GLYCOTECHNOLOGIES

IN VETERINARY DERMATOLOGY:

A NEW ERA FOR TOPICALS
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It has long been recognised that certain bacteria such as *Pseudomonas* spp. and some yeasts such as *Candida* spp. exploit sugars to adhere to cell surfaces. Recent studies suggest that many more microbial species may utilise sugars for cell adhesion.

While adherence is a highly significant part of microbial colonisation and infection it is now clear that sugar molecules have important roles in a wide range of biological processes. Sugars are acknowledged as central molecules in the communication business. Sugars acting as surface receptors facilitate cells to communicate with each other as well as with their surrounding environment.

Skin infections and inflammation are two major areas that the veterinary practitioner experiences in their clinic on a day to day basis. Sugars play key roles here. **Microbial adherence to skin and the modulation of inflammatory processes are influenced by sugars.**

The knowledge gained through glycotechnology studies has opened potential therapeutic gateways. This is both timely and apt as traditional antibiotic therapies come under threat by the increasing problem of microbial resistance. **Sugars as therapeutic agents against the common veterinary microbial skin infections and control of dermatitis offer new solutions to some old problems.**

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Sugars have long been limited to their role of energy-yielding substrates. In recent years however, scientists have begun to recognise their structural role on the cell surface, uncovering their functional value in cell-to-cell communication and signalling. These molecules are now beginning to exert their right as active components with beneficial effects on animal health.

« The last decade, has witnessed the rapid emergence of the concept of the sugar code of biological information. Mono-saccharides represent an alphabet of biological information similar to amino acids and nucleic acids but with unsurpassed coding capacity." Acta Anatomica 161, April 1998.

« Carbohydrate modifications of proteins and lipids are key factors in modulating their structure and function within cells. In the extracellular milieu, they exert effects on cellular recognition in infection and immune response. » Science 291, March 2001, Special issue on Carbohydrates and Glycobiology.
SUGARS: WHAT ARE THEY? (17, 39)

The sugars or carbohydrates are the most abundant class of organic compounds found in living organisms. They are called carbohydrates because the carbon (carbo), hydrogen (hydr) and oxygen (ate) they contain are usually in the proportion to form water with the general formula Cn(H2O)n.

Sugars can be divided into two major subfamilies:

- the simple sugars also called oses or monosaccharides such as glucose, galactose, mannose, fucose, etc. They are made of one single organic molecule.

- the complex sugars:
  - oligosaccharides composed of 2-10 monosaccharide molecules.
  - polysaccharides with more than 10 monosaccharide molecules. Many polysaccharides consist of repeated disaccharide units. They include glycosoaminoglycans (GAGs).
SUGARS: WHAT ARE THEY FOR? (17, 21, 39)

The many faces of sugars

Sugars are involved in various body functions.

Sugars have long been considered only for their role as major sources of metabolic energy (glucose) and energy storage molecules (glycogen).

They are also known to play a structural role, notably in cartilage (glucosamine, chondroitin sulphate), and as one of the three essential components of DNA (desoxyribose) and RNA (ribose).

Recent advances have unveiled the fundamental role of sugar molecules at the level of the cell membrane. Here, complex sugars link with proteins and fats to form glycoproteins and glycolipids with a very broad range of properties.

The cell surface sugars are the molecules most widely used in cell-to-cell recognition, interaction and communication. They play also an important role in immune system signalling and help the organism to fight against pathogenic micro-organisms.

So ubiquitous are these cell surface sugar molecules that cells generally appear to one another as sugar coated. Sugar receptors are of major importance in the epidermis where they are called “skin sugars”.

6/39
The role of skin sugars in surface microbe-host interactions (5, 8, 13, 17, 18, 28)

Skin infection depends on the ability of micro-organisms to adhere to host skin cells, colonise and proliferate and produce virulence factors. They do this by means of lectins, glycoproteins which recognise and bind to skin sugars exposed on host cell membranes. Keratinocytes in particular, the main cells of the epidermis, do harbour skin sugars on their surface. Microbial lectins are important virulence factors located on the surface of yeast or bacteria, either on the cell wall or on cell membrane developments (pili). Lectins are classified according to the type of sugar they recognise specifically, such as D-galactose, D-mannose, D-fucose, sialic acid and N-acetyl-galactosamine (17, 28). The interaction between microbial agents and the animal host cell is multivalent. The abundance of carbohydrates in various forms at the animal cell surface is one reason why microbes to a large extent have selected sugar receptor for colonisation and infection.

In healthy skin, an integrated array of defence mechanisms, related to the physical and chemical properties of the integument, allow to control microbial proliferation. Continual desquamation, the secretions of the cutaneous glands as well as the release from cells within the epidermis of peptides and lipid metabolites play an antimicrobial role and may also inhibit microbial adherence. (17) The cutaneous microflora further helps to prevent microbial proliferation by competitive growth and chronic stimulation of epithelial surfaces. (18)
Thus, although the skin is constantly exposed to potential pathogens, such as bacteria (*Staphylococcus intermedius*, *Pseudomonas aeruginosa*) and yeast (*Malassezia pachydermatis*), infection only occurs when the normal epidermal protective functions are disrupted. On the epidermis, the adherence of pathogenic bacteria and fungi is expressed on fully differentiated keratinocytes (corneocytes) and hence, any alteration of the skin cells differentiation process (wound, inflammation, seborrhea, etc.) can promote microbial adherence and trigger infection. (8, 17)

A “converse way” is also described in which microorganisms themselves harbour carbohydrates on their surface that are recognised by host cell surface lectins (17).

Microbial cell surface carbohydrates promote intercellular adhesion between micro-organisms, producing biofilm. Microbes aggregation increase their virulence and resistance to antimicrobial agents. Biofilm formation is dependent on adherence to the substrate, such as host cells or inert material eg catheters. (5, 13, 17)
The role of skin sugars in surface immunity (7, 10, 11, 17, 42)

The skin, and particularly its outer layer (epidermis), is the interface between the internal milieu and the external environment, usually providing an effective protective barrier. The keratinocytes, the most numerous cell population in the epidermis, can however become key cells for the initiation and maintenance of inflammation in skin.

In the healthy epidermis, keratinocytes are not activated. But many exogenous factors (skin exposure to ultraviolet radiations, infections, irritants, allergens, etc.) and/or endogenous stimuli (cytokines produced by cells of the immune system, etc.) may lead to keratinocytes activation.

Activated keratinocytes release a wide panel of cytokines (soluble factors mediating communication between cells), such as IL1, TNFα, Chemokines and Growth Factors. These cytokines act as “alert signals” meaning that something is going wrong in the skin or that external aggression is ongoing. The cytokines alert fibroblasts, endothelial cells, melanocytes, Langerhans cells and contribute to lymphocytes recruitment. Activated keratinocytes also express cytokine receptors on their surface, allowing them to respond to their own cytokine secretion. This is the source of a cascading inflammatory response.

Skin barrier alteration and keratinocytes activation
The biological response to cytokines involves specific cellular receptors associated with other transducing molecules transmitting the biological signal within the cell to the nucleus. It is now recognized that cytokines are bi-functional molecules i.e., they harbour two binding domains on their surface. One domain is called the receptor-binding domain (RBD) that binds to a receptor protein on the target cell membrane. The second domain is the carbohydrate binding domain (CBD) that binds specific carbohydrate epitopes on the target cell surface. This CBD therefore confers to cytokines a lectin-like activity. Once the CBD has recognised and interacted with the target carbohydrate, the immune signal is activated and the inflammatory cascade is perpetuated. Subtle changes in cell surface carbohydrates suppresses the cytokine activity. Several authors have already described the lectin-like activities of several cytokines including IL1α, IL1β, IL2, TNFα and TNFβ (7).

Immune signal propagation: the role of cytokines – carbohydrate receptors interactions
AN INNOVATIVE TECHNOLOGY BASED ON SKIN SUGARS

INTRODUCING THE GLYCO TECHNOLOGIES

Since sugars exposed on skin cell membranes are involved in microbial adherence and inflammatory reactions by the host, a promising approach in dermatology is to use similar exogenous carbohydrates to help control cutaneous infections and inflammatory disease. Glycotechnologies is the term applied to the exploration and exploitation of this concept.

GLYCO TECHNOLOGIES REDUCE MICROBIAL ADHERENCE

Principles of the anti-adhesive action of exogenous carbohydrates

Microbial lectins interact with the corresponding sugars located on the epidermal cell surface. Similarly, on the skin surface, microbial lectins will bind exogenous topically administered carbohydrates acting as “traps”.

Blocking of microbial lectins by exogenous sugars on the skin surface
The exogenous sugars saturate the lectin binding sites on the microbial surface, making impossible for the pathogens to adhere to host carbohydrates (anti-adhesive effect).

Proofs of the anti-adhesive action of exogenous carbohydrates: in vitro studies

*In vitro* studies \((15, 33)\) confirmed that specific saccharides, which mimic natural epidermal sugar moieties, can effectively inhibit microbial pathogen adhesion. These anti-adhesive properties have therapeutic potential in the management of corresponding infections.

**Inhibition of *Pseudomonas* adherence to canine corneocytes** \((25, 26, 40, 41)\)

*Pseudomonas aeruginosa* is arguably the most important pathogen involved in canine otitis and of major concern is the emergence of widespread and multiple antibiotic resistances.

Adherence is an established prerequisite for microbial colonisation and *Pseudomonas aeruginosa* is known to adhere to a variety of epithelial surfaces using lectins that bind target sugars \((40, 41)\).

**A study** \((25, 26)\) was performed at The University of Liverpool Department of Veterinary Clinical Science to evaluate the anti-adhesive properties of 3 monosaccharides (D-galactose, D-mannose and L-rhamnose) against 3 strains of *Pseudomonas aeruginosa* cultured from clinical cases of canine otitis (6 dogs). Initially the monosaccharides were tested individually, then a combination of all 3 test sugars was evaluated.
**STUDY PROTOCOL ON MONOSACCHARIDE INHIBITION OF PSEUDOMONAS ADHERENCE TO CANINE CORNEOCYTES** (25, 26, 27)

**INCUBATION**
- at 38°C for 45 minutes in moist chamber

**CORNEOCYTES WASHED AND STAINED**
- (crystal violet)

**QUANTIFICATION**
- Image analysis > calculation of corneocyte surface area covered by bacteria

6 healthy dogs tested:
- Canine corneocytes collected from inner pinna using adhesive discs (D-Squame®)

One of the 3 strains of *Pseudomonas*:
- P1
- P2
- P3

One of the 4 sugar solutions:
- 2 sugar concentrations: 0.05% and 0.1%
  - D-galactose
  - D-mannose
  - L-rhamnose
  - The 3 sugars in combination
  - Or sugar-free solution (control test)

*Corneocytes incubated with Pseudomonas* (x 1000 magnification, crystal violet staining)
- Image courtesy of Dr NA McEwan

- *Pseudomonas* stained in violet
- Corneocyte
The mean percentages of *Pseudomonas aeruginosa* adherence to canine corneocytes after incubation with the test sugar solutions, by reference to the level of adherence in the control group, are represented below.

Inhibition of *Pseudomonas* adherence by monosaccharides individually or in combination

D-galactose, D-mannose and L-rhamnose each decreased *Pseudomonas* adherence to canine corneocytes. Furthermore, when the three sugars were used in combination, adherence was further reduced by more than 50%.

In conclusion, the monosaccharides studied presented a marked anti-adhesive effect. The use of the combination of the test sugars is anticipated to play a significant role in the management of *Pseudomonas* infections in dogs.
Inhibition of *Staphylococcus* adherence to canine corneocytes (23, 24)

*Staphylococcus intermedius* are bacteria of the normal canine skin flora but they can proliferate under favourable conditions to cause skin infection, particularly pyoderma secondary to various underlying dermatoses. Pyoderma is one of the most frequent skin disease in dogs.

Adherence is an established prerequisite for microbial colonisation and *Staphylococcus intermedius* is known to adhere to corneocytes using lectins that bind target sugars. (24)

A preliminary study was performed at The University of Liverpool Department of Veterinary Clinical Science to evaluate the anti-adhesive properties of 3 monosaccharides (D-galactose, D-mannose and L-rhamnose) against 3 strains of *Staphylococcus intermedius* cultured from clinical cases of canine pyoderma. The protocol used in this study was similar to that described before for *Pseudomonas*. The monosaccharides were tested individually, then all 3 test sugars in combination were evaluated. Unfortunately the results were not those expected. The level of *Staphylococcus intermedius* adherence remained unchanged after incubation with the test sugars.

Additional more complex sugars were tested then (23). The polysaccharides fructooligosaccharide (FOS) and alkylpolyglucoside (APG) at 1% concentration were evaluated under the same protocol.

The mean percentages of *Staphylococcus intermedius* adherence to canine corneocytes after incubation with the polysaccharide solutions, by reference to the level of adherence in the sugar-free control group, are represented below.
The results showed that, while the fructooligosaccharide (FOS) failed to block coci adhesion, the alkylpolyglucoside (APG) exhibited significant anti-adhesive properties. The APG solution was able to reduce *Staphylococcus intermedius* adherence to canine corneocytes by 50%.

In conclusion, this study suggested that APG might have therapeutic potential in the treatment of staphylococcal infections in dogs.

Inhibition of *Malassezia* adherence to canine corneocytes

*Malassezia pachydermatis* are yeasts of the normal skin flora of dogs. The factors that predispose to yeast proliferation are not completely understood but it is involved in many dermatological problems, such as *Malassezia* dermatitis and otitis in dogs, frequent complications of canine atopic dermatitis. These represent awkward inflammatory, pruritic conditions for affected dogs. *Malassezia* infections have also been associated with the development of chronic lesional signs of atopic dermatitis and may act as perpetuating allergens. Topical and systemic anti-fungal medications are widely used to control *Malassezia* infections. *Malassezia* are, however, commensal organisms, and re-colonisation and infection from mucosal reservoirs is common. Preventing colonisation is therefore an important goal in the long-term control of yeast complications.

Colonisation requires adherence to the corneocytes of the superficial *stratum corneum* and *Malassezia pachydermatis* is known to adhere to a variety of epithelial surfaces using lectins that bind target sugars. (3)

A recent study (45) was performed at The University of Liverpool Department of Veterinary Clinical Science to evaluate the potential of 3 monosaccharides (D-galactose, D-mannose and L-rhamnose each at 0.1%) and 1 polysaccharide (the alkylpolyglucoside APG at 0.5%) to inhibit the adherence of *Malassezia pachydermatis* to canine corneocytes. The protocol used was similar to previous studies on bacteria.
The mean percentages of *Malassezia pachydermatis* adherence to canine corneocytes after incubation with the sugar solutions, by reference to the level of adherence in the sugar-free control group, are represented below.

![Graph showing percentage of Malassezia adherence](image)

**Inhibition of *Malassezia pachydermatis* adherence by mono and polysaccharides**

(R & M & G = Rhamnose + Mannose + Galactose)

The results showed a moderate inhibitory effect of D-galactose, D-mannose and L-rhamnose taken altogether in combination on *Malassezia* adherence (25% reduction vs control). The best efficacy was achieved by the polysaccharide APG that reduced *Malassezia* adherence to canine corneocytes by 42%.

**Image courtesy of Dr NA McEwan**

![Corneocytes incubated with *Malassezia pachydermatis* strain 3 and without sugar. (x 1000 magnification, crystal violet staining)](image)

**In conclusion**, this study suggested that APG plus the 3 monosaccharides tested might have a therapeutical interest in *Malassezia* infections in dogs.
Principles of the immunomodulatory action of exogenous sugars

When keratinocytes are activated by exogenous or endogenous factors, they release cytokines such as IL1 or TNFα. These cytokines are soluble factors that initiate the inflammatory cascade. The biological response to cytokines involves two specific cellular receptors associated with transducing molecules. Cytokines possess both a receptor binding domain (RBD) and a carbohydrate binding domain (CBD), the later being a lectin. The CBD recognises specific sugars and this interaction is essential for the production of the immune signal. Exogenous sugars can interfere by competitive action on the CBD-carbohydrate interaction and consequently reduce cytokine stimulation.

![Diagram illustrating the immunomodulatory role of exogenous sugars](image)

The immunomodulatory role of exogenous sugars
Proofs of the immunomodulatory action of exogenous sugars: in vitro studies

In humans, specific monosaccharides, such as L-rhamnose, have been reported to exert inhibitory effects on cytokine activity in vitro, and are effective in suppressing in vivo manifestations of cellular immunity (1, 31).

In dogs, keratinocytes can be activated in vitro by a cytokine produced by T cells (interferon-γ). Such stimulation is evidenced by the release of the pro-inflammatory cytokine TNF-α by activated keratinocytes (6).

A study (12) was conducted at the National Veterinary School of Nantes, Unit of Dermatology, Clinical Parasitology & Micology, to evaluate the modulation of canine keratinocyte activation by L-rhamnose. The quantity of TNFα produced by activated keratinocytes incubated in 3 different solutions: a sugar-free control solution, a rhamnose solution (10 mg/mL) and a dexamethasone solution (2 x 10⁻⁵ mol/L) was recorded. The incubation of stimulated canine keratinocytes with L-rhamnose, or with dexamethasone, decreased TNF-α release by 75%, and 56%, respectively.

A specific monosaccharide therefore demonstrated inhibitory properties on cytokine signals mediating inflammation in canine epidermis. The role of epidermal cell surface sugar-coating in cell-to-cell signalling and communication is the basis for such effects.
APPLICATIONS OF GLYCOTECHNOLOGIES IN THE FIELD OF VETERINARY DERMATOLOGY

BENEFITS IN DERMATOLOGICAL THERAPY

The control of both microbial proliferation and inflammation is mandatory in many skin diseases. One classical method to manage microbial proliferation is the use of antibiotics or antiseptics, while inflammation is often addressed by systemic (oral, injectable) glucocorticoids. The prolonged use of these drugs, however, is not harmless. Long-term use of systemic steroid therapy may affect the way the immune system can deal with microbial challenge and the issue of antibiotic resistances is an increasing concern. New therapeutic options, that do not depend on antibiotics, are a welcome addition to the armoury of the treatment of skin infections. In this context, the microbial anti-adhesive and immunomodulatory effects of glycotechnologies represent a high tech new avenue that opens promising perspectives.

Opportunities offered by Glycotechnologies in veterinary dermatology
GLYCOTECHNOLOGIES IN VETERINARY TOPICAL PRODUCTS

Inspired by advances in human dermatology research, and after patient exploration in dogs, Virbac once again makes the innovation by introducing the high tech skin sugars in veterinary topical therapy. This new concept, the Glycotechnologies, is in line with the company long-term commitment to provide veterinarians with the most effective and updated dermatological products.

The choice of the sugars newly introduced in Virbac topical products was based on the results of proof-of-concept studies as exposed before. The sugars selected are the following:

- **3 monosaccharides:**

  ![D Mannose](image1)
  ![D Galactose](image2)
  ![L Rhamnose](image3)

- **1 polysaccharide:**

  ![Alkylpolyglucoside (APG)](image4)

These are precisely the sugars proved to be effective against the 3 most common pathogens found in infectious dermatological cases: *Staphylococcus intermedius*, *Pseudomonas aeruginosa* and *Malassezia pachydermatis*. Virbac has consequently filed a proprietary international patent on the veterinary dermatological applications of these compounds.

Glycotechnologies are a ground breaking innovation in veterinary topical therapy, but their target is not to replace existing efficient molecules. Rather, they have been developed to complement and optimise current formulas in order to improve their efficacy.

Therefore by now, the main therapeutic Virbac shampoos and ear cleansers, include this new technology, in addition to previous components that have made the success of the range.
GLYCOTECHNOLOGIES ARE ASSOCIATED WITH ANOTHER VIRBAC INNOVATION, THE NON-IONIC SPHERULITES®

The value of non-ionic Spherulites® in topical therapy

**Spherulites®: what are they?** (16)

**Spherulites®** are microvesicles made of multiple layers of surfactants. They represent a new generation of delivery systems for the encapsulation of active ingredients.

The Spherulites® were developed by the CNRS (Centre National de la Recherche Scientifique, the equivalent of the NIH in France) and veterinary dermatological applications are protected by a VIRBAC patent.

**The structure of Spherulites®** (16)

Spherulites® are composed of tensioactive molecules (surfactants). These molecules feature two opposite poles: one hydrophilic (the head) and one lipophilic (the tail). Consequently, surfactants tend to locate at the interface between substances with different polarities, such as oil and water.

Spherulites® diameter may vary between 0.2 and 20 µm, depending on the number of concentric layers, ranging from 10 to 1000.
Characteristics of non-ionic Spherulites®\(^{(2, 16)}\)

**Encapsulation of multiple active ingredients**

Spherulites® are multilamellar structures allowing the encapsulation of different molecules into the same microvesicle. The surfactant constituents allow the incorporation of hydrophilic, as well as lipophilic ingredients. This is not the case of alternative encapsulation technologies (nanocapsules or liposomes).

**Penetration in skin deeper layers**

The surfactants used in non-ionic Spherulites® do not contain electronic charge so that the resulting microvesicles do not bind to negatively charged hair and skin. Non-ionic Spherulites® therefore are able to penetrate into deep skin structures and this favours the flow of active ingredients to their site of action. This property was demonstrated using radioactive non-ionic Spherulites® on dog skin biopsies \(^{(2)}\).

**Progressive release of active ingredients**

Each layer of the multilamellar structure acts as a barrier limiting the diffusion of active molecules outside the microvesicle. The breakdown of the first, or even the first few, outer layers does not lead to complete structure destruction. Therefore, as opposed to liposomes, Spherulites® display great intrinsic stability. As the layers slowly breakdown, progressive release of active ingredients occurs into the skin. Consequently, their efficacy is prolonged.
Some of the sugars encapsulated in Non-ionic Spherulites® are conveyed in the skin deeper layers, where they can exert their immunomodulatory properties.

The other non-encapsulated sugars and the remaining encapsulated sugars on the skin surface provide quick and prolonged microbial anti-adhesive effects.

Glycotechnologies and Spherulites® therefore work together in synergy.
As an ongoing effort to improve its products, Virbac introduces the latest innovation in topical therapy. The “Glycotechnologies included” label is the privilege for veterinarians of prescribing the most advanced solutions.
Virbac developed a large medicated topical range, organised around the 4 main therapeutical challenges in veterinary dermatology:

- Allergies: Class A
- Keratoseborrheic disorders: Class K
- Cutaneous Infections: Class I
- Otitis: Class O

Unlicensed topicals, shampoos or ear cleaners, provide a valuable aid in the management of the above skin disorders.

Products in each Class that benefit from the glycotechnologies innovation include:

<table>
<thead>
<tr>
<th>Class</th>
<th>Brand name</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><strong>Allermyl® shampoo</strong></td>
</tr>
<tr>
<td>K</td>
<td><strong>Sebolytic® shampoo</strong></td>
</tr>
<tr>
<td>I</td>
<td><strong>Pyoderm® shampoo</strong></td>
</tr>
<tr>
<td>O</td>
<td><strong>Epiotic® Advanced ear cleanser</strong></td>
</tr>
</tbody>
</table>
What goes wrong in the skin of atopic dogs?

**NORMAL DOG STRATUM CORNEUM**

**ATOPIC DOG STRATUM CORNEUM**

Electron microscopic observations of the *stratum corneum* intercellular lipids in normal and atopic dogs (30).

The results of this study (30) revealed structural abnormalities in intercellular lipid deposits within the *stratum corneum* of atopic dogs.

These defects can explain the IMPAIRED SKIN BARRIER FUNCTION OF ATOPIC DOGS.
**Allermyl® response: the triple action**

<table>
<thead>
<tr>
<th>TARGET ACTIONS</th>
<th>ACTIVE INGREDIENTS</th>
<th>PROPERTIES</th>
<th>BENEFIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Reinforcement of the cutaneous barrier</td>
<td>Skin lipid complex (32) = Ceramides + EFAs + cholesterol</td>
<td>Multi-lamellar system which mimics the structure &amp; composition of intercellular lipids in the stratum corneum</td>
<td>Restore skin integrity</td>
</tr>
<tr>
<td>2. Control of microbial proliferation</td>
<td>Piroctone olamine (44)</td>
<td>Efficient at low concentrations</td>
<td>Antimicrobial activity</td>
</tr>
<tr>
<td>3. Reduction of skin inflammation</td>
<td>Glycotechnologies (11,23,26,45)</td>
<td>Natural sugars present in epidermal cell membranes</td>
<td>Respect of the skin / microflora</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of cytokine signalling</td>
<td>Immunomodulatory properties reduction of inflammation</td>
</tr>
</tbody>
</table>

**An optimised formula**

A formulation for optimal tolerance

- Physiological pH.
- Glycotechnologies complement the antimicrobial activity of piroctone olamine while respecting the cutaneous ecosystem.

A formulation for optimal distribution and penetration in skin

**MICRO-EMULSIONED SHAMPOO**

- Very small droplets: high power of solubilisation & diffusion
- Oily phase: introduction of liposoluble ingredients (fatty acids)

A formulation for more satisfaction

Allermyl® new formula improved cosmetic properties:

- Better lathering power
- Optimal viscosity
**SEBOLYTIC ® SHAMPOO**

**PROPERTIES:**
- Antiseborrheic
- Keratolytic
- Keratomodulating
- Antibacterial
- Antifungal

**APPLICATIONS:**
Cleansing shampoo as an aid for the control of keratoseborrheic disorders in dogs and cats

What goes wrong in keratoseborrheic disorders (KSD)?

**HYPERSEBORRHEA**
Alteration in sebum and epidermal lipids secretion / composition

**KERATINIZATION DEFECTS**
Excessive scale formation = Hyperkeratosis
Abnormal differentiation
Basal cells hyperproliferation
Epidermal barrier deficiency

**INFLAMMATION AND PRURITUS**
Inflammatory infiltrate
Neutrophils
Lymphocytes
Mastocytes

**MALODOR**

**MICROBIAL PROLIFERATION**
Staphylococcus intermedius
Malassezia pachydermatis
### Sebolytic® response: a multi-site action

<table>
<thead>
<tr>
<th>TARGET ACTIONS</th>
<th>ACTIVE INGREDIENTS</th>
<th>PROPERTIES</th>
<th>BENEFITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Correct keratinisation defects</td>
<td>Salicylic acid</td>
<td>Solubilises intercellular cement</td>
<td>Keratoplastic effects on the intercellular cement</td>
</tr>
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<td></td>
<td></td>
<td>Exfoliant action</td>
<td>Decrease horny layer thickness</td>
</tr>
<tr>
<td></td>
<td>Linoleic acid</td>
<td>Incorporation in the intercellular cement</td>
<td>Restore the epidermal barrier</td>
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<tr>
<td></td>
<td>linolenic acid</td>
<td>Major structural component of cell membrane phospholipids</td>
<td>Maintain functional integrity of keratinocytes and promote their normal maturation</td>
</tr>
<tr>
<td>2. Regulate sebum production</td>
<td>Zinc gluconate</td>
<td>Inhibits the enzyme 5α reductase which stimulates sebaceous secretion</td>
<td>Reduce sebum secretion</td>
</tr>
<tr>
<td></td>
<td>Vitamin B6</td>
<td>Synergistic inhibition of 5α reductase</td>
<td>Reduce sebum secretion</td>
</tr>
<tr>
<td>3. Control microbial proliferation</td>
<td>Piroctone olamine</td>
<td>Large antimicrobial spectrum (Gram + &amp; Gram – bacteria, yeasts)</td>
<td>Antimicrobial activity</td>
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<tr>
<td></td>
<td></td>
<td>High affinity for keratines</td>
<td>High persistence on skin and hairs</td>
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<tr>
<td></td>
<td>Glycotechnologies</td>
<td>Microbial anti-adhesive effect</td>
<td>Antimicrobial activity</td>
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<td></td>
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<td>Natural sugars present in epidermal cell membranes</td>
<td>Respect the skin / microflora</td>
</tr>
<tr>
<td>4. Reduction of skin inflammation</td>
<td></td>
<td>Reduction of cytokine signalling</td>
<td>Immunomodulatory properties reduction of inflammation</td>
</tr>
<tr>
<td>5. Control malodor</td>
<td>Tea tree oil</td>
<td>Natural pleasant odor</td>
<td>Reduce malodor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interact with microbe membranes</td>
<td>Antimicrobial activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of histamine and inflammatory mediators production</td>
<td>Reduction of skin inflammation and itching</td>
</tr>
</tbody>
</table>
An optimised formula

A formulation for optimal tolerance

- Mild cleansing agents
- Physiological pH.
- No coal tar, therefore:
  - No skin irritating, drying and staining effect
  - No unpleasant shampoo odor, appearance and color
  - Can be used in cats
- Tea Tree Oil provides a natural pleasant herbal scent therefore no perfume was added to the shampoo, reducing hypersensitization hazards.
- Glycotechnologies complement the antimicrobial activity of piroctone olamine while respecting the cutaneous ecosystem

A formulation for deep targeted action in skin

To exert their inhibitory action on sebaceous gland secretion, topical zinc and vitamin B6 should penetrate and diffuse into deep skin structures. For this purpose they were incorporated, together with monosaccharides, in Non-ionic Spherulites®, that favour the flow of active ingredients to their sites of action.
**TARGET ACTIONS** | **ACTIVE INGREDIENTS** | **PROPERTIES** | **BENEFITS**
--- | --- | --- | ---
1. Control of microbial proliferation | Chlorhexidine gluconate (3%) | Large antimicrobial spectrum (Gram + & Gram –, yeasts) | Antimicrobial activity
 | | Adapted and efficient formulation | Excellent tolerance

2. Reduction of skin inflammation | Glycotechnologies | Microbial anti-adhesive effects | Antimicrobial activity
 | | Reduction of cytokine signalling | Immunomodulatory properties
 | | Natural sugars present in epidermal cell membranes | Respect the skin / microflora

**PYODERM® SHAMPOO**

**PROPERTIES:**
- Antibacterial
- Antifungal

**APPLICATIONS:**
Cleansing shampoo as an aid for the control of bacterial and fungal infections in dogs and cats

What happens in skin infections?

**Pyoderm® response: the dual action**

CHRONIC INFLAMMATION AND PRURITUS

**Inflammatory infiltrate**
- Neutrophils
- Lymphocytes
- Mastocytes

**Microbial Proliferation**
- Staphylococcus intermedius
- Malassezia pachydermatis

**Surface proliferation (BOG)**
- Folliculite
- Furunculosis
- Cellulitis

MALATTIA INFLAMMATIVA E PRURITICA

**Inflammator y infiltrate**
- Lymphocytes
- Mastocytes

**Glycotechnologies**
- Natural sugars present in epidermal cell membranes

**Malassezia pachydermatis**
- Staphylococcus intermedius
An optimised formula

A formulation for optimal tolerance

- Mild cleansing agents.
- Physiological pH.
- Moisturising agents
- Glycotechnologies strengthen the antimicrobial activity of chlorhexidine while respecting the cutaneous ecosystem balance.
- Chitosanide is a filming agent that has healing properties.
- The quality of chlorhexidine formulation (1) provides efficiency on bacteria and yeast at 3% concentration (20), with excellent dermal tolerance.

A formulation for deep targeted action in skin

To exert its inhibitory activity on deep microbial infections, some chlorhexidine should penetrate and diffuse into deep skin structures. For this purpose, chlorhexidine molecules are also incorporated, together with monosaccharides, in Non-ionic Spherulites® that favour the flow of active ingredients to their sites of action.

A formulation for long lasting action

- Spherulites provide a progressive release of chlorhexidine and Glycotechnologies onto the skin.
- Chitosanide exerts its filming action on the skin and hair surface, thanks to its positive charges. This film keeps active ingredients in contact with target sites.
**Epiotic® Advanced response: the triple action**

<table>
<thead>
<tr>
<th>TARGET ACTIONS</th>
<th>ACTIVE INGREDIENTS</th>
<th>PROPERTIES</th>
<th>BENEFITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Emulsification and removal of cerumen, debris, pus, exsudates, etc.</td>
<td>Dioctyl sodium sulfosuccinate (DSS)</td>
<td>Emulsify and disperse wax and debris</td>
<td>Remove cerumen, debris, etc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Makes greasy dirt easily soluble</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salicylic acid</td>
<td>Solubilise intercellular cement</td>
<td>Keratoplastic effects on the intercellular cement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exfoliant action</td>
<td>Decrease horny layer thickness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Astringent agent</td>
<td>Dry the ear canal surface and prevent maceration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mildly bacteriostatic</td>
<td>Help to control bacterial proliferation</td>
</tr>
<tr>
<td>2. Control of microbial proliferation</td>
<td>Parachlorometaxylenol (PCMX)</td>
<td>Bactericidal</td>
<td>Antimicrobial properties</td>
</tr>
<tr>
<td></td>
<td>EDTA Sodium</td>
<td>Permeabilise the outer membrane of Gram-negative bacteria</td>
<td>Potentiate PCMX activity</td>
</tr>
<tr>
<td></td>
<td>Glycotechnologies</td>
<td>Microbial anti-adhesive effect</td>
<td>Antimicrobial activity</td>
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<tr>
<td></td>
<td></td>
<td>Natural sugars present in epidermal cell membranes</td>
<td>Reduce inflammation</td>
</tr>
</tbody>
</table>

**PROPERTIES:**
- Cleansing
- Antibacterial
- Antifungal

**APPLICATIONS:**
Ear cleanser as an aid for the management of bacterial and yeast otitis

**What happens in otitis externa?**

**EMULSIFFICATION AND REMOVAL**

- Cerumen, debris
- Dead cells
- Cocci (Staphylococcus)
- Rod (Pseudomonas)
- Yeast (Malassezia)
- Inflammation
- Pus

**EPIOTIC® NEW GENERATION**

**ACTIVE INGREDIENTS**
- Dioctyl sodium sulfosuccinate (DSS)
- Salicylic acid
- Parachlorometaxylenol (PCMX)
- EDTA Sodium
- Glycotechnologies

**PROPERTIES**
- Emulsify and disperse wax and debris
- Makes greasy dirt easily soluble
- Solubilise intercellular cement
- Exfoliant action
- Astringent agent
- Mildly bacteriostatic
- Bactericidal
- Permeabilise the outer membrane of Gram-negative bacteria
- Microbial anti-adhesive effect
- Reduction of cytokine signaling
- Natural sugars present in epidermal cell membranes

**BENEFITS**
- Remove cerumen, debris, etc.
- Keratoplastic effects on the intercellular cement
- Decrease horny layer thickness
- Dry the ear canal surface and prevent maceration
- Help to control bacterial proliferation
- Potentiate PCMX activity
- Antimicrobial activity
- Immunomodulatory properties
- Reduce inflammation
- Respect of skin / microflora
An optimized formula

A formulation for optimal tolerance

- Mild cleansing agents.
- Physiological pH.
- Glycotechnologies strengthen the antimicrobial activity of PCMX and EDTA while respecting the cutaneous ecosystem balance.
- Non-irritant formula: in tolerance tests conducted in compliance with the most stringent legal requirements for topical products, the Primary Cutaneous Irritation Index (CPI) of Epiotic Advanced was 0. Products are classified according to the following CPI scale:
  - 0 to 0.5: non-irritant
  - 0.5 to 2: slightly irritant
  - 2 to 5: irritant
  - 5 to 8: very irritant
- Good preservation: repeated microbial challenge of Epiotic Advanced bottles was performed according to the procedures of the 4th European Pharmacopea (2002). All tests qualified for the best criteria (A) of antimicrobial preservation recommended for topical formulations.

A formulation for enhanced satisfaction

Improved product perception.

Includes a patented anti-odor technology for neutralizing unpleasant smells, based on the reactivity of aldehyde compounds with volatile molecules (neutralisation).
CONCLUSION:
THE IMPORTANCE OF TOPICAL THERAPY
IN DERMATOLOGY

Why topicals matter?

Topical therapy plays an important role in veterinary dermatology for many reasons:

- Unlike many organs, the skin is readily accessible to medications. **Topicals convey active ingredients in direct contact with the skin, without prior dilution, to produce immediate action on target sites.**

- As the outer barrier of the body, the skin surface accumulates material (allergens, dust, foreign bodies,..), secretions and organisms that may become harmful. **Topical products, such as shampoos, have a mechanical cleansing effect that removes detrimental material (scales, crusts, debris, exudate..) or organisms (invading pathogens) from the skin surface.** (27)

- The cleansing effect of shampoos (first application) moreover enhances the ability of active ingredients to get in direct contact with deeper structures or sites (second application).

- The water content and moisturising agents in the shampoo have a great **beneficial effect on skin hydration**, an important parameter to maintain an effective epidermal barrier.

- **Topicals act locally, minimising the risk of general adverse events.**

- **Topicals are unique in improving skin and coat aspect**, resulting in increased owner satisfaction.

- **Topicals act in synergy with systemic treatments**, helping to reduce the dose or frequency of the later, or providing a quicker or stronger response. (22)

- On the long term, topical therapy is often helpful to **prevent relapses and control chronic skin diseases.**

Therefore topical use should be the MAINSTAY of ANY dermatological treatment. It can be associated with a systemic medication to increase overall efficacy and safety.
RECOMMENDATIONS FOR THE BEST USE OF SHAMPOOS (27)

PROCEDURE:

1. 1st application = cleaning
2. Rinse
3. 2nd application = treatment
4. WAIT (at least for 5 minutes, better if 10 minutes)
5. Final rinse (thorough)

SHAMPOO FREQUENCY (general rule):

1. Initially 2-3 times a week for 2 weeks
2. Then reduce gradually to longest interval over which treatment is still effective (usually 1 to 2 weeks) for maintenance

To be adjusted according to the severity of the skin problem, response to treatment & owner's possibilities (compliance)


41. Virbac SA internal study #690.64/40002. Pharmaceutical Development Department. Animal Unit.

42. Hoechst internal data. Antimicrobial spectrum of piroctone olamine.