H. Summary

In vitro models of proliferating and differentiating epidermal keratinocytes from the bovine hoof

This thesis describes an *in vitro* model of epidermal keratinocytes originating from the bovine hoof and an organotypic *in vitro* model of hoof tissue for the first time. Both models have been established and characterised.

Keratinocytes were isolated from the complex multilayered strongly cornifying squamous epithelium of bovine claws. Because of the specific morphology of the claw a number of modifications of isolation techniques described for epidermal cells of the skin were required. The isolated cells were cultured and subcultivated under standard conditions, i.e. 37 °C, 5% CO_2, 90 % humidity. Because of the intimate spatial interdigitation of dermis and epidermis of the claw the primary isolates of keratinocytes were always contaminated by dermal fibroblasts to a certain degree. It was possible to establish cell lines of keratinocytes by using different separation methods. Pure keratinocyte cultures grew very slowly and only incomplete differentiated colonies were formed. Co-cultures of keratinocytes with up to 30 percent fibroblasts formed three dimensional, organotypic colonies showing a tissue specific differentiation. A region specific differentiation, however, was not detectable in these oganotypic cultures. The high degree of differentiation is related to the fibroblast growth factors synthesised by dermal fibroblasts.

Using immunohistochemical methods the expression of tissue specific keratin proteins was detected. These findings were verified and individual keratins identified by separation of tissue protein extracts by SDS-PAGE electrophoreses and subsequent Western blotting.

Cultured keratinocytes expressed the following keratin proteins: K 1-3, K4/5, K6; K 13/14 and K 16. K4/5 and K 3/14 are the keratin pairs specific for bovine skin.

Light- and electron microscopic examination of the organotypic cultures revealed morphological features characteristic of hoof epidermis. As in hoof tissue, three layers with different stages of differentiation were present. Their spatial arrangement resembled the *in vivo* situation. A basal layer was located directly on the surface of the culture plate followed by up to 18 layers of spiny cells. The superficial stratum consisted of several layers of completely as well as incompletely cornified cells.

The electron microscopic examination demonstrated intermediate filaments establishing a three dimensional cytoskeleton associated with the desmosomes in a typical manner. Immunogold labelling identified the filaments as keratin proteins. Membrane coating granules, the specific organelles of epidermal cells, were present in the intermediate

layers. Their contents, the intercellular cementing substance (membrane coating material - MCM), was demonstrated in the intercellular spaces after extrusion by exocytosis. Formation of a cornified envelope and cornification were demonstrated in the superficial layers indicating terminal differentiation.

The organotypic culture described here for the first time is a helpful tool for studying dermo-epidermal interacions, which have been reported frequently to play a key role in the pathogenesis of claw diseases and laminitis in particular. The cell lines will be of great importance for studies on the influence of single bioactive molecules and growth factors on proliferation and cellular responses. Furthermore, adhesion and cytopathic effects of bacteria involved in the aetiology of infectious claw diseases can be studied. Initial experiments have been carried out already. Cultured keratinocytes were successfully infected with different Treponemes isolated from digital dermatitis lesions in dairy cows.

Using the models established and described here biological processes within the claw epidermis and dermal influences on epidermal proliferation and differentiation will be studied. The results of these *in vitro* studies will contribute to a better understanding of the pathomechanisms of laminitis and other claw disorders. The *in vitro* models are of particular interest to study the underlying mechanises of the well examined and described clinical lesions.