Zonulae occludentes in the epidermis of the snake *Natrix natrix* L.

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Summary. Zonulae occludentes of a very tight type were identified between the uppermost stratum germinativum cells of the grass snake *Natrix natrix* L. by means of the lanthanum tracer technique. Since zonulae occludentes alone are not capable of preventing transepithelial water flow, an additional barrier mechanism is postulated, responsible for the low rates of cutaneous water loss measured in squamate reptiles. It is suggested that the observed zonulae occludentes are involved in the sloughing process.

Tight junctions in the form of continuous zonulae occludentes are a common feature of the epidermis of anamniotic vertebrates. They are present in fishes[2] as well as in amphibia[3,4]. In mammals, however, tight junctions have been identified only in the form of single fusion points, spotlike maculae occludentes or single strands, whereas complete zonulae occludentes were never encountered[5-7]. Since zonulae occludentes occur regularly in the epidermis of the mainly aquatic anamnia and are absent in the terrestrial mammals, it seemed worthwhile to investigate their presence in the skins of reptiles which, phylogenetically, lie between the lower and the higher vertebrates.

Materials and methods. Skin biopsies were taken from the backs of several specimens of the grass snake *Natrix natrix* L. during the perfect resting condition of the sloughing cycle[8,9]. The outer scale surface as well as the hinge region were examined. After fixation in cacodylate-buffered 2% formaldehyde-2% glutaraldehyde, specimens were postfixed in 1.3% s-collidine-buffered osmium tetroxide, dehydrated in acetone and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate.

For tracer studies 15 mM $LaCl_3$ in saline was injected s.c. 45 min before biopsy. In order to improve penetration of the tracer, lanthanum was added in the same concentration to all agents up to acetone 90% in some specimens.

Results. The use of lanthanum as a tracer demonstrated that the intercellular space of the stratum germinativum forms a continuous system, surrounds the cells from all sides and separates them from each other (figure 1). The width of the intercellular space was fairly constant: apart from occasional dilatations, it measured 20–30 nm between the outer leaflets of apposing plasma membranes. Invaginations into the keratinocytes were frequently seen. The impregnation was not impeded by desmosomes or gap junctions. Arrow in figure 1 points to the barrier for outward flow of the tracer, where labelling of the intercellular space was interrupted; it was encountered at a level just beneath the lowermost keratinized layer, the alpha-layer. This result was the same in specimens injected with lanthanum as in specimen treated with fixatives containing $LaCl_3$. No difference in the localization of the barrier was observed between the outer scale surface and the hinge region.

High-power micrographs (figure 2) revealed that a further penetration of the tracer was prevented by tight junctions, localized between the distal portions of the lateral plasma membranes of the most superficial stratum germinativum cells. Since no tracer leaked into the intercellular space between stratum germinativum and alpha-layer, it is concluded that the tight junctions form belt-like zonulae occludentes. Suitable sections of the junctions (figure 3) showed an average depth of 0.35 µm and about 8 fusion points or strands.

It is admitted that transmission electron micrographs are not very suitable for measuring the depth of a zonula occludens or for counting the number of strands therein. Adequate information is to be expected only from sections cut normally through a zonula occludens. In order to avoid oblique sections, the micrographs showing the zonulae occludentes with the smallest extent were the only ones taken into consideration. Although definite proof by freeze-fracturing has still to be obtained, the values presented above seem to be approximately accurate.

Discussion. It is known that tight junctions allow steep ionic and osmotic gradients and also block the diffusion of small electrolytes and water[10]. Zonulae occludentes with a depth of 0.35 µm and containing about 8 strands belong – according to the classification of Claude and Goodenough[11] – to the very tight type. Therefore it is concluded, that the 'very tight' zonulae occludentes around the uppermost stratum germinativum cells seal off the intercellular space very efficiently, thus preventing water and solutes from penetrating through the intercellular space into the keratinized layers.

Zonulae occludentes in the frog epidermis, which occupy a similar position just beneath the stratum corneum[3,12], are known to establish a high transepithelial resistance[13] and thus to be very tight. But amphibian skin also shows a very high rate of water loss as well as large water influx by osmosis[14]. Total flow through an epithelium is composed of 2 components, one taking a paracellular pathway, the other a transcellular one[13,15]. Since the paracellular pathway is sealed by zonulae occludentes, the observed water movement has to take the transcellular pathway. From this it is concluded that zonulae occludentes in snake epidermis prevent penetration of molecules and ions through the intercellular space, but do not in themselves protect the animal from desiccation. Therefore an additional effective permeability barrier, responsible for the low rates of cutaneous water loss measured in squamate reptiles[16], is postulated. It has to be localized above the level of the zonulae occludentes, presumably in the alpha-layer[17] or in the mesos-layer[18].

During vertebrate evolution, the integument had to cope with different environments and to meet various requirements. In aquatic animals such as fishes and amphibians, preventing transepithelial flow of solutes and electrolytes is more important than protection from desiccation. This goal is adequately achieved by zonulae occludentes and the semipermeable plasma membrane of epidermal cells. In terrestrial animals, however, reduction of transcutaneous water loss becomes crucial. In mammals this seems to be brought about by membrane coating granules, which are extruded into the intercellular space and form a continuous system of hydrophobic lipid lamellae in the upper stratum granulosum and lower stratum corneum[19,20]. Due to the fact that this system extends over several cell layers, enveloping the individual cells, it has the capacity of blocking the paracellular as well as the transcellular pathway. Zonulae occludentes are no longer a necessity and their formation is suppresed.

Since the zonulae occludentes in snake epidermis cannot be correlated with the establishment of the permeability barrier, it is suggested that they play a role in the complex formation of 3 distinct keratinized layers as well as in the preparation of the sloughing process. Backing this sugges-

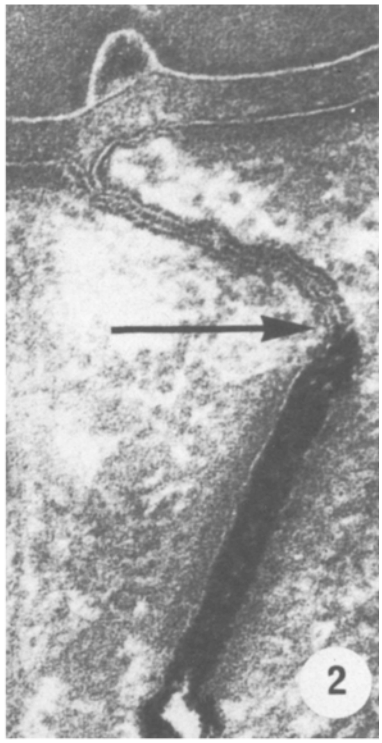

Fig. 1. Stratum germinativum (sg) of the Natrix epidermis showing tracer-penetrated intercellular space. Upward flow of lanthanum is blocked by zonulae occludentes between the uppermost living cells (arrow). × 13,000.

Fig. 2. Zonula occludens stops tracer abruptly (arrow). × 170,000.

Fig. 3. Zonula occludens revealing fusion points or strands (arrows). No tracer was applied in this specimen. × 170,000.

tion is their presence just beneath the lowermost keratinized layer, regardless of its keratin type, during the whole sloughing cycle and between the lowermost cells of the outer epidermal generation (i.e. the part of the epidermis which, eventually, will be shed) in the later stages of the cycle[18].

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Ultrastructural evidence for the innervation of human pulmonary alveoli

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Summary. Electron Microscopic observations of the biopsied human pulmonary alveoli showed the occurrence of unmyelinated axons in the interstitium near the type I pneumocytes. These axons very likely have sensory functions.

Previous light microscopic studies of the innervation of the lungs in various animal species presented different results concerning the innervation of the alveoli. Honjin[2] and Spencer and Leof[3] showed nerves in the subpleural alveolar walls; Larsell and Dow[4] found nerve fibres in the alveolar ducts; and Elftman[5] and Hirsch and Kaiser[6] demonstrated nerves to be generally distributed in the alveolar walls; but Gaylor[7] and Fillenz and Woods[8] did not observe sensory nerves beyond the respiratory bronchioles. Despite such discrepancies of the histological studies, there was an increasing physiological evidence suggesting the presence of the sensory nerves in the alveolar walls[9]. In support of the physiological findings, recent ultrastructural investigations have revealed the presence of unmyelinated axons located in the interstitium of the alveolar walls of the rats[10] and mice[11,12]. Axons containing various types of vesicles have also been shown to supply the pulmonary capillaries in the dogs[13]. This paper presents electron microscopic observations of nerves in the human pulmonary alveolar walls.

The human lung tissues were obtained from a lung biopsy taken near a tumor under the pleural surface. They were

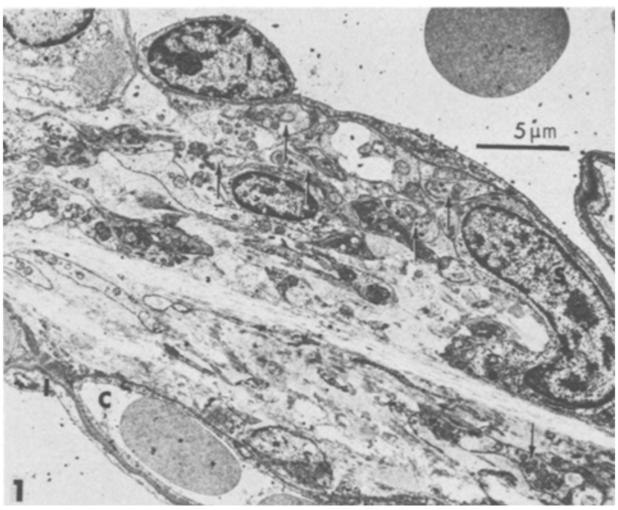

Fig. 1. This is a section through the alveolar septum which is covered on both surfaces by the type I pneumocytes (I) and contains a pulmonary capillary (c). Numerous unmyelinated axons (arrows), partially or completely covered by the Schwann cell (S) are located in the septum, and some of these are immediately under the type I pneumocyte. Calibration: 5 µm.

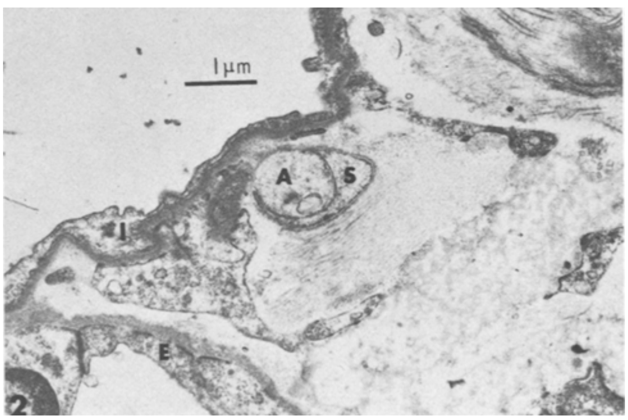

Fig. 2. A single axon (A) partially surrounded by the Schwann cell (S) is near the type I pneumocyte (I). E: Endothelium of the pulmonary capillary. Calibration: 1 µm.