APPLICATION OF SKIN ENZYME ACTIVITY TESTS TO THE SAFETY AND EFFICACY ASSESSMENT

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SUMMARY

Enzymes are biological catalysts, present in all cells which might be useful for cell metabolism or may act elsewhere outside the cell.

Enzymes intervene in dozens of chemical reaction and without them there is no reaction between the substrate or anyhow it does not happen in correct time. Any chemical capable to inactivate an enzyme is potentially toxic.

Enzymes can be classified as glycolytic, hexosemonophosphate shunt enzymes, Krebs cycle enzymes, nitrogen and fat metabolism enzymes.

Enzymes in the skin might be endocellular and extracellular, they are essential in order to maintain skin good conditions, contribute to the right skin pH and maintain skin protective capability against pathogens**.**

To inactivate an enzyme, or the trace elements primary for its activity, may result in metabolic disorders.

Since 1976 Vevy Europe raw materials for skin application undergo trials to determine their possible enzyme toxicity or activity through checking of the different sequential steps of sugar, fat and protein metabolism. Several examples of studies on ATPase, alfa-naphtyl esterase, leucine-aminopeptidase, succinic-dehydrogenase and acid phosphatase following the topical application of a specific ingredient will be presented and discussed.

INTRODUCTION

Enzymes are biological catalysts and their synthesis is regulated in the nucleus being stimulated or inhibited, according to physiological needs, by a repressor protein linked to DNA. Enzymes are found in all living cells, animal or vegetable, skin or hepatic ones. Enzymes may act either within (e.g. cellular respiration) or outside it (e.g. digestion). All substances introduced in our body first must be degraded, their size reduced and converted into another substance beneficial for the normal functioning of our body. Enzymes are essential for such a transformation process, keeping it at the right pace. If an enzyme is lacking the transformation still occurs but it will take longer and basically will lose its effectiveness. As an example of enzyme activity we can refer to a cell containing glucose-6-phosphate. Cell needs dephosphorylated glucose for its energy; an hydrolytic enzyme (phosphatase) introduces a water molecule and separates the phosphoric radical allowing the cell to have its much needed pure glucose.

Anabolism and catabolism constitute cell metabolism and each involved chemical reaction has to occur only at the proper moment and correct rate. Failure in the production or activity of a single enzyme may result in metabolic disorders.

These few considerations evidence that any chemical capable to inactivate an enzyme is potentially toxic and, since 1976, prompted Vevy Europe to check the safety of its raw materials for skin application also through a series of tests evaluating their potential skin enzyme toxicity then to be related to the results of the customary toxicity tests. Moreover these histochemical controls turned out to be also very promising in determining the performance of specific ingredients in relation with the attested activity on skin enzymes.

Vevy Europe raw materials for skin application undergo standard trials to determine their possible enzyme toxicity or activity through checking of the different sequential steps of sugar, fat and protein metabolism.

SKIN ENZYME STUDIES

In Vevy Europe skin enzyme trials the separate activity of 12 enzymes of the different sequential steps of carbohydrate, fat and protein metabolism is usually checked following the application of the tested material.

The regular controlled enzyme are:

Glycolytic enzymes: hexokinase, glucosephosphateisomerase, glucomutase, aldolase, lactic dehydrogenase;

Hexosemonophosphate shunt enzymes: glucose-6-phosphate dehydrogenase;

Krebs cycle enzymes: malic dehydrogenase, isocitric dehydrogenase;

Nitrogen metabolism enzymes: GOT, GPT, glutamic dehydrogenase;

Fat metabolism enzymes: hydroxyacyl-CoA dehydrogenase.

The screening on energetic metabolism has been favoured because, if it is deeply compromised, the remaining of the metabolic pathways is blocked by lacking of ATP. Therefore if an ingredient is toxic for these enzymes automatically becomes toxic for the cell as such too.

Table 1 is an example of the effect on skin enzymes of a distinctive molecule commercially available.

As the 100 value is considered as the enzyme normal activity, it is clear that this molecule enhances skin enzyme activity and therefore becomes beneficial in formulation containing enzyme-depressing chemicals (e.g. surfactants).

TABLE 1

Enzyme	**% activity**
L-Alanine:2-oxoglutarate aminotransferase	**100**
L-Glutamate:NAD^+ oxidoreductase	**100**
L-Lactate::NAD^+ oxidoreductase	**100**
ATP:D-gluconate 6-phosphotransferase	**101**
D-glucose-6-phosphate ketol-isomerase	**300**
alfa-D-glucose-1,6-biphosphate: alfa-D-glucose -1-phosphate phosphotransferase	**115**
D-fructose-1,6-biphosphate: D-glyceraldehyde -3-phosphate-lyase	**100**
D-glucose-6-phosphate:$NADP^+$ 1-oxidoreductase	**200**

Several endocellular and extracellular enzymes are found in the skin and are essential in order to maintain skin good conditions, contribute to the right skin pH and maintain skin protective capability against pathogens. Why is important to keep a normal enzyme activity avoiding to use products depauperating it?

The consequences of an impairment of enzymes, even through the inactivation of trace elements (e.g. magnesium and manganese for leucine aminopeptidase) primary for enzyme performance, cannot be evidence after one application but only following repeated treatments, as is the case for cosmetics which might be applied several times a day for years.

In the epidermis we find the enzymes of the glucidic metabolism (phosphorylases, which increase if we are wounded); oxydases (in the germinative layers); alkaline phosphatases (not

evidenced in the normal epidermis, but only if an alteration occurs); acid phosphatases (in the stratum corneum, granulosum and spinosum); esterases (stratum corneum, hair follicles, sebaceous glands); peptidases (proteolytic enzymes); beta-glucuronidases (connected to the metabolism of hyaluronic acid); collagenases (catalyst for collagen hydrolysis).
Histochemical studies revealed that the skin of an 80-year old volunteeer is, enzimatically, as young as the skin of a 25-year old volunteer.
Here follow some example of the accomplishments in evidencing both the skin enzymatic activities as such and the influence of specific cosmetic actives on them.

ATPase
This enzyme is involved in ATP dephosphorilation to ADP, a process which frees the energy needed for each new synthesis. ATPase is predominantly found in: epidermal basal elements (high metabolic activity); hair medulla (growth phase) and sweat glands.

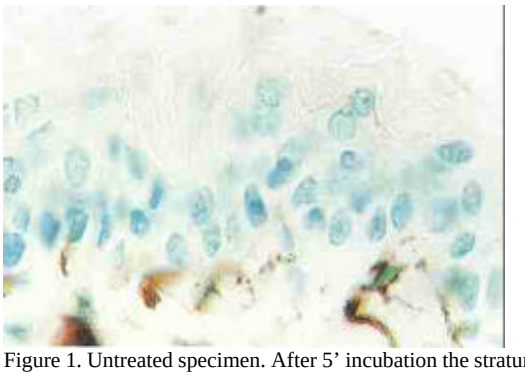
Figure 1. Untreated specimen. After 5' incubation the stratum granulosum is not yet black stained (1250x).

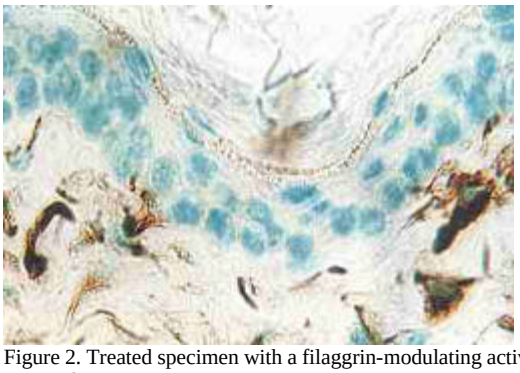
Figure 2. Treated specimen with a filaggrin-modulating active. After 5' black-stained granules are evidenced in the stratum granulosum as an index of increased ATPase activity (1250x).

ALPHA NAPHTYLESTERASE
It is an hydrolytic enzyme active at pH 7.0 - 7.5 predominantly found in: keratinising epithelial elements;
stratum granulosum and hair germinative epithelium when a new production starts.

Figure 3. Enzymatic activity stained reddish-brown is located in the epitheliocytes above the hair in which the hair duct is being excavated. It testifies the intensive cell activity during hair eruption (500x).

LEUCINE-AMINOPEPTIDASE

A peptide hydrolase splitting the - CO-NH2 bond and actively involved in protein catabolism and anabolism. It is not found in the epidermis but only in the dermis (fibroblasts), in the sweat glands and the adventitia of blood vessels.

Figure 4**.** The dark red colour clearly shows that there is no LAP in the epidermis, whereas it is strongly present in the fibroblasts of the dermis (1250x).

SUCCINIC-DEHYDROGENASE

Involved in the transformation of succinic acid into fumaric acid, a Krebs cycle reaction.
It is predominantly found in: striated muscle cells, epidermis (basal layer), hair and glandular epithelium.

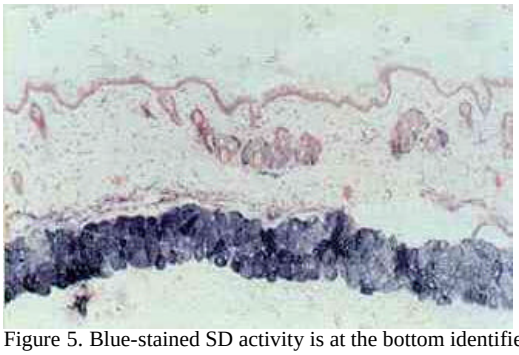

Figure 5. Blue-stained SD activity is at the bottom identified in the layer of muscle tissue and at the top in the pilosebaceous complex. The different blue intensity in the muscle cells is due to a non simultaneous contraction of muscle fibers. The deeper blue stains identify contracting fibers requiring more energy and enzyme activity (125x).

ACID PHOSPHATASE

It catalyzes phosphoric esters hydrolysis at pH 5.0, is present in the keratinization process when prekeratinic molecules are trasformed into mature keratin. It is useful for studying skin barrier properties being typically found in the stratum corneum and the sweat gland.

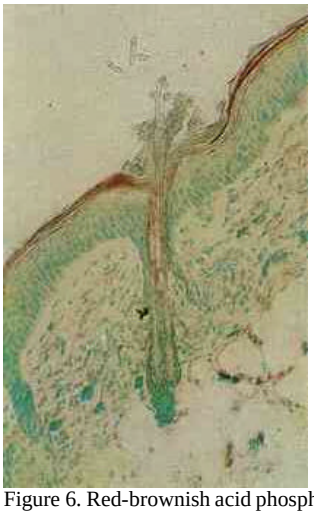

Figure 6. Red-brownish acid phosphatase activity is localized in the most superficial layers of the epidermis. Cell nuclei are stained in green. Acid phosphatase activity is also present along the shafts of two hairs originating from the same dermal papilla. Being close to surface this activity usually disappears if it comes into contact with unsuitable skin care ingredients or chemicals (300x).

CONCLUSIONS

Dermo-epidermal enzymes are important both for their metabolic significance and in the case of their impairment because the latter could be an indirect parameter in the tests to control cosmetic and pharmaceutical ingredients.

Several times the histochemical studies on enzyme activities allowed to study the effects of actives apparently useful, but actually unsuitable for skin application due to their enzyme inhibition.

Conversely to study the influence on specific enzymes of an ingredient may be helpful in supporting its efficacy.

It is therefore important that raw materials and actives for skin application be checked for their potential enzyme toxicity or activity in order to attain further information on both their safety and efficacy.

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