THE MORPHOFUNCTIONAL SIGNIFICANCE OF THE CLEAR LAYER OF SKIN AND THE RUMEN MUCOSA IN ANIMALS THE EPIPLASMAL BARRIER

VICTOR DRĂGOLICI

C.S.V. Crevedia, Dambovita county

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By using histochemical and histoenzimatic methods I have revealed an enzimatic molecular layer between the last layer of live cells (granular layer), and the corneous layer of the rumen mucosa and the skin. This extracellular molecular layer, secreted by the cells of the granular layer, is composed of energy enzymes (alkaline and acid phosphatase, determined through the Gomori method), ATP (Wachstein method), cytochromoxidase (G-Nady) and SH tiolitic groups (through Chevremont and Frederic reactions). This layer has the role to protect the epithelium of the rumen and the skin from the substances that penetrate from the outside, to divide them into their subcomponents, to select and transport them to the internal environment through an active biological process even against the osmotic gradient. I named this layer "The epiplasmal barrier", which is essentially a biochemical buffer and a selective filter where complex molecular reactions take place through energy expense (ATP, phosphatase) under the genetically-fixed impulse and control of nucleic acids.

Key words: The epiplasmal barrier of the rumen mucosa and skin.

INTRODUCTION

The clear layer from the mammals' epidermis, placed between the last cell layers of the mucous malpighian body (the granular cells) and the corneous layer, was thus named due to its clear aspect, which cannot be stained by common histological dyes or argentic impregnation.

Histologically, two distinct areas have been described in the clear layer[1, 8]:

1. Ranvier's intermediate layer, which does not contain granules of Keratohialina and does not show the characteristic refractivity of Keratin[1] and

2. The clear layer as such, where one can distinguish the outlines of degenerated, flattened, translucent cells, with a cytoplasm filled with a homogeneous substance, which appears like eleidin in the cells of the corneous layer.

Why does the clear layer remain refractory to common histological staining?

We start from the premise that nature does not create unfunctional structures, especially in a multifunctional organ, that sits at the boundary between two different environments such as skin.

In similar structures, the stratified pavimentous epithelium of the rumen has the role to absorb water, ions and the first fractions of the rumenal digestion, such as volatile fatty acides[6–13]. Their transport through the component layers of the mucous membrane was attributed to the phenomenon of diffusion, because of the differences in concentration between the ruminal lumen and the internal environment. Still, the transport through the mucous membrane against the osmotic gradient is not justified when concentration in the rumen is increased.

W.Von Engelbardt[6] presumes that in the deeper layers of the epithelium there is an area with high osmotic pressure that prevents the net flow of water due to normal osmotic gradients. Also, D.H. Steven and A.B. Marshall suggest an active transfer of Na+ in the rumen mucosa from lumen to blood, involving a three-compartment system[13], (Fig. 13). The experiments on the absorption of

volatile fatty acids the author has made, by using an original method, have shown that their absorption is normally done in 6 hours, with a rising curve in the first 3 hours, and then a decreasing lysis at sheep (lambs and reformed sheep subjected to intensive fattening), fed with fibers and 30% concentrates. If the fibers in the fodder ration are chopped or ground, and the ground concentrates exceed 60%, the absorption of volatile fatty acides decreases notedly, and after 25–30 days of feeding it almost becomes zero. Increasing the percentage of ground concentrates, as well as chopped or ground fibrous fodder, leads to the disruption of the absorption process at the level of the rumen mucosa.

– By what certain mechanisms is absorption done at the level of the rumen mucosa, against an osmotic gradient?

– What are the structures that attend to the formation of these mechanisms?

In this paper I try to clarify this very complex biological mechanism using histochemical and histoenzymatic methods.

MATERIAL AND METHODS

I collected rumen fragments through laparotomy from lambs older than four months and adult reformed sheep, subjected to intensive fattening, as well as skin samples from dogs, guinea pigs, rats and lab rats.

The specimens were divided into 4 groups on which histochemical and histoenzimatic reactions were tested as follows:

– The first group was processed freshly-frozen by sectioning it under the freezing microtome and transferring the sections directly into the incubating environments, prepared for the following histoenzimatic reactions: alkaline phosphatase (Gomori), acid phosphatase (Gomori), ATP (Wachstein & Meisel) and cytochromoxidase (G-Nady).

– The second group was immersed in Carnoy's fluid, fixed, embedded in paraffin and sectioned into 3–5 microns. These sections were tested through the PAS reaction (McManus), to reveal neutral mucopoly-saccharides, and Feulgen's DNA reaction.

– The third group was immersed in 10% neutralized formol, paraffin-fixed and sectioned into 3–5 microns. These sections were tested through Sudan Black B reaction for lipids, Chevremont and Frederic reaction for sulphidrilic groups SH.

– The fourth group was exposed to staining with both Eosin hematoxylin and Masson trichrome.

FINDINGS

The analysis of the specimens under the microscope led to the observation that an active molecular layer exists both in the rumen mucosa and in the skin, secreted by the epithelium's granular cells, made of large cytoplasmic granules as well as granules on the external surface of the plasmic membranes of the granular layer, forming a more or less compact continuous layer above it. I also noticed that there is a direct connection between the degree of development for the granular cell layer and the clear layer. Regardless of the species and the sampling place, the clear layer is highlighted, whether more or less clearly, where the granular layer is clearly obvious with 2–3 cell layers, having a granular slightly basophilic cytoplasm.

The histoenzimatic reactions that I observed revealed certain functional mechanisms that connect these structures, namely:

– Alkaline phosphatase is positive in the cytoplasm of the base cell layer, as well as at the level of the plasmic membranes of the granular layer cells, staining both large cytoplasmic granules and granules on the external surface of the superior plasmic membranes of the granular layer, forming a continuous layer (Figs. 1–3).

Fig. 1. Alkaline fosphatasis in a section of dog skin. Observe the intense positive reaction at the level of the intermediary lucidum layer. Gomori method, OB 10×.

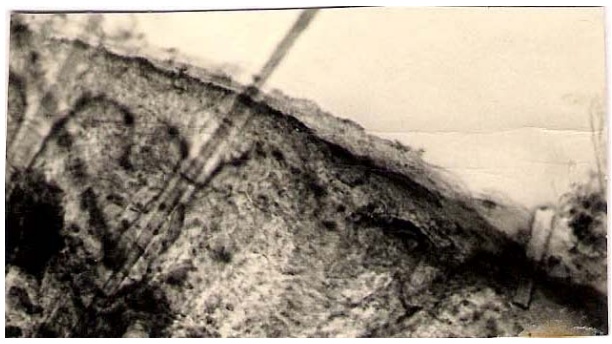

Fig. 2. Alkaline fosphatasis at the level of the bazal and intermediary lucidum in a section of white rat epiderm. Gomori method, OB 10×

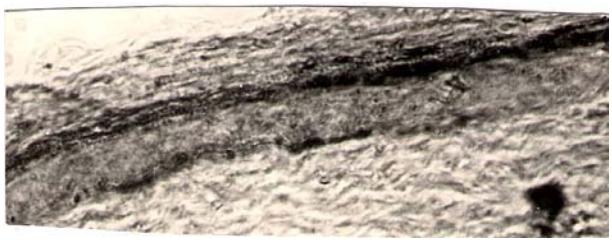

Fig. 3. Alkaline fosphatasis in a section of dog skin (detailed). Positive reaction at the level of the bazal layer, in the plasmatic membranes of the poliedric cells and especially in the granular cells and in the intermediary lucidum layer. Gomori method, OB 10×.

– Acid phosphatase, negative in the cells of the epithelium's base layer and in the thorny cells, appears as reactive granules in the cells of the granular layer, accumulated by lysosomes, but is also present as fine granules at the superior edge of the granular cell layer along the superior plasmic membrane outside it, forming a continuous band (Fig. 4a,b).

– ATP is present in the cells of the base layer and the granular layer, and in its membranes, as well as outside the granular layer, belonging to the extracellular enzymatic layer (Fig. 5).

Fig. 4a. Acid fosphatasis in mouse skin. Positive reaction in the liosomes of the last granulary cell, at the level of the upper plasmal membrane and at the intermediary lucidum layer. Barka-Anderson method, OB 24 000×, pH 5, EPON, uranil-aceatte contrast and Raymonds.

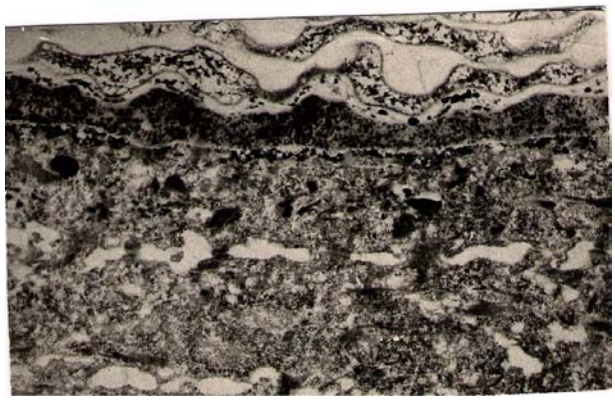

Fig. 4b. Acid piplasmal fosphatasis – detailed.

– Cytochromoxidase is positive in the cells of the base layer, the thorny and granular cells, but is negative at the level of the clear layer, being strictly intracytoplasmic enzymes, generated by mitochondria and connected to the electron transport within the cellular respiratory chain.

– SH groups appear intensely positive in the cells of the base layer, sometimes also being diffused in the intercellular paces. They are more intense in the granular cells, connected to cytoplasmic extracytoplasmic granules, forming an intensely reactive band at the level of the clear layer (Figs. 6, 7).

– PAS reaction is negative in the clear layer.

– Feulgen's reaction reveals the presence of a considerable amount of DNA in the superior area of the cytoplasm of the last layer of granular cells and at the level of plasmic membranes without crossing this limit (Figs. 8, 9).

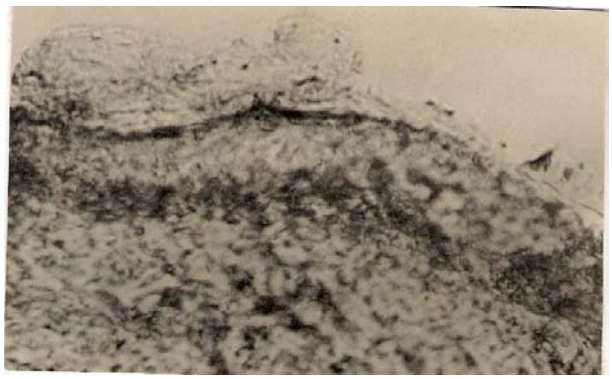

Fig. 5. A.T.P. – asis in mice akin. Positive reaction at the epiderm base in the poliedric and granular cells and in the intermediary lucidum layer, as a continuous stripe. Eachstein and Meisel method, OB 10×.

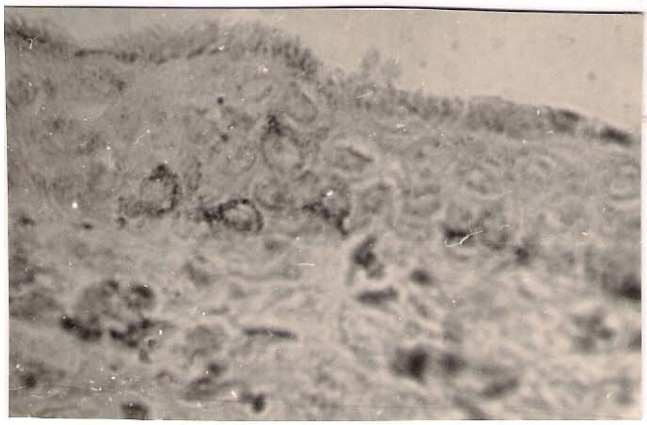

Fig. 6

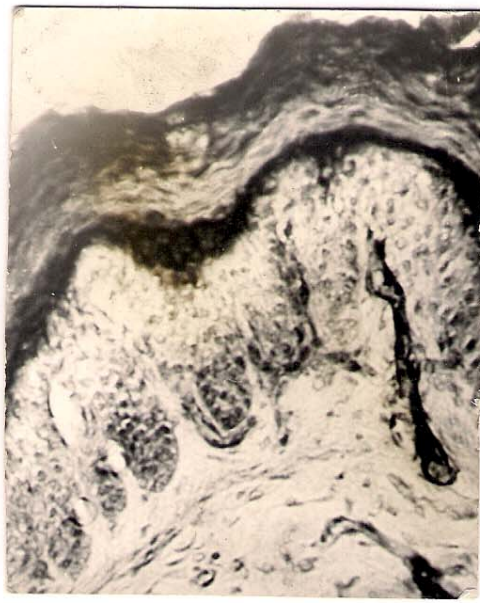

Fig. 7

Fig. 6 and Fig. 7. Reaction for SH groups in mice epiderm and rumen epithelium. The reaction is granulary in the intermediary lucidum layer or dense and continous. Chevremont and Frederic method. OB 10×.

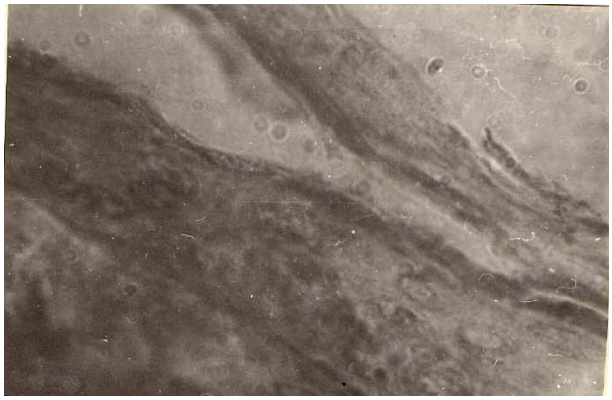

Fig. 8. Positive D.N.A. at the border zone between the vital epiderm cells and the cornos layer. Section of dog skin. Feulgen reaction. OB 10×.

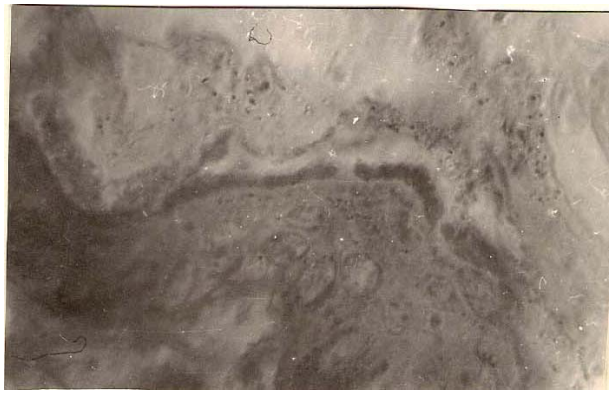

Fig. 9. Feulgen reaction in mice epiderm. Accumulated A.D.N. at the upper limit of the granular cells. Feulgen reaction. OB 90×.

CONCLUSIONS

The material I examined, comparing and contrasting the microscopic aspects of the respective reactions on both sections of rumen mucosa from ovines and skin sections from different mammals attests the fact that the distribution of the histochemical and histoenzimatic reactions is similar in all the analysed sections, proving the unity of the mechansims of functional action in similar structures.

The slight morphological differences that appear between species related to the thickness of the mucous Malpighian layer or the corneous layer, to the presence or the absence of pilose follicles and annex glands, does not affect the functional aspects of these structures. Two of these aspects are worth mentioning in particular:

– The intense enzimatic and metabolic activity in the cells of the granular layer of both the epidermis and the rumen mucosa, where besides keratohyalin a large quantity of energy enzymes (phosphatase, ATP), DNA and SH tiolic groups;

– The presence of a continuous layer, outside the plasmic membrane of the last granular cells, made of intensely reactive enzymes and SH groups, secreted by these granular cells.

This layer located between the last vital cells of the epidermis (the granular cells) and the first cornous cells corresponds histotopographically with the clear layer from classical histology. In particular, the extracellular chemical structures that were revealed by the reactions are in the middle area of the clear layer, where histologists were not able to distinguish any structure (Fig. 13); which may be explained through the fact that tiolic enzymes and groups have no affinity with regular histological dyes (Fig. 10).

By contrast, the specific histoenzimatic reactions reveal at this level the presence of active proteic molecular groups of energy enzymes and sulphydrils, which have a great mobility and endless possibilities of reacting in a certain order, in space and time[3, 9] (Figs. 11, 12).

Undoubtedly, like other enzymes and substrates I did not determine in the current research they are part of the molecular structure of this extracellular layer which I named « The epiplasmal barrier ». This "buffer" layer poses itself between the cells of the epithelium and the corneous cells to prevent live cells from coming into direct and brutal contact with the substances that enter the large spaces between the corneous cells by diffusion. The presence of enzymes from the hydrolase and SH groups in this layer proves that chemical reactions of molecule division in subcomponents and configuration through free radicals take place here, achieving an active transport of substances by "carrier-transport" like in the case of plasmic membranes.

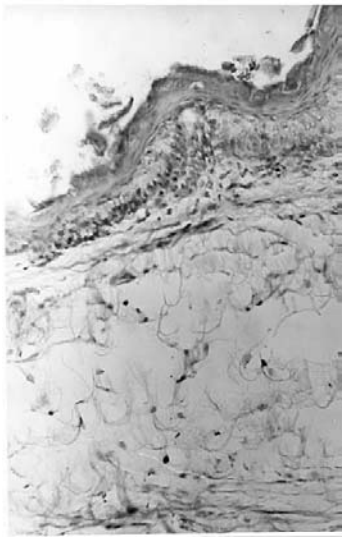

Fig. 10. Histological section of mucous rumena of a lamb. Hematoxylin eosin staining and Masson Thricomes. Ob. 10×.

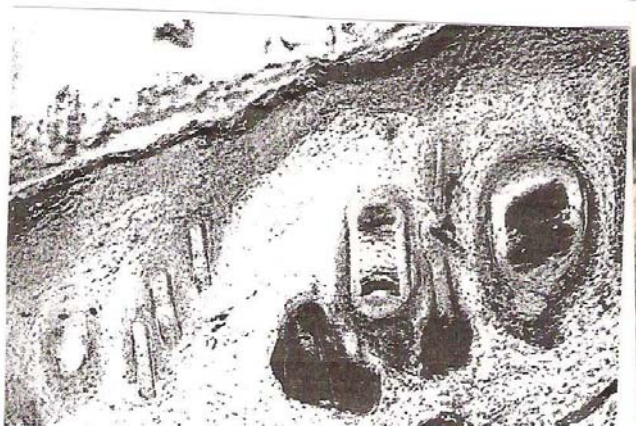

Fig. 11. Alkaline phosphatase on a section of dog skin. Highly positive reaction, at the level of the clear intermediate layer. Met. Gomori. Ob. Iox – Epiplasmal barrier.

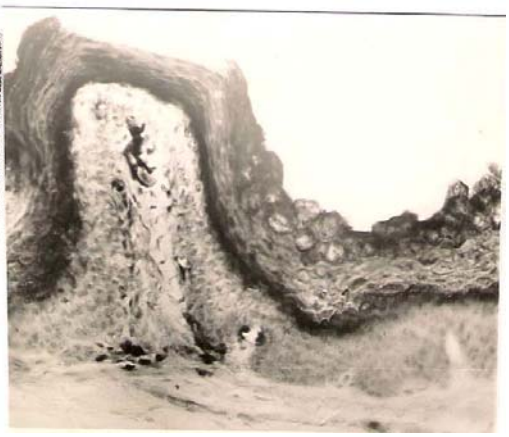

Fig. 12. Alkaline phophatase on a section of the mucuous rumen of a lamb. Highly positive reaction, at the level of the clear intermediate layer. Met. Gomori. Ob. Iox – Epiplasmal barrier.

D.H. Steven and A.B. Marshall

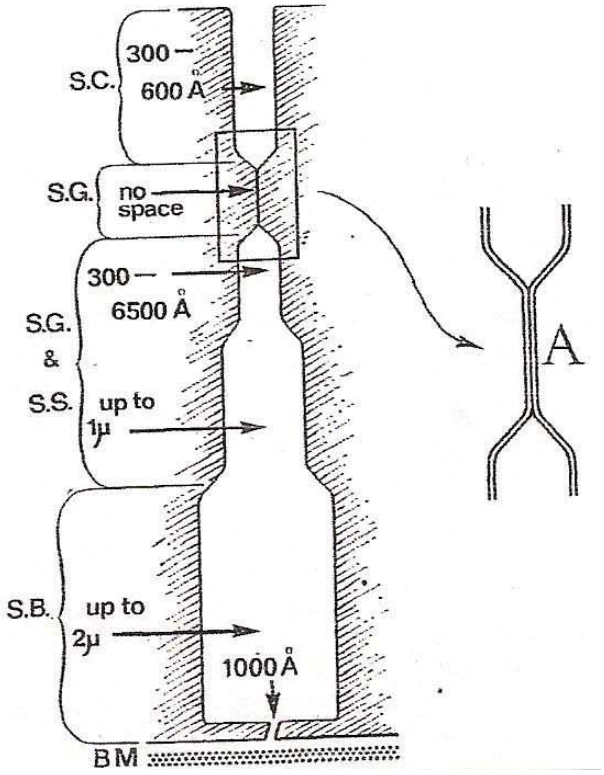

Fig. 13. In "The Organization of the Rumen Epithelium", as seen under an electronic microscope, the authors [13] state that there is a small space between the corneous and the granular layer that belongs to the granular layer, where no structure can be distinguished (this would be a junction between the membranes of the granular cells). In reality this junction (A) corresponds to the middle area of the clear layer where [4, 5] histochemical and histoenzimatic methods have revealed the presence of energy enzymes (alkaline and acid phosphatase, ATP, cytochromoxidase and SH tiolic groups), secreted by the cells of the granular layer, the (A) area which I have named "The epiplasmal barrier".

From a functional perspective, « The epiplasmal barrier » plays the role of mediator between the organism and the environment, the free molecules it is made of being highly mobile and prompt in biochemical action. By connecting or rejecting the substances that enter the interlamelar spaces of the corneous layer from the outside or leave the epithelial structures, « The epiplasmal barrier » becomes an active semipermeable biological layer, a biochemical buffer and a selective filter, where complex molecular reactions take place, with energy expense (ATP, phosphatase), under a genetically-fixed impulse and control (DNA), of the nucleic acids[11, 14].

Therefore the physical and chemical theories on substance transport elaborated so far[7, 10] are explained by the identification of « The epiplasmal barrier », the biological basis of continuous exchanges between the organism and the environment. Thus through energy expense, the physical force of diffusion is transformed into an active biological process, programmed to capture, select and transport the exchange substances between the rumen and the internal environment, against the concentration gradient.

Theoretically, this barrier should be present at the level of all protection and absorption epithelia. The epiplasmal barrier takes on different morphological aspects according to local functional necessities. The presence and the variations of « The epiplasmal barrier » in all the other epithelia remains to be verified, in relation to the substance transport between the organism and the environment.

I am now underlining the presence of this active barrier in the clear layer of skin (in dogs, guinea pigs, rats and lab rats) and in the rumen mucosa in ovines, in its functional relationship with the granular layer of the epidermis and the rumen mucosa, where it is secreted.

In this sense, I consider that the clear layer must be limited only to Ranvier's so-called intermediate area, which corresponds to « The epiplasmal barrier » (Figs. 11, 12).

The pressed cells with a degenerated nucleus and eleidin-filled cytoplasm, which are right above this area, can be interpreted as belonging to the corneous layer proper.

From this point of view, the clear layer would morphologically constitute a limiting factor between the vital cell elements of the epidermis, the ruminal epithelium and the underevolved cell elements, and functionally, the biological basis of the information transfer, selective exchanges and defence that take place uninterruptedly between the organism and the environment.

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