

## **G. SUMMARY**

### **Wound-healing of the RUSTERHOLZ Ulcer in the Bovine Claw**

Analysis of a Controlled Clinical Study on Wound-healing and the Influence of Biotin on the Healing Process with the Light and Transmission Electron Microscope

25 cows were selected for the examination of the healing of the RUSTERHOLZ ulcer (and the possible influence of biotin supplementation upon the healing). The light and transmission electron microscope were used for examination. The animals selected for the study were all affected by moderate RUSTERHOLZ ulcers. The cows were housed in the veterinary surgical clinic of the University Zurich throughout the entire study (50 days). 12 animals received 40 mg of biotin per day with their feed, 12 other animals served as a control group. The samples were taken at three different dates and were examined thoroughly with the light and transmission microscope. The gel electrophoretic separation of the cytoproteins in the cornified epidermis of the biotin and control animals serves, on the one hand, as a basis for comparison of the expressed cytoprotein cluster of individual animals during the various healing stages. On the other hand, it serves as a comparison between the biotin and the control animals.

During the transformation of the papillary body in the RUSTERHOLZ ulcer, the microcirculation is disturbed by the thrombosis and the dilation of hyperaemic capillaries in the dermal papillae. Thus, the nutritional supply of the avascular epidermis is hindered. The epidermal cells react by becoming dyskeratotic and changing their process of differentiation. Their synthesis products, cytokeratins and intercellular cementing substance, change their structure. Therefore, the intracellular and intercellular quality and the cytoarchitecture of the tissue undergo a change in structure. Characteristics of these processes are a cloudy swelling of the basal and lower spinous cells, a disturbed synthesis of the keratinproteins (filaments and Intermediate Filament-Associated Proteins = IFAP), their association and biochemical binding. The synthesis, structure, and exocytosis of the intercellular cementing substance (membrane coating material, MCM) into the intercellular space are also disturbed during malfunctioning synchronisation of the cornification.

During the wound-healing processes inflammatory cells (granulocytes and lymphocytes) amass in the dermis and also migrate into the epidermis. The collagen microfibrils of the papillary dermal connective tissue lose their regular pattern and

clear contours by inflammatory influences. In addition, their ends split. The basal membrane reveals thickening, doubling, and looping of the lamina densa at various locations, as well as the loss of the lamina rara externa. Furthermore, an inflammatory exsudat from the dermis reaches the expanded epidermal intercellular spaces to fill these, in some cases in addition to the present intercellular cementing substance. This process leads to an increasing separation of the epidermal cells and the cell contacts become more rare. The appearance of supra-basal mitosis in the epidermis is a clear sign of compensatory proliferation with the goal of closing the wound with new epidermal cells as quickly as possible.

Melanocytes and Merkel cells arising in the epidermis are a sign of advanced healing.

According to the results presented, the phases valid for cutaneous healing are not directly transferable to wound-healing of the claw tissue. Events required for the definition of these phases are missing or temporarily displaced. Therefore, another classification must be found. This is not possible on the basis of the results presented.

Practitioners frequently find structural weak points of the claw after a RUSTERHOLZ ulcer is clinically healed. The morphological results explain that these structural weak points are traced back to the microcirculatory disturbances and the dyskeratotic changes in the epidermal matrix. Regarding the influence of biotin on the wound-healing of claw ulcers, this study is not suited to make a statistically significant statement.

On the basis of morphological evaluation the following pattern results. There is a balanced proportion between animals with improvement, deterioration and constant quality in the biotin group. The control group shows a corresponding picture.