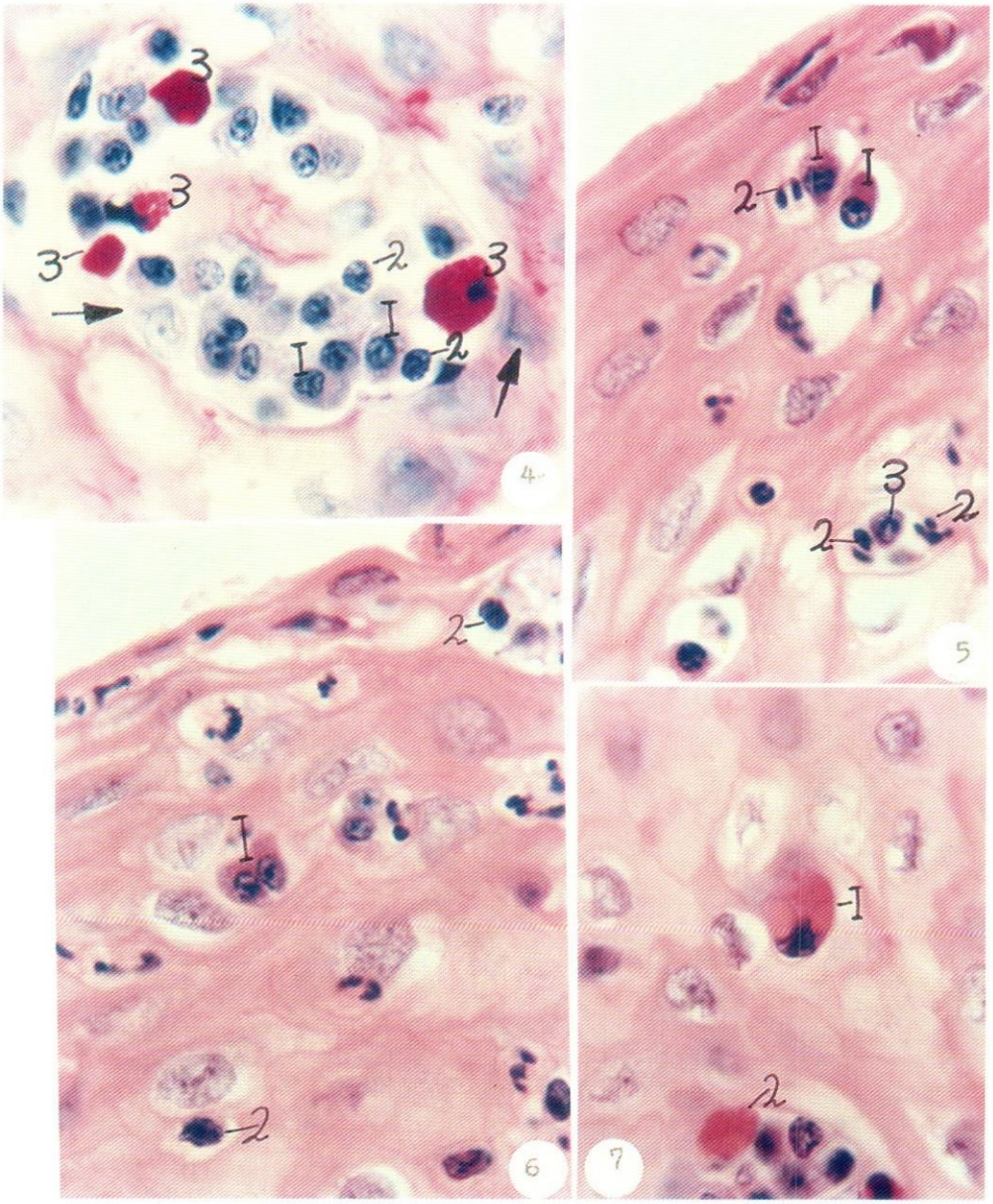
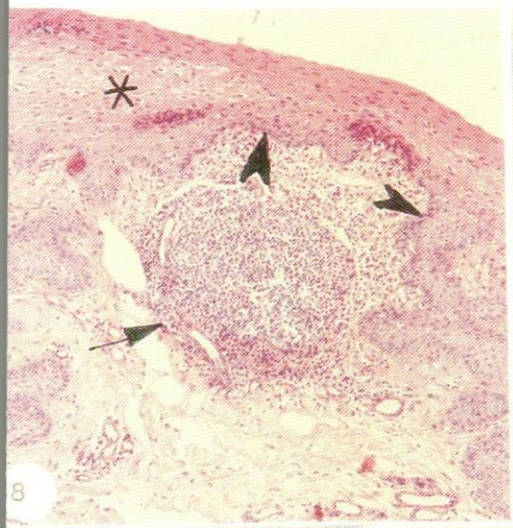


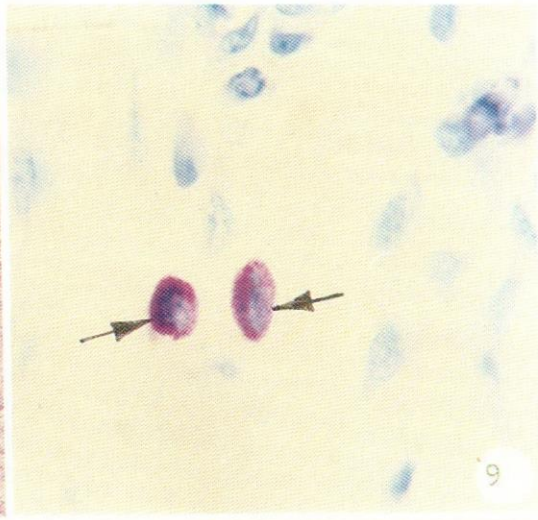
Fig.4: Penile skin of buffalo showing crossly cut dermal papilla. (1) Plasma cells Lymphocytes. (3) Macrophages filled by lysosomes and the left middle one sho vacuole; they bordering the keratinocytes (arrows). (PAS; X1000).

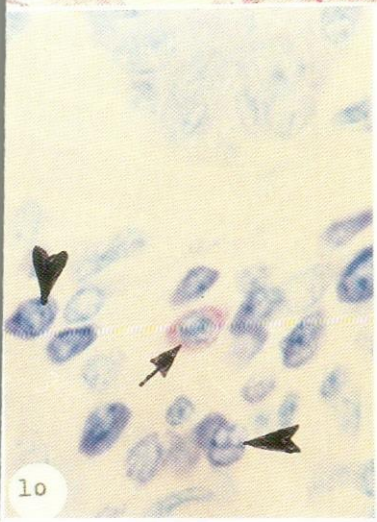
Fig.5: Penile skin of buffalo. (1) Singular plasma cells in lacunae between the keratinocytes Degraded nuclei of keratinocytes. (3) Lymphocyte in lacuna. (H&E;X1000).

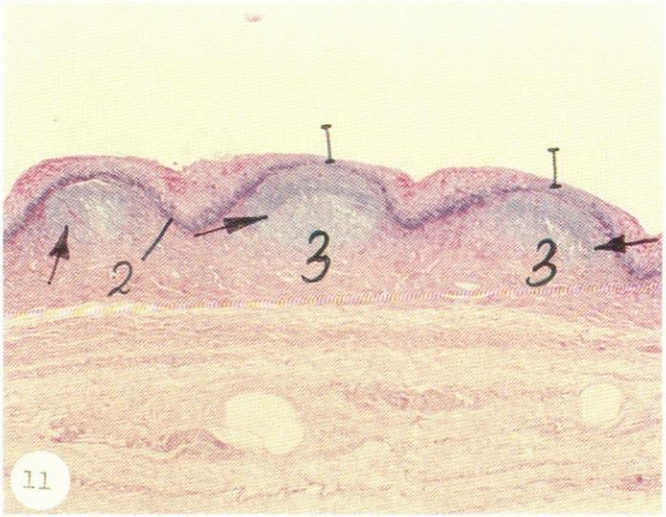
Fig.6: Penile skin of buffalo. (1) Two plasma cells in lacuna. (2) Lymphocytes in lacu (H&E;X1000).

Fig.7: Penile skin of buffalo . (1) Macrophage in lacuna. (2) Macrophage partially embe toward the keratinocytes. Note the lysosomes. (H&E;X1000).









8: Penile skin of buffalo showing thick epidermis; stratum basale (arrows heads); stratum spinosum (asterisk); lymphoid follicle (arrow). (H&E;X100).

9: Penile skin of buffalo showing mast cells in the dermis (arrows). (Toluidine blue; X1000).

10: Penile skin of buffalo showing mast cell (arrow) between plasma cells (arrows heads). (Toluidine blue; X1000).

11: Penile skin of dog showing lymphoid follicles (arrows), (1) Thin epidermis at the central free part of the dome. (2) Well identifiable stratum basale. (3) Erroneously the germinal centers. (PAS, X40).

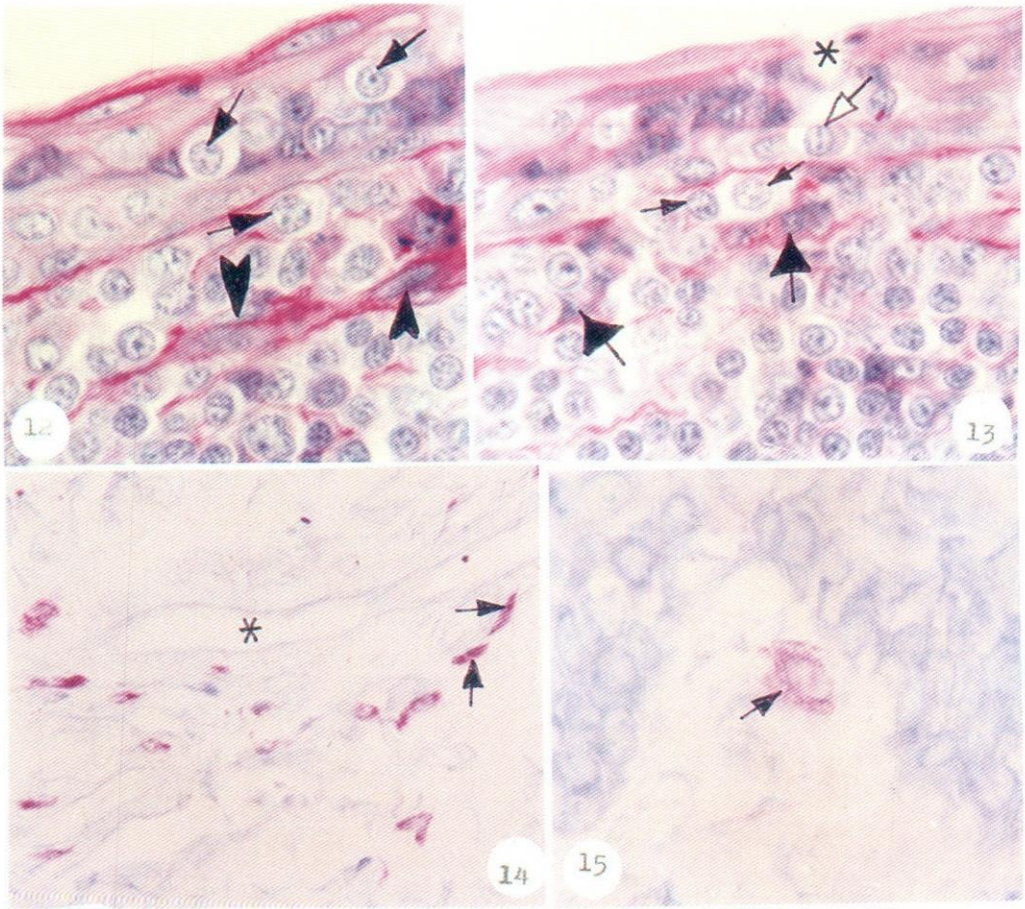


Fig.12: Penile skin of dog showing the central free part of the dome of lymphoid follicle with epidermis infiltrated by lymphocytes (arrows) and interrupted keratinocytes (arrows heads). (PAS; X1000).

Fig.13: Penile skin of dog showing the central free part of the dome of lymphoid follicle with epidermis infiltrated by lymphocytes (small arrows), interrupted keratinocytes (large arrows), plasma cell in lacuna (open arrow) and so loose keratinocytes at the central free point of the dome (asterisk). (PAS; X1000).

Fig.14: Penile skin of the dog showing mast cells in larger amount (arrows) associating blood vessel (asterisk). (Toluidine blue; X400).

Fig.15: Penile skin of the dog showing poorly granulated mast cell (arrow) in the simple dermal papilla. (Toluidine blue; X1000).

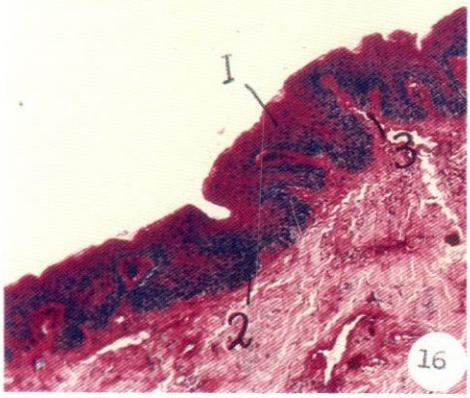


Fig. 16: Penile skin of dog. (1) Thin epidermis. (2) More crowded cells of stratum basale. (3) Slightly branched dermal papilla. (PAS;X100).