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AN ULTRASTRUCTURAL AND CYTOCHEMICAL ANALYSIS OF THE GLABROUS BORDER OF THE BOVINE LIP

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Summary—Glabrous border of the lip of cattle had a malpighian epithelium 800 μm thick. The horny layer was about 200 μm thick and there was no granular layer visible with light microscopy or electron micrography. The more evident morphological features were: high concentration of tonofilaments, mitochondria in basal as well as in spinous cells; few small keratohyaline granules. Blood supply, as shown by the histochemical demonstration of TPPase, was composed of capillary loop which penetrated up to near the horny layer. The oxidative enzymes reactions of bovine lip epithelium were compared with those of rat lip epithelia. Microphotometric evaluation of histochemical preparations showed a greater activity of SDh and Gl-6-ph Dh in bovine glabrous border; LDh, on the contrary, revealed lower activity. Different structural and metabolic characteristics between epithelia of both species were noted.

INTRODUCTION

The so-called vermilion or red margin of the lip, which is a feature of the human face, is a hairless smooth or glabrous zone which resembles mucosa in colour, though in pigmented people its redness tends to be obscured by pigment. However, it differs from the oral mucosa in being without mucous glands. As far as is known uniquely to man, this marginal zone of the lips frequently contains sebaceous glands unassociated with hairs (Miles, 1958). A similar marginal zone is present in many mammals, if not all, though it rarely forms a feature of the face as in man.

The term red margin of the lip is obviously not an entirely suitable term even for the human state. The term transitional zone, which has been used because the zone has resemblances to both skin and mucosa, seems only suitable in a histological context. We shall call it the glabrous margin of the lip as an improvement on vermilion margin in the present context of comparative anatomy.

The epithelium of the glabrous border of cattle shows morphological characteristics that justify its baseline analysis in order to acquire data about the biology of epithelia. Applying stereological methods, we have demonstrated (Lanfranchi and de Rey, 1978) that, in spite of the great thickness of glabrous border epithelium of cattle (1000 μm), the various component layers maintain the same proportion as in the glabrous border of human beings and rats. The presence of a thick orthokeratinized layer (200 μm) and the absence of a definite granular layer make this tissue a good model to compare with other epithelial tissues in order to obtain data on epithelial metabolism, especially those concerning keratin production. We therefore studied the ultrastructural and metabolic characteristics of the glabrous border epithelium using light and electron microscopy and histochemical techniques.

MATERIAL AND METHODS

Segments of lip were taken from 5 cows (*Bos taurus*) 15 min after slaughtering and from lips of 5 Wistar rats, fixed in formaldehyde, embedded in paraffin wax and sectioned. Sections were stained with haematoxylin and eosin. In addition, small samples (approx. 2 mm^3) of cow lip were processed for electron microscopy using Palade (Palade, 1952) and Karnovsky (Karnovsky, 1965) solutions as fixatives. After dehydration, pieces were embedded in Epon 812 orientated flat at the bottom of reversed Beem capsules (Ladd Research Industries, Burlington, Vt., U.S.A.) in order to obtain sections cut perpendicular to the epithelial surface. Sections were obtained with a Reichert OmU_2 ultramicrotome, stained with uranyl acetate and lead citrate and photographed with a Philips M 300 electron microscope.

A segment of the lip of each animal, containing skin and glabrous border, was fixed in neutral 4 per cent formaldehyde at 4°C for 24 h. Frozen sections were cut and treated for tiaminopyrophosphatase (TPPase) demonstration, according to the technique described by Goldfischer, Essner, and Novikoff (1964). TPPase was selected as a marker enzyme for vascular walls in order to study the subepithelial vascularization.

Another unfixed specimen of the lip of each cow was mounted together with a rat lip specimen in a single block on the stage of a thermo-electrically cooled cryostat. Serial sections were mounted on coverslips, allowed to dry at room temperature for 10 min and processed for succinic dehydrogenase (SDh), glucose-6-phosphate dehydrogenase (Gl-6-ph Dh) and lactic dehydrogenase (LDh) according to Nachlas *et al.* (1957) and Hess, Scarpelli and Pearse (1958).

The enzyme reaction product in the tissue sections was measured in a Cytoscan Zeiss microspectrophotometer provided with an automatic scanning stage.

The methodology and validity of the microspectrophotometric quantitation of histochemically demonstrated oxidative enzymes were previously discussed (Cabrini, Viñuales and Itoiz, 1969; Cabrini, Klein-Szanto and Itoiz, 1970).

Four scans from basal membrane to the horny layer were performed with a 1 μm diameter luminous spot on the skin and the glabrous border of each section under study. Blank measurements were taken from similar scans in unstained adjacent sections mounted on the same slide. In this way, it was possible to eliminate errors due to light absorption by substances different from enzyme reaction product, such as the melanin pigmentation which is nearly always present in bovine basal epithelial cells.

The optical density values obtained during scanning were recorded and integrated, thus obtaining information about total enzyme content (TEC) registered through the whole epithelial thickness. The mean enzyme concentration (MEC) which shows the amount of reaction per unit of tissue and is obtained by dividing TEC by the length of epithelial scans, was used to compare reactivity of different thicknesses of epithelia. Percentage relation between bovine and rat epithelia measured on the same slide was used in order to average values of enzyme reaction obtained from different animals. For each slide, the rat epidermis value was considered as 100 per cent. Using this relationship, the errors arising from variations in thickness of sections and technical processing of the material were eliminated.

Variations of enzymic reaction shown by different strata within each epithelium were also considered by dividing epithelial width arbitrarily into four equal parts, named for convenience basal, deep spinous, medium spinous and superficial spinous. Percentage relationship between different areas in the same epithelium was recorded, taking the value of the basal fourth as 100 per cent.

RESULTS

Morphology

The outstanding feature of the orthokeratinized stratified squamous epithelium of the bovine glabrous border was its great width. Hair follicles and sebaseous glands were absent in the corium. The basal epithelial cells were large with conspicuous elongated nuclei. The spinous layer consisted of large polyhedral cells, interconnected with intercellular bridges and was up to 800 μm thick. The horny layer consisted of several strata of horny cells and was up to 200 μm thick. Noteworthy was the absence of a granular layer underneath the anucleated horny layer.

Because TPPase is very active in the pinocytic vesicles of endothelial cells, the histochemical demonstration of this enzyme clearly shows vascular distribution observed with the light microscopy if sections of proper thickness are employed. In these preparations, subepithelial vessels of cattle glabrous border appeared as a dense network from which capillary loops passed into the thin rete pegs which extend into the epithelium up to the sub-horny layers (plate Fig. 1).

In the electron micrographs, the undulating basal lamina was associated with a wide band of collagen fibrils (Plate Fig. 2). Basal cells usually had oval nuclei perpendicular to the skin surface, with nuclear pores, abundant homogeneous chromatic material and one or two nucleoli. Many perinuclear mitochondria, little endoplasmic reticulum and plenty of free ribosomes were present. The cells of the spinous layer contained similar quantities of mitochondria but fewer other organelles. Upper spinous cells showed conspicuous keratinosomes, 0.1 to 0.3 μm in diameter, usually located near the cell membrane. These organelles, oval or circular in shape, had external membranes and an internal lamellar organization. These cells contained a high concentration of tonofilaments (Plate Fig. 3) which formed a dense network which gradually changed to constitute the horny cell matrix; the filamentous pattern remained to some extent in the amorphous intracellular substance (Plate Fig. 4).

In the superficial spinous layer that would correspond to the granular layer in other malpighian epithelia, the few elongated cells with long axes parallel to the tissue surface contained some small and isolated keratohyaline granules (Plate Fig. 5).

Oxidative enzymes

The total reaction product (TEC) of the three analyzed enzymes was proportional to the number of cells in each epithelium, and was considerably larger in cattle than in rat epithelium.

The mean enzyme concentration (MEC), which shows the amount of reaction product per unit of tissues, of SDh was strikingly higher in the glabrous border than in the skin in both species (Text Fig. 6). LDh, on the contrary, was lowest in bovine glabrous border and highest in bovine skin. The values for rat tissues were intermediate but higher in the glabrous border than in rat skin. Reaction for Gl-6-ph Dh was markedly more intense in bovine epithelia than in rat epithelia, glabrous border values being slightly higher than those of the skin.

LDh activity was low in the deep spinous strata, increased in the superficial spinous area and decreased again in sub-horny layers of all epithelial types (Text Fig. 7). Gl-6-ph Dh gradually increased from deep spinous layers and was maximal in the superficial spinous areas of the four epithelia studied. SDh decreased from the first layers of spinous strata towards the surface in all epithelia, with the exception of bovine glabrous border in which SDh increased in the deep spinous area and slowly decreased to the surface.

DISCUSSION

The great thickness of glabrous border epithelium in cattle accords with the greater thickness of other epithelia in cattle compared with other mammalian species. We have already shown (Lanfranchi *et al.*, 1978) that the ratio between the thickness of the glabrous border and lip skin epithelia is constant in man, cattle and rat. Within each epithelium, thickness values of the various strata are also proportional.

Nutrition of superficial layers is maintained by numerous capillary loops. The very thin and large rete pegs which contain the capillaries are sometimes difficult to recognize in conventional preparations but they become evident when vascular marker techniques are used. The subepithelial vascularization is

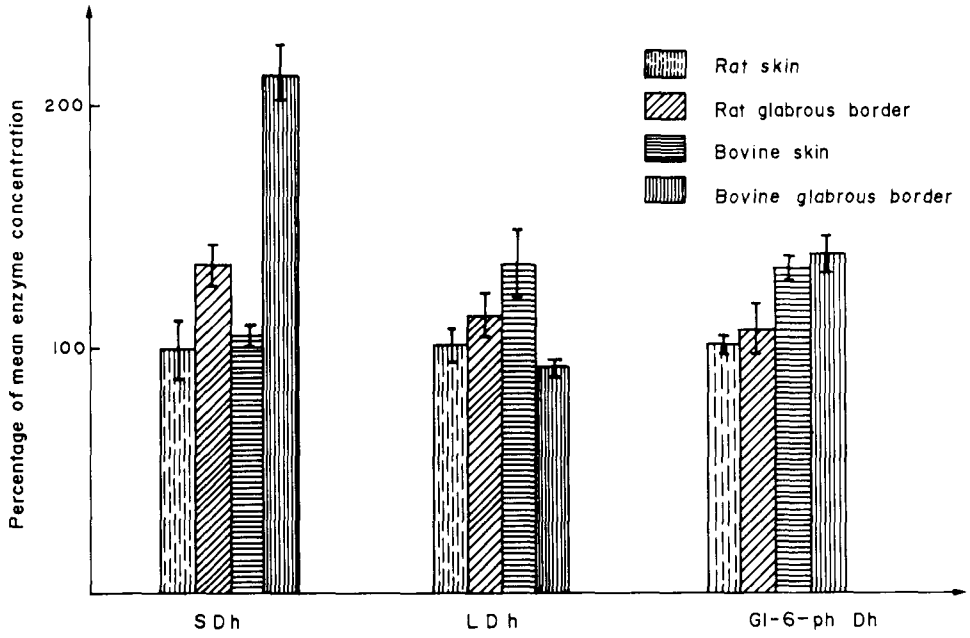

Fig. 6. Mean enzyme concentration (or activity per unit tissue) in the four epithelia. Percentage values were obtained for comparison of different slide measurements, rat skin value taken as 100 per cent. SDh: succinic dehydrogenase; LDh: lactic dehydrogenase; Gl-6-ph Dh: glucose-6-phosphate dehydrogenase.

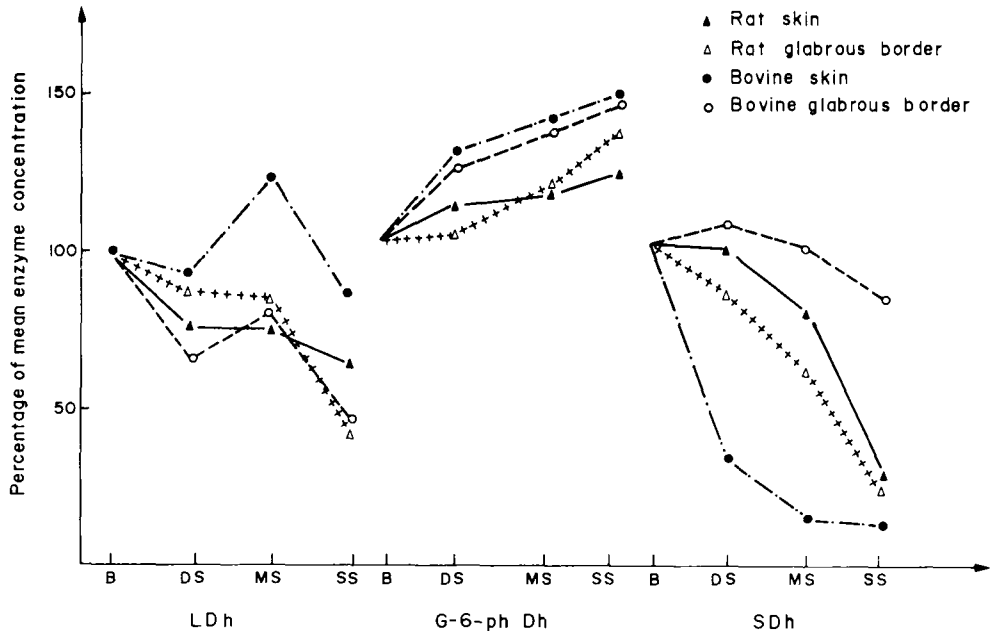

Fig. 7. Enzyme concentration in the various epithelia strata. Values are expressed as percentage, taking basal stratum value as 100 per cent. B: basal; DS: deep spinous; MS: medium spinous; SS: superficial spinous; SDh: succinic dehydrogenase; LDh: lactic dehydrogenase; Gl-6-ph Dh: glucose-6-phosphate dehydrogenase.

proportionally higher in cattle than in laboratory animals (Carranza *et al.*, 1966) and would allow higher oxygen consumption; it would thus explain the large number of mitochondria found in the superficial layer, as well as the greater use of Krebs Cycle pathway, as shown by the high SDh reaction, and the reduced energy supplied by the anaerobic glycolysis, as revealed by the lower LDh reactivity.

The pentose shunt in malpighian epithelia is not only an alternative pathway of energy supply but is also a way to produce intermediate products for the RNA synthesis, which in turn is necessary for protein synthesis. Accordingly, Gl-6-ph Dh activity may be related to the production of horny substance (Itoiz, Gimenez and Cabrini, 1974). In normal epithelia, the greater activity of this enzyme is localized in the most keratinized areas. (Itoiz *et al.*, 1972). In hyperkeratinized lesions of varied aetiology, it is greatly increased (Itoiz *et al.*, 1974). The intense reaction of this enzyme in highly keratinized cattle epithelia also supports the hypothesis that the enzyme is concerned with keratinization.

The great amount of protein synthesis which is necessary to maintain the thick horny layer is also evident in electron micrographs where a striking density of tonofibrils is present in all layers of the cattle glabrous border.

The isolated small keratohyaline granules revealed by electron microscopy are not visible with light microscopy in the glabrous border. If keratohyaline material is a stage in the keratinization process, the absence of large quantities of this material from this thick horny layer, which does show plenty of membranous formations, suggests that keratinization in the glabrous lip epithelium is a process different from keratinization in other orthokeratinized epithelia.

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Plate 1.

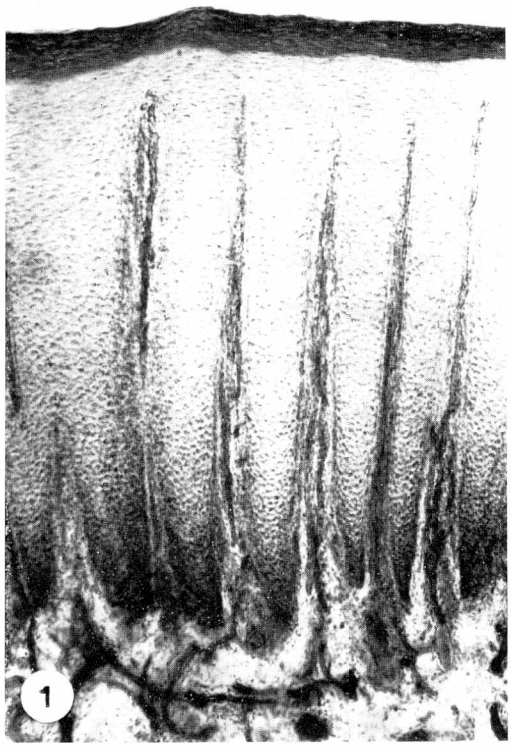
Fig. 1. Histochemical demonstration of TPPase as a marker of vascular walls in bovine glabrous border. Thin rete pegs contain capillary loops which reach subcornified layers. × 80

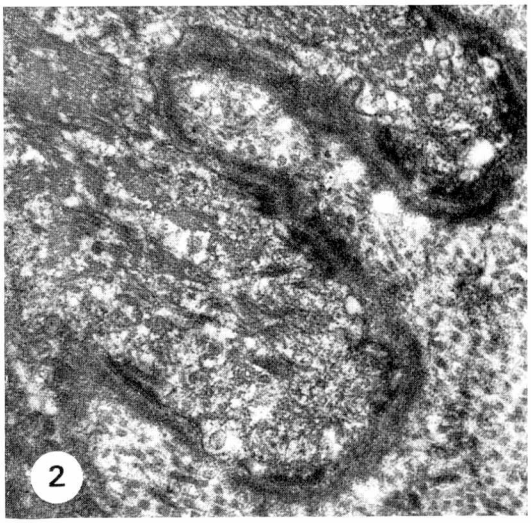
Fig. 2. Bovine glabrous border. The winding basal lamina is accompanied by a wide dense band of collagen fibers. × 21,000

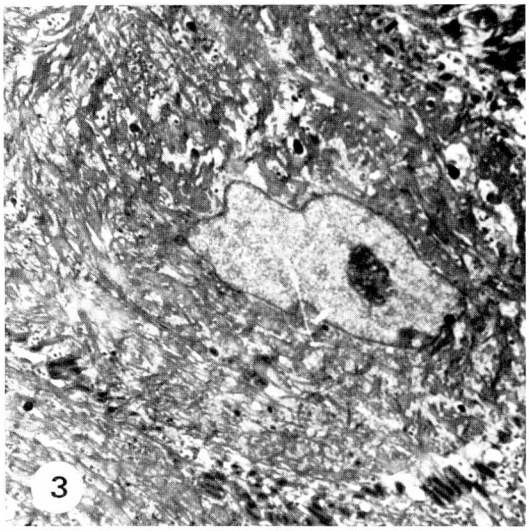
Fig. 3. Spinous layer cells in bovine glabrous border dense network of tonofilaments comprising the nucleus. Some small granules are visible in the network. × 3000

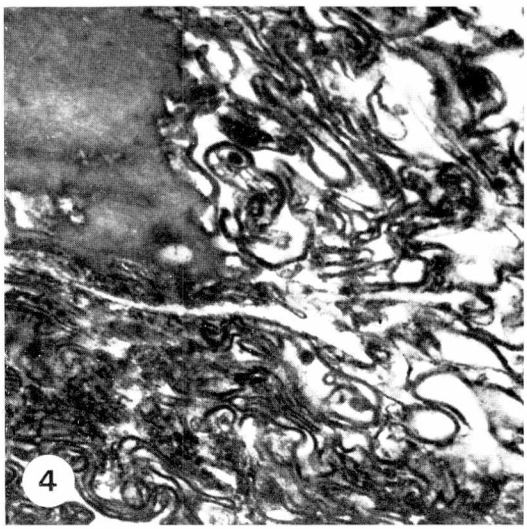
Fig. 4. Membranous areas are frequently evident, constituting an important portion of the horny layer. × 6000

Fig. 5. Small and isolated granules of keratohyaline in the granular layer of bovine glabrous border. × 3500









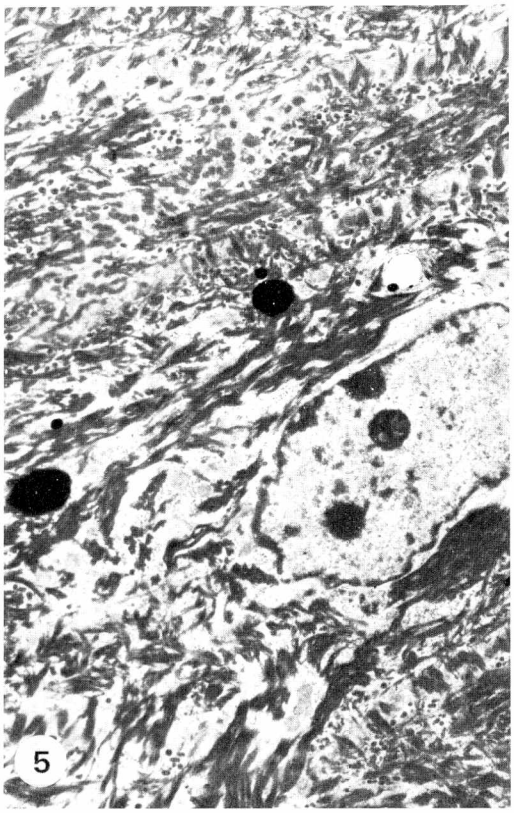


Plate 1.