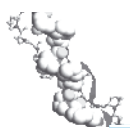




# COLWAY PRODUCTS



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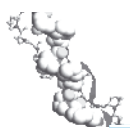


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## INTRODUCTION

This document has brought together all of the most interesting material currently available on the products distributed by **COLWAY**. We are freely able to rewrite, quote and publish with the express consent of the authors and with no clear conflict of interest regarding competing companies. Nor are we in danger of violating the norms of so-called good practice which apply, especially, within the field of medicine.

**COLWAY** has at its disposal a significant amount of clinical research data proving the effectiveness of fish collagen in both the fields of cosmetology and medicine. Yet within an environment of intense commercial rivalry, where the competition is lying in wait for any misstep, we cannot make all of it available to those in our network. As this would leave us open to inevitable legal conflict.

The echoes of this data however, even some of it is still classified, have found their way into the text you will find below. Read it carefully.

We would like to use this introduction to clear up one question of particular importance.

Collagen extracted from the skin of fish is, without a doubt, a great discovery of Polish science. The trouble is, that it is not a discovery made by one specific person but rather, the perfected work of a team, unfolding over the course of several years. There would be no collagen were it not for the work of the (still at the time communist, yet one must admit, world renowned) Polish School of Biochemistry.

In the 1970s Polish science shone in the field of extracting protein from marine organisms. Poland was one of the few countries to have a unit fully dedicated to marine research – the RV (Research Vessel) Profesor Siedlecki which was world renowned for among other things, preliminary trials in the extraction of consumer protein from the boundless resource available in plankton - e.g. Antarctic Krill.

As the years went by the world solved the problem of protein deficiency through transgenic crops. China and India became rice exporters. The Profesor Siedlecki was out of business. There were now 60 wonderfully trained biochemists, 12 of whom had attained professorships, who did not really know what to do with their expertise. These people knew an awful lot about the biochemistry of fish protein, but had no real opportunity to profit from their knowledge. Among those worthy of mention are Ilona Kolodziejska, Maria Sadowska, Edward Krajewski and Mieczyslaw Skrodzki.

In the meantime Polish scientists got to work at the Gdansk center on such things as a method for extracting food gelatin from the waste products of the seafood production industry. Waste that developed countries would pay handsomely to be able to utilize.

The political and economic realities of Poland mean that while possessing unique technology for obtaining genetic food gelatins in a completely safe manner and for a nominal fee – Poland is an importer of gelatin, or funds a gelatin affair. A very similar thing happened with collagen. Though first extracted in the 1980s it could attract no capital support. The first “collagen patent”- no. 167114 - whose description you will find at [www.uprp.pl](http://www.uprp.pl) (click on *PPO's databases* – check the *inventions* box then click on *quick search* – check the *Applicant/Holder* box and then write “skrodzki” in the search box) was never to be exploited commercially until its protected legal status expired.

The inventors at this time lacked two elements; an ideal fish species as a source and a perfected final filtration stage.

This was finally achieved by Prof. Jozef Przybylski of the Chemistry Institute at the University of Gdansk by using salmon as a source and silk fibers as a filter. His crowning achievement was the first Polish fish collagen able to be extracted commercially. On the 31<sup>st</sup> of January 2006 Prof. Przybylski was awarded a patent that allowed him to develop collagen he had obtained while avoiding certain methods of extraction.

The company 3-Helisa, current producer of fish collagen according to the Przybylski method, and other firms buying it at a reasonable price, have nonetheless been left in the dust by the competition. It seems that the market success of Polish fish collagen will not be decided by ideological certainty, but by something far more prosaic – the products resistance to temperature.

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Fish collagen is a hydrate, a concoction of proteins, which keeps its gelatinous consistency and its biological activeness up to a certain temperature. The limit of this is based on its degree of hydration, on cross-linking and above all else, on the temperature at which the fish (our collagen “donors”) were raised.

Human collagen “leaves” its interspiral relationship at a temperature of 107.6°F (42°C) and then it dies along with the rest of the organism.

Fish collagen differs from the human variety only slightly. Oddly enough it is biochemically closer to bovine collagen, which is wonderful in implants but disappointing in cream supplements.

One of the differences lies in resistance to temperature.

Fish vertebrates of seasonal waters align the temperature of their organisms to the environment. The competitive battleground among Polish producers has focused on temperature resilience in the hydrates they produce, as this is the criteria determined by demand in countries with the greatest potential. That is... countries where it is hot not only in the summer. Poland, the Czech Republic and Ukraine are only training grounds.

Inevitably the global success of this sensational product will rely not on quality certificates or sensational clinical research, but rather on the possibility of guaranteeing a storage temperature to the end users in their home environment.

Market demands are not governed by the lauds of opinion makers, but by actual effectiveness, according to the real life conditions of the consumer.

With this goal in mind COLWAY selected Inventia as its collagen producer and shipper in 2004, because they were able to beat the competition. Specifically in terms of the length of collagen storage time resilience. The collagen of other companies, although biologically active, maintained its spiral structure to a maximum of 68° F (20°C) and demanded storage on ice. Also it is a disgraceful and poorly smearing product.

We sell about 65% of the collagen produced in Poland. COLWAY is sponsoring, experimental for the moment, attempts to extract collagen from Panga, African Catfish and Tropical Carp using a polymer filtration method (more effective than silk).

All this is toward the goal of ensuring the network stays one step ahead of the competition. By the time this text is printed, we will already have our hands on an alternative to paraben preservatives!

If our temporary product supplier is loyal to us we will stay with them always. For the strength of any organisation stems from its unity. If however, they were to attempt to break away from the network, then we will go our own way. We have, after all, a wealth of knowledge, biotechnological potential and capital.

**COLWAY's** management priorities are: building a good network, ensuring its development, full connection to the products and absolute competitiveness; regardless of media, patent or other disputes.

We have undertaken a policy to withdraw from publication all brochures regarding the medical value of collagen, as well as with all material noted from articles, information, reports, research analyses, interviews and quotes from the worlds of cosmetology, biotechnology, bio-organics and biochemistry – rather than the field of medicine. This is simply what is politically correct. Any discussion about the relationship between collagen and medicine should come strictly from the mouths of doctors. While collagen is helpful in the treatment of Multiple Sclerosis and cancer – we shall leave it to the competition to announce these facts through chemists and not doctors.

***The Editors***



## THE MOST IMPORTANT THINGS TO KNOW ...

Collagen is the most important protein, not only for humans, but for all the vertebrates. It makes up more than 30% of the protein mass in the human organism. It is the main component of the most important tissue in our lives – connective tissue.

Everyone knows how important blood is to the organism, but few are aware that as an organism, we are literally swimming in collagen. The cellular fluid, in which our tissues are submerged, is actually collagen. The bone hair and dental matrices are likewise collagen.

What is the largest human organ? This question is a tough one and often draws the wrong answer. The answer is the skin, which covers, protects and adorns us. It also serves our organism in excretion, emission and respiration - 70% of skin protein is also collagen.

Collagen is not given to us once and for all. It is constantly undergoing change. It dies and is simultaneously created and constantly supported by chondrocytes, keratinocytes and fibroblasts – the cellular production and renovation works for collagen. How does this work? Well, in the teeth, eyes and hair there are biosynthetic processes underway which join at least 19 amino acids in a cyclical pro-collagen sequence. The peptide sequence chain forms a polipeptide chain which contains as many as 1000 amino acids. This “factory” releases cellular fibroblasts which once in extracellular space, join into spirals, usually laevorotatory superhelices.

These are the basic aspects of our knowledge on biochemical proteins. We can often hear opinions on the “impossibility” of spreading collagen onto the skin and having it penetrate to the dermis and, in particular, creating fibroblasts or keratinocytes from it.

This is a misunderstanding. There will be much more on the penetrability of collagen later in the text. However, no one has ever demanded that high-molecular fish protein, upon being spread onto the skin, should reach the cells naturally producing collagen in the human skin. And no one has ever stated that this is the case. First of all, this is clearly impossible. Secondly – what for?

The transdermal mechanism of **Natural Collagen** relies on something much different. Upon encountering the five layer barrier of the epidermis, fish collagen spirals break down into amino acids, which are constructed the same way as human collagen. These are the same amino acids as in human collagen: hydroxylene, glycine, proline and hydroxyproline.

This last one is worthy of special attention, as it is relatively easy to measure it in micrograms per cubic millimeter in all research materials. The same is true with comparative trials in the extracellular matrix.

The extracellular matrix is a gelatin network of proteins and sugars which act as a framework for the three layers of skin: the epidermis and hypodermis which are connected by the dermis.

The extracellular matrix is composed mainly of collagen, elastin, glycoprotein and carbohydrates. Its consistency and appearance would remind one of **Collagen Natural Silver** if we were to evaporate two thirds of its water.

If we are striving to improve the condition of collagen in our dermis through diet and the application of creams (with biologically active substances and vitamins with carriers) as well as caring for it with natural collagen - we are in fact striving to increase the amount of collagen in the extracellular matrix! This forms a protein network, whose density determines the elasticity, firmness and degree of skin wrinkling. For the extracellular space this is a collagen “factory”, of fibroblasts, chondrocytes and keratinocytes freeing the polipeptide amino acid chains. It is here, with the help of ascorbic acid (vitamin C) as an activator, the amino acid sequence twists into helices. We already know that this happens much more enthusiastically when the extracellular matrix is reinforced by a shower of peptides which, are absorbed and dissolved, on their way through the high capacity layers of the dermis - fish collagen spirals.

That is what, in basic terms, the transdermal process of **Natural Collagen** looks like. Today this process can be revealed in five ways: by a tissue biopsy measuring the number of hydroxyprolines before and after application of protein hydrates onto the skin, by administering a radioisotope preparation and tracking its movement through the organism, by observing the decreased suppression activity of T lymphocytes isolated with collagen in vitro, through the immunofluorescent marking method and finally by determining the level of the densitometric

percentage in a peptide preparation accompanying the so-called alpha chains. No one has questioned the transdermal nature of these peptides. What we don't know exactly however, is why amino acid tissue simulations of fish collagen hydrates activate the function of fibroblasts and keratinocytes. This question requires research of the highest level which, at the moment, is doubtful to be carried out in Poland.

In any case, the frequent questioning of the benefits of Natural Collagen from a "scientific standpoint" is clearly off the mark. None of collagen's critics can question the basis of the research because no one is undertaking this research. Collagen protein is still a grey area in the field of bio-organics, and those who think they know it well, are quickly humbled. We are, at present, not even able to precisely describe all of varieties and sub-varieties of collagen.

Triple helix collagen has a very similar "appearance" to human DNA spirals, and it turns out to be even more mysterious than that "rather well known" spiral. Human DNA however, is doomed to the cell, while superhelices live extracellularly.

If you read or hear the following statement: "collagen as a protein is high-molecular and it cannot penetrate the skin...etc." you know that they are being logically and technically correct, but are speaking off the subject. They are preaching about something which has never been stated.

**Natural Collagen** does not penetrate, as it does not need to penetrate to the dermis. It is totally sufficient for the stimulation of reconstruction of the organism's own collagen. If hydroxyl peptide chains or hydroxyproline remaining from the fish protein spirals are torn away due to penetration of the epidermis, they will enrich the extracellular space of the layer connecting the epidermis and the dermis.

Relatively speaking, nothing much happens during this process, aside from the original formation phase of peptides, which are "pushed" out of the cell anyway.

Here, in the extracellular space, the pro-collagen formed in the fibroblasts is synthesised into helices. Thus "our" collagen NEED NOT penetrate to the nucleus of the cell.

The fish collagen applied to the skin does not adhere miraculously to our collagen fibrils. Nor does it penetrate the skin in whole form, nor does it reach the interior of the fibroblasts. No one has ever said it does.

It immediately enriches the extracellular matrix of all the layers of the skin with amino acids, which causes an, as of yet clinically undefined mechanism, facilitating fibroblast activity. At the same time increasing the organism's production of its own collagen.

Cosmetologists and dermatologists questioning the a priori activity of **Natural Collagen** do so strictly on the basis of the books from which they learned - 100% of which were written in the 20<sup>th</sup> century.

They wrote in the late 60s that collagen cannot dissolve in water (which is obviously not true), at the end of the 80s that it can only possess a spiral structure within a living organism (also untrue) and at the close of the 90s, and even later, that it was "too large" molecularly to move about in the epidermis (also no longer current).

Normal immunological research has currently shown how collagen molecules are formed, and eventually migrate into the extracellular matrix of the epidermis, where peculiar enzymes lead them to connections with several hundred amino acid peptide constructions, created in keratinocytes.

All this goes on in an epidermal layer, "too dense" according to modern cosmetology, to fit in any high-molecular proteins.

One major firm on the Polish market recently publicized pre-EU accession research from the SCCNFP (an EU Scientific committee) showing that compounds, with a molecular weight greater than 1000, "get stuck" in the epidermis. Yet developments in biochemistry are encouraged with the pace allowed by the speed of the information age.

Thus collagen created in the keratinocytes (and then in the epidermis) proceed with great ease to extracellular space. An identical thing happens in the extracellular matrix of the membranes of the stratum basale.





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Fish collagen hydrates (**Natural Collagen**) which is conformed to a spiral structure of 1.4 nanometers, clearly can reach extracellular space. Its penetration of the epidermis and connection to the epidermal-dermal extracellular matrix is, today, no longer in dispute. No clinical research is required. Today the theory of an absolute ceiling on epidermal capacity is scoffed at by students practising on electron microscopes.

Yet even now in Poland many doctors and cosmetologists have not the slightest idea about the extracellular matrix, nor the objective fact that it exists in all layers of the skin. There is much fascinating literature on the subject of the extracellular matrix, which is also known as ECM.

There are other questions which likewise need to be understood and empirically accepted as important phenomena, such as: collagen's ability to retain a helix structure outside its natural environment (that is inside a vertebrate organism), its ability to achieve a metabolic process once there as well as defining to what degree, and why, we speak at all of a "living" biologically active protein.

One thing we can say about the ingenious discovery of fish collagen by Polish scientists is that those, who learn of its existence, are almost never indifferent. On the contrary, it produces enormous enthusiasm, bounding on euphoria or, conversely, extreme denial.

Then who, instead of being sincerely happy that such a wonderful thing has come into the world and seeking to widen knowledge of it – would seek to criticize and deny?

- those who believe collagen will ruin their business
- those who receive the information in good faith, but not good knowledge
- those who receive the information, but not well enough or completely enough
- those who are immediately turned off by the collagen distribution system.

Lets begin with the last one. There are many groups of experts who would shout the praises of the collagen phenomenon as loudly as they denounce it, had they heard about it from a doctor, pharmacist or even the advertising media. Anyone instead of a sister-in-law or neighbor who (oh the horror!!!) actually sells it.

It really is hard - but necessary - to tell people who are so hooked on the stereotype that, no... this isn't wine made by your sister-in-law, or your neighbor or even the distributor – this is true collagen. It is just that this particular product is a "living" protein which dies in the heat. One summer's day in a shop with no air-conditioning, or even on the road to the shop and...

Since, owing to its properties, it is not such a good idea to place collagen in shops, or even pharmacies, it makes no sense to entrust it to advertisers or pharmacies. Whether or not someone likes direct selling or not, sometimes the nature of the product demands this form of distribution. After all no one is forcing this product.

Its exactly the same with collagen. No advertisement is able to effectively present its benefits and importance as well a distributor who also uses it. Therefore it is necessary to present it to the receptive sceptic. A separate issue is the "quality" of knowledge about collagen.

That is precisely the idea behind this...

Armed with the valuable knowledge and very strong relevant arguments contained in this text, as the proponents of Polish fish collagen, you will be able to change the opinion of people imprisoned in stereotypes or limited by viewpoints which have been determined by out of date information.

You will even change the minds of those who see collagen as the competition.

How? By making them obsolete.

So then, what's the idea of this "living"collagen? Isn't that overdoing it?

It turns out that some people seek to trivialize the collagen molecule, by imagining it as something comparable to a sperm cell, breaking its way through the barrier protecting the epidermis, to eventually fertilize the fibroblast with a family of wee little *collagenies*.

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Such a presentation is bound to arouse laughter or offense in anyone who has even a smattering of biochemical, anatomical or cosmetological knowledge.

The truth however, is otherwise.

We all know that when a tree dies its leaves keep growing and its sap flows through its trunk, right up until the chain saw starts cutting. When a person dies their hair and nails (skin fragments) live, and even grow, for several more days.

The development of life on Earth is based on proteins. They die completely when their peptide chains break down. Yet some primitive organisms are able to return their proteins to full vitality after long periods of complete hibernation. Bedbugs after two days and some bacteria after even two hundred years! How? Because the proteins that allow for their revival do not submit to degradation.

When defining collagen it is clearly easy to start abusing semantics. But only in the case of the unschooled presenter.

The fact is that what we are holding is actually living collagen. Living because, despite being sealed in this cold glass, nearly dehydrated to a pulp, they still retain the same spiral structure which they obtained in the metabolic matrix process of the organism..

Not many people gasp the essence of this phenomenon of Polish biochemistry. And it all comes down to this: the protein extracted from the skin of a fish eaten two years ago, continues to possess the identical conformation, according to its rank, as a fish just pulled from the net.

All due to a process involving the lightning fast removal and freezing of the skin, (before the collagen molecules break down) followed by an extraordinarily delicate mechanical process, a slowly finessed chemical reaction, multiphase filtration and finally - something no laboratory in the world, outside the one in Gdansk, has been able to do – collagen which doesn't lose its helical structure, and which comes out clean (though brittle and defenseless). Next... into a water solution and obediently into the jar.

What could be better than several dozen tons of beef collagen, produced every year at varying quality, in different locations around the world?

The differences are many – but the most important is that this is “living” collagen. It has a roughly lower molecular mass than bovine collagen, and reaches deeper after being applied to the epidermis. At the moment of contact with the human organism, it is not just a static bundle of “dead” amino acids, but a constantly ordered, three-core spiral. It isn't until it reaches the stratum corneum of the epidermis that it is sliced into peptide chain parts. But this is another, and unusually fascinating story.

***The Editors***

## EXPERT TESTIMONY – 1

Special information for those selling and recommending **Natural Collagen– Q 5-26.**

### 1. The advantages this natural cosmeceutical has over other cosmetic collagens

- a transdermal gel form (i.e. is absorbed by skin),
- contains natural fish protein,
- contains a biologically active (“living”) protein of spatial form with an unparalleled similarity to human collagen, which as the only one in the world, maintains its biologically active form, within a temperature range of between 41°F– 78°F (5°C – 26°C) and keeps it (as long as it is not cooled) until it reaches the skin’s extracellular space,
- the first preparation in the world to attack the causes, not merely the effects of aging on the skin. And what’s more - the organism,
- the absorption process does not require the use of additional methods nor techniques resulting in side effects (e.g. injecting wrinkles as with bovine based collagen),
- replacing faulty collagen in human skin renews the pace of fibroblast function. A return to function which does not leave the skin dependent on cosmetics,
- it is odorless, non-allergenic, bacteria resistant, soothing and relaxing.

### 2. Storage procedures

- although tolerant to temperatures up to 78° F (26° C), it is best to store collagen in its original, textured packaging (with Styrofoam insulation) and either refrigerated (during hot spells) or outside of the refrigerator.

### 3. Precautions

- low-protein diet, e.g. in kidney dialysis,
- if suffering from one of the group of collagen disorders e.g. sclerodermia and chronic rheumatoid disorders,
- eczema with a protein allergy background,
- allergy to fish protein,
- between the 4th to 8th month of pregnancy (this is due to legal issues),
- more than 6 weeks after finishing chemotherapy, radiation therapy or phototherapy for psoriasis.

### 4. Application (general terms)

- prepare skin for collagen application using (enzyme peeling or gommage) exfoliating preparation, must be adjusted to type of complexion and skin,
- collagen must be applied directly onto clean wet skin!!!
- wash skin with water and soap equivalent which does not irritate the skin,
- apply collagen once or twice daily.

## 5. Application of collagen on the face and neckline (collagen **PLATINUM**)

- before using collagen remove make-up (with milk, cream or olive oil) then wash face, neck and neckline,
- after cleaning and drying skin, moisten skin again with boiled or mineral water. Do not use tonics with alcohol!
- message a very thin layer of collagen (3 – 4 pumps from applicator) onto the skin; rub with motions along the grain of the muscles using downward movements,
- daytime application: under make-up and toning cosmetics, under skincare products, UV protection products (sunblock) and under cold, wind and air-conditioning protection products,
- night time application: as the only cosmetic if slight facial contractions stop a few minutes after application, if contractions continue for longer - apply cream or lotion (the **COLWAY** series is excellent),
- in cases of complexion problems (i.e. allergies, dermatitis or acne rosacea) use specialist cosmetics or medications (a necessity)! If these cosmetics or medicines contain ingredients weakening the biological activity of collagen (see pt. 6) apply them 30 minutes after application of collagen.

## 6. Adjusting cosmetics to **Natural Collagen**.

- apply right away after quick absorption (only non-gel cosmetics); the **COLWAY** set of products are ideal,
- acid free cosmetics (e.g. fruit, salicylic) without zinc, sulfur and alcohol which weaken the biological activity of collagen,
- biologically active cosmetics with retinol, cytoxins, ceramides etc. are not recommended (that's why I fully recommend cosmetics based on **COLWAY** collagen),
- I also recommend medical cosmetics containing fewer ingredients (e.g. cold creams) moisturising and revitalising without artificial ingredients, preservatives, colors or fragrances. Natural sun blocks, cosmetics for sensitive or problem complexions containing indispensable unsaturated fatty acids (i.e. evening promise oil and medicinal cucumber),
- all cosmetics used should be appropriate to the complexion.

## 7. Application of collagen to the body (collagen **SILVER**)

- proceed use with application of peeling one week prior to collagen body application,
- before using collagen wash skin with coarse sponge, bath glove or brush (for extra skin massage),
- rub thin layers of collagen into wet skin, moving in upwards direction toward the heart  
or
- bathe in water, 86°F/30°C ( ) in temperature, with 20 – 30 milliliters of collagen **SILVER** or **GRAPHITE** dissolved into the bath. More efficient absorption of the collagen in a bath can be aided by using a water beading sprayer with ozone if available.
- after using collagen on the body its effects can be increased through use of balsams or preparations for problem skin. Collagen not only rejuvenates skin but has positive effects in the areas of widening the blood vessels, cellulite, stretch marks and bloating and swelling of the legs.

## 8. Applying collagen to the hands (collagen **GRAPHITE**)

- after each washing of the hands apply onto moist skin,
- rub intently into fingernails requiring regeneration,
- polish may be applied directly onto nails smeared with collagen,
- collagen not only rejuvenates hands but also decreases discoloration.



## 9. Applying collagen to the scalp and hair (collagen **GRAPHITE**)

- after intense washing of hair rub collagen into moist scalp (some of the collagen stays in the hair follicles) do not rinse,
- blow dry hair after a few dozen minutes, at low setting, from a significant distance.

## 10. Applying collagen to the feet (collage **GRAPHITE**)

- rub collagen into moist, washed feet,
- peelings and pedicures increase collagen absorption,
- use of collagen on the skin is effective in softening the epidermis, strengthening the nails, healing cracked skin on the heels and reducing foot sweating.

## 11. Additional affects of collagen

- regenerates skin after burns, cuts, chaffing, rashes, bruises and dermal haematoma (varicose veins),
- effective against seborrhoeic skin diseases, reactions resulting from excessively calloused skin, and death of the sebaceous and sweat glands,
- effective in caring for skin with allergic reactions, psoriasis, juvenile acne and acne rosacea, dandruff, calloused follicles and so-called fish scales. In cases of allergic reactions, dandruff, psoriasis, juvenile acne and acne rosacea , it is necessary to consult a doctor or cosmetologist,
- scars, scar tissue becomes flatter, less visible and more elastic.

**Natural Collagen** is not a medication. Nor is it only a cosmetic. Owing to the fact that it not only beautifies, but also influences the functioning and structure of tissue (activity typical of medications) it has been justifiably recognised as a **cosmeceutical**. The name cosmeceutical, as a term for preparations combining the functions of cosmetics and medicines, was proposed for years by the profesor of dermatology Albert M. Klingman.

It has been propagated for many years to include among others, dermo-cosmetics and transdermal preparations with healing properties.

*Aleksandra Izykowska*

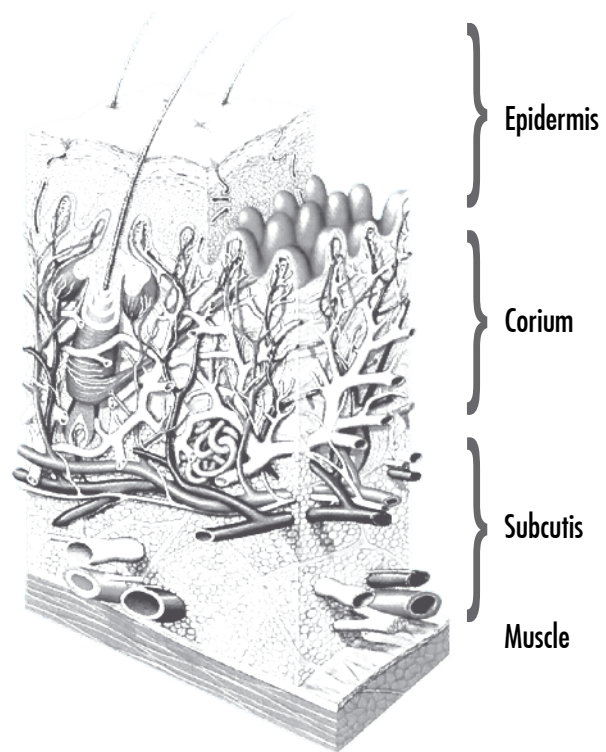
*Master of Cosmetics*

*Warsaw, August 2006*

## WHAT WE SHOULD KNOW ABOUT THE SKIN

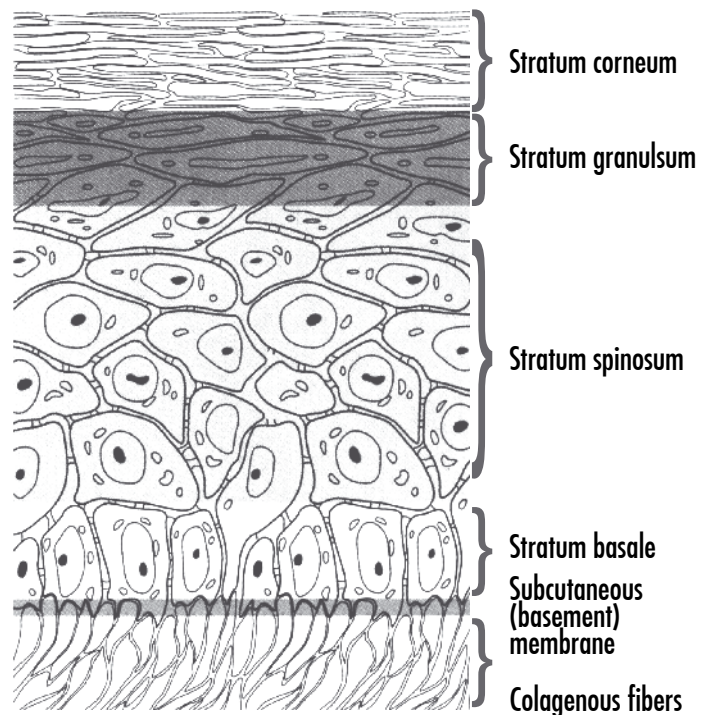
If we don't include the deepest layer of the hypodermis, the human skin has an approximate thickness of a few millimeters. The thinnest is on the eyelids while the thickest is on the back. Much thicker on men than on women.

The most popular anatomical division of skin is, epidermis, dermis and hypodermis. Sometimes however, the spaces found at the junction of the epidermis and dermis are isolated into individual parts joining the epidermis and dermis, or dividing the hypodermis into the: papillary region, reticular region, stratum lucidum. The skin weighs between 2.5 – 4.5 kg (5.5 – 10 lbs.) while its area can extend up to 2.2m<sup>2</sup> ( 23.6 ft.<sup>2</sup>).



It is similar with the epidermis. Here we can talk about four or sometimes even six layers, adding the stratum lucidum (between the corneum and granulsum).

The cells forming the epidermis are 90% keratinocytes. Their role is to defend the system against intruders ( they also have Langerhans cells to help them). The keratinocytes in the base (reproductive) layer produce inferior collagen. Cytokines formed in the keratinocytes awaken fibroblasts which in turn produce superior collagen. As living cells the keratinocytes weaken in the next layers. They become completely calloused in the stratum corneum. In the stratum corneum layer they form about half the thickness of the epidermis, aren't packed tightly and are fully alive but without the ability to multiply (divide mitotically). In the stratum granulosum, the keratinocytes are arranged in one to four rows of cells cemented together with keratin.



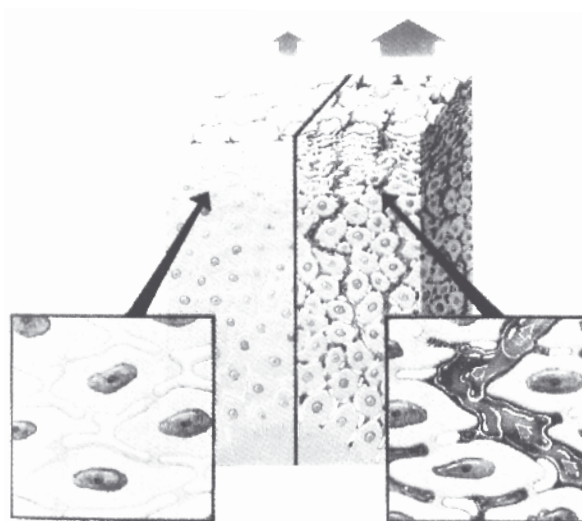
The undamaged stratum corneum is an ideal physical barrier. It defends its appearance against mechanical injury, chemicals, bacteria, fungus, viruses and other micro-organisms and the by-products of their transformations. From the other side it retains water. If we were to completely remove the corium, the body would lose as much as 20 liters (5 gal.) of water daily!

Despite this however, the tissues of the stratum corneum are not as water tight as once believed. They possess physiologically natural gaps indispensable for the exchange of gas (5% of respiration occurs through the skin) and liquid and allow for the permeation of substances such as arsenic, hydrogen sulfide, estrogen, ichthol and unfortunately allergens (even those as large as heavy metals). They also allow water, fat, acid, liposomes, and, of course protein by-products such as extracorporeal collagen, through. The truth is however, (and a cause of much misunderstanding regarding the transdermal nature of externally applied collagen) that almost no non-gaseous substances can reach the keratinized cells. Absorption occurs almost entirely in the extracellular space along the keratinocyte fibers.

For all other cosmetic substances the dilemma is; how to get beyond the stratum corneum without simultaneously giving up their buffering function. Mechanical or chemical peeling obviously ease the job significantly.

The stratum corneum lies under the epidermis. Its internal layers are for the most part collagen as well as elastin, loop vessels and touch receptors.

***The barrier of the corium layers prevent, among other things, the evaporation of water from the deeper layers of skin. If we remove the fat and water binding substances, the evaporation of water increases dramatically, leading to dry skin.***



The dermis is furnished with collagen and elastic connective tissue fibers that protect the appearance from mechanical factors. These fibers also form fibroblasts known as connective tissue cells.



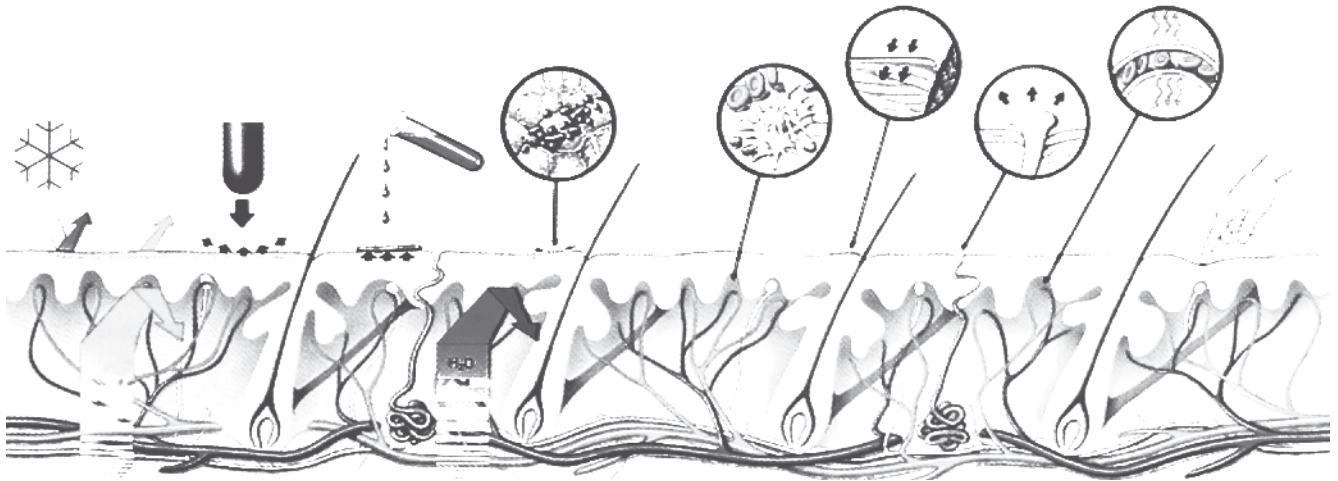
***The Collagen Net  
(taken under electron microscope)***

### Passive skin functions:

1. Protection against cold, heat and radiation	2. Protection against pressure, impact and friction	3. Protection against reaction to chemical substances	4. Protection against effects of micro-organisms, thanks to creation of a lipid coating
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### Active skin functions:

1. Protection against micro-organisms that make their way into the skin	2. Absorption of certain substances	3. Excretes sweat, cooling function. With sebaceous glands form a lipid coating	4. Regulates circulation and thermoregulation of blood via blood supply to skin	5. Sensory organ detecting stimulus, vibration, pain and temperature
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### Other functions of human skin

The complexion is determined by several factors, including color shade, texture, elasticity, mechanical tone and oil supply. Complexion can be evaluated subjectively or with the help of the following instruments: shadow-free lamps, diasscopes, evaporimeters, corneometers, cutometers, profilometers, and sebumeters. The following methods can be used: roller method, silicon casting, photometry, chromatometry, microscopy, and ultrasound, including Doppler ultrasound and microscopes.

Recent diagnostic advances in the fields of dermatology and cosmetology have allowed us to establish such procedures as auto-peeling (complete self-replacement of the stratum corneum). This takes a few days in people in their teen years to their twenties, about 28 – 30 days for those from 25 – 30, while depending on the degree of aging and degradation of collagen, the process can last up to 70 days.

The most important effect of ageing and wrinkling of the skin is degradation (disimilation). It is more or less the sudden “disappearance” of collagen from our skin. Precisely encapsulated it is the loss of the gel-like substances filling the space between the fibers of the connective tissues. This changes the arrangement of the collagenous membrane, which then loses its endurance over time. The process of collagen degradation, it makes up 70% of all proteins, proceeds not so differently than in other organs. The production of polipeptide fibroblast chains (which then twist into helices and finally fibrils) begins to decrease about 1% a year from the 27<sup>th</sup> to 30<sup>th</sup> year of life. The foremost problem is rather with the defective “service” of collagen. So-called collagen filtration, or cross-weaved formations, advance resulting in increasingly stiff and brittle collagen. Under the microscope we can see how the collagen fibers decrease in number, and in precise level of organisation and density. This of course leads to a decreased level of active fibroblasts, which also create elastin, a sister protein of collagen (3% - 5% of the skin’s protein). The elastic fibers of elastin, similar under a microscope to a flexible out-stretched ribbon, are responsible for the ability of skin to return to its original shape after stretching or wrinkling. They now become very broken and less elastic.

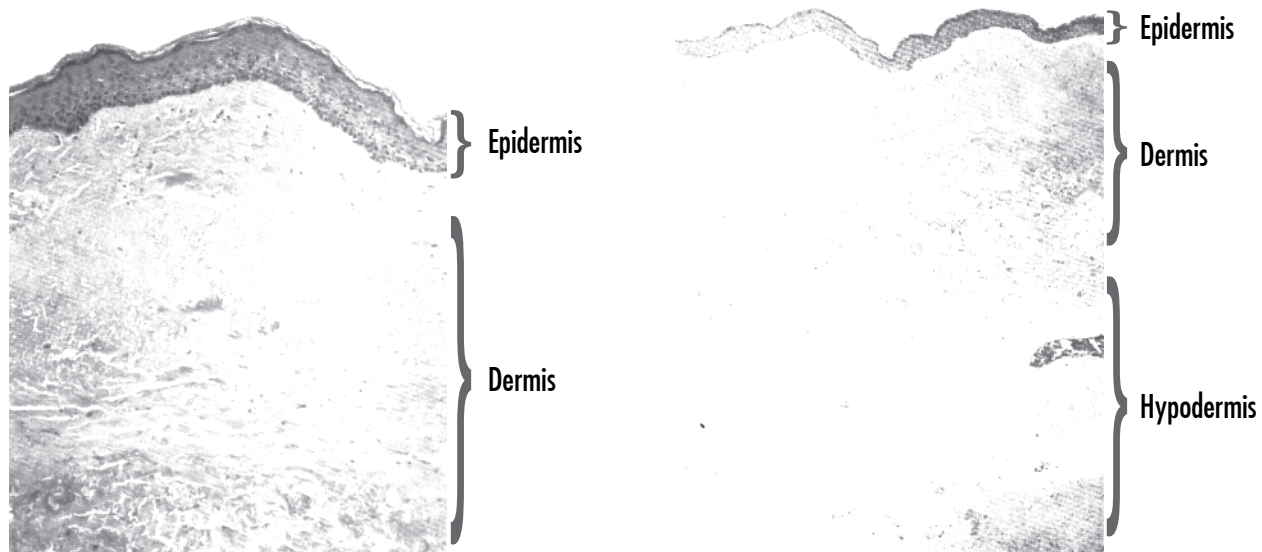
If we add to this the thinning and stiffening of the collagen fibers, the reduction of the mitotic division of keratinocytes, which is related to the advancement of chaos in the base layers of the epidermis – we now have the whole complicated process of skin wrinkling.

This is genetically programmed, yet collagen in the skin can be “aided” by avoiding the sun and not dehydrating the organism. By not smoking tobacco and more importantly through proper nutrition which halts the appearance of fat cells in the skin. Some dermatologists also recommend facial gymnastics and massage as well as sleeping on your back. We can also help the fibroblasts and keratinocytes



by supplementing the skin with collagen both externally and internally or else using protective creams (appropriate to age and skin type) which exfoliate, rejuvenate and moisturize our tissue. Also by maintaining a sufficient amount of vitamins C, E and A .

### Microscope photo of aged skin.



**Cross-section of a fiber cluster**  
Collagen and coarse elastic fibers

**A cluster cell of fat cells**  
squeezed into the dermis

### Types of wrinkle

1. Foetal wrinkles – they appear at birth - for example on the neck – and are there to stay.
2. Movement furrows – they don't depend on the state of our skin, they occur naturally at deep points of the body, in overweight people they reproduce and cannot be changed.
3. Laugh/worry lines – they occur as a result of movement of the facial muscles, influence over them is not minimal, i.e. Botox injections.
4. Dry wrinkles – occur as a result of dry skin, 100% liquidated through application of **Natural Collagen**.
5. Bags under the eyes – they are not exactly wrinkles but result from the weakening of facial muscles, to a large degree they are unconditionally genetic, application of **Natural Collagen** can only temporarily alleviate them, never eliminate them.
6. Oval face wrinkles – occur in people in advanced age as a result of tooth loss, atrophy of the jaw bone, bags under the eyes, etc. The effect of **Natural Collagen** is scant.
7. Age wrinkles – occur as a result of degradation of the skin's fibers, collagen and elastin, in hundreds of cases the systematic application of Natural Collagen contains their progress and significantly shallows their appearance.

### Types of skin

1. Dry skin
2. Sensitive skin
3. Problem skin
4. Mixed normal skin
5. Oily skin

Selected cosmetics such as creams, masks, peelings and tonics can have a significant impact. **Natural Collagen**, in pure gel form, is a preparation beneficial to every type of skin – a cosmetological phenomenon!

**The Editors**

# APPLICATIONS OF TRANSDERMAL COLLAGEN IN MODERN COSMETOLOGY AND SKIN DISEASES

*Wanda Brajczewska-Fisher MD, PhD, specialist in dermatology and immunology*

At the present time, while considering the physiologic processes taking place within healthy skin and skin diseases, one should apply so called “intelligent cosmetology”, which is based on molecular biology and takes into account the role of immunological phenomena. Maintaining proper immunity of the skin tissue depends on the effective cooperation between the bone marrow derived lymphoid cells residing there. There are for example: granulocytes, monocytes, macrophages and lymphocytes, which are the so called T lymphocytes - thymus dependent and thymus-maturing lymphocytes (thymus being the central lymphoid organ) as well as thymus independent, or so called B lymphocytes, which differentiate under the influence of the lymphoid tissue of the digestive tract.

If working efficiently, cells of the immune system can destroy pathogenic substances directly (known as local cytotoxicity) or indirectly by producing inactivating proteins – antibodies.

Successful defence in the skin tissue can be attained only if the following conditions are met: a key role is taken by the T lymphocytes and so called memory cells are produced for the pathogens encountered, moreover, if there is effective cooperation between helper T lymphocytes and B lymphocytes in the process of antibody production. The function of suppressor T lymphocytes is also important, as they play an essential role in immunosurveillance.

During cell cooperation, intercellular signals called cytokines (which are substances of low molecular weight) are sent. This name is applied to a wide range of proteins: cellular growth factors, interleukins, monokines and chemokines. Cytokines promote cell proliferation and maturation, the migration of cells to infected areas, the destruction of malignant cells as well as the production of various substances (including other cytokines and collagen).

From a cosmetology perspective, cytokines are present in the skin and worthy of note as they carry out a protective function including: renewal of the epidermis, collagen synthesis and a reconstructive process related to the length of the skin’s degeneration.

The most important skin cytokines are produced by keratinocytes – live cells of the stratum basale. They promote or inhibit the activity of fibroblasts (cells produced in the tissues as a result of transformation from macrophages) as well as the skin’s vascular endothelial cells and pigment cells (melanocytes).

The following cytokines stimulate fibroblast reproduction and the production of collagen:

- FGF (Fibroblast Growth Factor) – fibroblast growth factor produced by keratinocytes, it stimulates the maturation of fibroblasts and the production of collagen - creating a skin supporting substance,
- TGF (Transforming Growth Factor) – a group of growth factors which transform cells while at the same time stimulating angiogenesis (the formation of new blood vessels in the skin, through its influence on the vascular endothelial cells) also produced by keratinocytes in the epidermis cells.

The following cells may also produce substances promoting fibroblast growth:

- PDGF (Platelet-Derived Growth Factor) – it is a factor regulating growth and maturation of platelets; it promotes fibroblast proliferation and production of collagen during inflammation processes – it is secreted by platelets.
- IL 1 (Interleukin 1) is produced by blood monocytes and skin macrophages, it is called proinflammatory cytokine. It intensifies the skin’s inflammation processes while at the same time activating fibroblasts at a high level.
- IL 6 (Interleukin 6) is produced by, among others, fibroblasts and macrophages. Interleukin 6 stimulates fibroblast growth and intensifies collagen production; it belongs to the group of cytokines intensifying inflammation reactions.

In some conditions, where Interleukin 6 takes a form connected with serum proteins inhibiting its function, it inhibits fibroblast proliferation and the production of collagen.

Adding type IV and V collagen to a skin cell culture inhibits the function of fibroblast growth factor, also responsible for increased growth of new vessels.

Fibroblasts themselves also produce and secrete such cytokines as epidermis cell growth factor (Interleukin 6) thus regulating the maturation and proliferation of fibroblasts and the production of collagen.

The above described functional interdependence of epidermis cells, lymphoid cells and fibroblasts, intermediated by informative protein substances (cytokines) forms a rough presentation of a complicated network of interconnections resulting in the undisturbed physiological production of collagen by the fibroblasts of the skin.

The balance between creating (production) and decomposition (destruction) of collagen is disturbed as a result of skin aging and some forms of dermatosis. The decomposition of collagen and elastin structures, which progresses with age, lowers the value of the support structure of the dermis-epidermis line and in the dermis, while also lowering the permeability of the blood vascular walls feeding the dermis and hypodermis. Supplying additional doses of transdermal collagen to the skin surface in the form of well-absorbed gel has become the basis of modern non-invasive cosmetology. Regular application of collagen derived from fish skin preserves the structure of the triple helix. As a result of its filtration through silk fibroin, it is well absorbed after a few minutes, allowing a quick effect of well moisturized skin, skin elasticity and general reconstruction of the extracellular matrix of the skin. Positive effects preserved in the skin tissue include renewal of elasticity and skin tension. These can be obtained after only a few months of application and moreover, in cases of skin irritation, a clearly soothing effect can be observed.

Applying transdermal collagen accelerates the occurrence of positive cosmetic results, as opposed to local application of vitamin A derivatives (retinoids) – where positive effects were noticeable after ten months of application. Therefore it is understandable that transdermal collagen should attract growing attention.

What is more, expectations connected with the occurrence of excellent cosmetic results from preparations containing a group of cytokines derived from bovine colostrum (applied in the past few years) have fallen short.

### **Applying transdermal collagen to skin diseases**

According to recent clinical observations, positive results can be obtained by applying collagen to various forms of allergies and diseases connected with dyskeratoses (psoriasis, follicular hyperkeratosis) and in skin diseases connected with the malfunction of the sebaceous glands (juvenile acne and acne rosacea); except for the occurrences of deep phlegmonous changes, which require anti-infectious treatment first.

In some forms of progressive psoriasis, where the intensified pathological changes cover a wide surface of the skin and are accompanied by exudate, the application of collagen may fail to bring the results expected.

Likewise, transdermal collagen is not advisable in cases of therapeutic long-term exposure to ultraviolet rays (known as phototherapy) as treatment for psoriasis received after previous sensitization of the skin to light by plant substances (psolarens). This is due to the inhibiting of cell division and a cell disintegration processes in the epidermis which takes a longer time for regeneration. Transdermal collagen application can bring excellent results in chronic vascular diseases (shank varicose veins) in which trophic changes occur, caused by dermal and hypodermal ischemia.

In other cases of dermal and hypodermal defects in collagen structure, such as congenial and acquired striae of the skin and in the irregular distribution of adipose tissue under the skin, called cellulites syndrome; a several-month, regular application of transdermal collagen gradually restores physiological conditions. Transdermal collagen applied in order to accelerate treatment of non-infected wounds appearing after skin injuries, applied on the skin around the wound, brings excellent results. Similarly, regular and long-term application of collagen on various types of post-traumatic and post-operative scars, brings an excellent cosmetic result. Should a benign hyperplasia, called a colloid, appear around the scars, collagen should be applied at three to four weeks after

finishing therapy with topical agents such as 5-fluorouracil in the form of creams and ointments. These precautions must be taken due to the danger of intensifying the inflammation around the keloid while simultaneous treatment limits proliferation of malignant cells due to the application of transdermal collagen. The collagen layer applied on the keloid is most likely, to prevent change, while gradual disposal of the metabolic products from keloid cytolysis by the epidermis occurs under the therapy, leading to inhibition of cell division in the pathogens and their final destruction.

Excellent results are also obtained after application of collagen on first degree-burns of the skin.

Initiation of collagen application should, nevertheless, be delayed in burns resulting from radiotherapy of the skin or malignant neoplasm in other organs where extensive skin erythema and exfoliation of the epidermis appear. The early sharp skin reaction after radiotherapy is a side reaction by the surrounding tissues, and takes place as a result of the use of ionization.

The final target of radiation is the DNA molecules in the nucleus of the neoplastic cells and its eventual disintegration resulting directly from radiotherapy. Free radicals on the target cells may occur as a by-product. Free radicals, as unstable molecules, may also damage the DNA, intensifying destruction of the neoplastic cells. This early post-radiation reaction lasts up to 6 weeks after radiotherapy is finished. The application of transdermal collagen should start after this period, so that the skin's integument tissues may rid themselves of cytolysis metabolites. Whereas in cases of older post-radiation scars, transdermal collagen may always be applied with satisfactory cosmetic results.

The most important problems connected with the establishing of safe and effective procedures in some of the dermatological diseases encountered by modern cosmetology were presented above. New issues connected with beneficial effects of transdermal collagen in medical cosmetology and the strategy of its application, come as a result of the constantly growing scope of new discoveries in the field of biomolecular medicine.

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## THE BENEFITS OF NATURAL COLLAGEN AS ATESTED TO BY RESEARCHERS AND CONSUMERS:

- covers epidermis with a water retaining film
- transfers polypeptides and amino acids to the extracellular space of the dermis
- has activational function, stimulates fibroblasts and keratinocytes
- makes epidermis elastic and soft
- smooths out minor wrinkles
- firms and moisturises skin
- slows skin aging
- aids in most cases of juvenile acne
- supports the treatment of acne rosacea
- supports the treatment of cellulitis
- has fantastic effect on insect bites and stings
- successfully reduces the effects of minor burns, scrapes and knocks
- removes chafes, bruises and early bedsores wonderfully
- cures symptoms of majority of nail diseases
- regenerates and strengthens hair
- prevents greying of hair
- aids in the immunological protection of the reproductive organs
- removes calcanean spurs
- shallows trapezoid furrows
- smooths blackheads
- cures systemic sclerosis
- lightens senile melanoderma and pigmented naevus
- relieves neuralgia and chronic osteomyelitis
- lowers skin reactivity
- accelerates burning of lactic acid in the muscles
- supports the treatment of shank varicose veins
- softens the edges of old scars and smooths new scars
- supports the treatment of skin allergies
- prevents striae of the skin
- relieves osteodynia, arthralgia, arthritic pain and rheumatic pain
- helps in supplementation of bone matrix
- slows osteoporosis in its early stages

- eliminates swelling
- eliminates inflammatory skin conditions
- eliminates dilatation of capillary vessels and spider veins
- eliminates trophic changes
- is a prophylaxis for melanoma - one of the most malignant neoplasms
- accelerates rehabilitation after broken bones, sprains and childbirth
- improves sight when applied on eyelids
- regenerates mucous membrane of the vagina
- prevents colpomycosis
- cures annular psoriasis
- stops alopecia areata
- highly effective against paradontosis
- supports treatment of scleritis
- improves venous and arterial circulation
- symptomatically relieves rachialgia and radiculalgia
- stops a running nose better than nasal drops
- excellent effect on frostbite
- regulates hyperhidrosis
- is the best aftershave and after depilation balm in the world

COLWAY does not guarantee that all of the above mentioned effects are certain and realistic. We have gained this information from our correspondence with consumers and contacts with doctors on the Internet. Yet, we show common sense. Despite for example, a few cases of hair returning to some hairless heads, we still do not feel confident enough to officially raise the hopes of millions of bald men... We also maintain similar scepticism towards, for example, gargling with the use of collagen solution or applying collagen rectally against haemorrhoids.

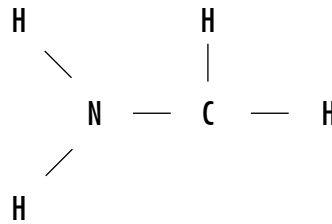
This product has been registered as a cosmetic and is a big enough sensation, without making it into a panaceum as well.

For the sake of balance, we have also published a list of precautions for using Natural Collagen. However, there are no literature to support the contraindications specified below. The list was made based on suggestions from friendly doctors:

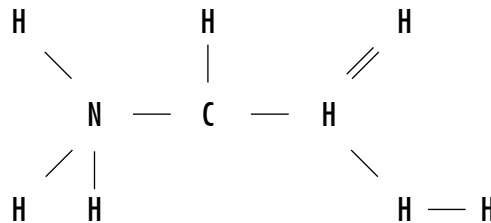
- low protein diet in serious (dialysed) kidney diseases
- chemotherapy
- radiotherapy
- diseases from the group of collagen diseases
- 4th to 8th month of pregnancy (only due to legal reasons)
- eczema based on protein allergy
- allergy to fish protein (for approximately 0.9% of the population)

## MAYBE THAT'S ALL THERE IS ABOUT COLLAGEN...

First, you need hydrogen, carbon and nitrogen...  
They create what is called the amide group...



which is joined by the carboxyl group...  
that is - carbon, oxygen and hydrogen and  
one more hydrogen...



...and so is born a very simple amino acid – glycine. For the purposes of the example to follow - this is one soldier in an army.

Should this “lower H, the one under N” be replaced with  $\text{CH}_3$ , then we obtain alanine and, for example, by replacing  $\text{CH}_2$  with  $\text{SH}$  we obtain cysteine. A slightly longer formula, with additions to the group  $\text{NH}_2$  and  $\text{COOH}$  (amide group and carboxyl group) would create even more complex amino acids, i.e. lysine, valine, hydroxylysine, proline and hydroxyproline. These amino acids can come together by adding the amide group ( $\text{NH}_2$ ) and one amino acid from the carboxyl group ( $\text{COOH}$ ) from a completely different amino acid.

Two amino acids make dipeptide

Three amino acids make tripeptide.

Ten amino acids make oligopeptide (like a squad in the army).

A hundred and more amino acids make polypeptide (like a company).

Polypeptides consisting of a hundred and more amino acids are considered proteins.

This now gives us a single-strand collagen. The name collagen is applied in biochemistry to proteins which include 19 types of amino acids and where 12 of them are endogenous amino acids (produced solely by vertebrata).

Therefore, the use of the term collagen with reference to vegetable proteins, for example, is an obvious misuse and marketing gibberish. It results from jealousy by the cosmetic companies of a very magic word... collagen. There is no collagen other than the one produced in the organism of a vertebrate.

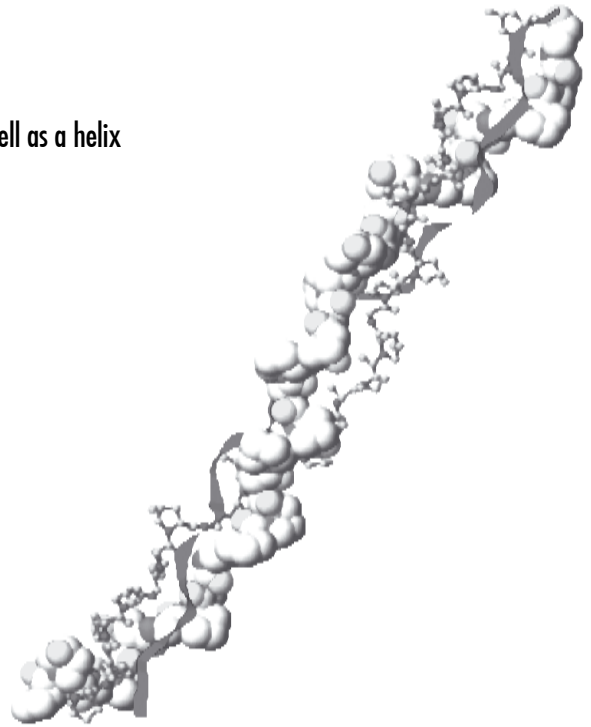
Altogether there are 25 types of amino acid in the organism of homo sapiens.

The joining of amino acids to form even bigger peptides, until they obtain the strength of a company or even a battalion (i.e. a polypeptide), still takes place only in the barracks (i.e. a productive cell, such as a fibroblast cell). Later on however, the battalion (about 1000 amino acids in one chain) is called out from its barracks (fibroblast) onto the military training field (extracellular space). There the battalions, now called helixes, join into a regiment (superhelix) and the whole formation now consists of up to 3000 amino acids.

But this is not the end. Regiments (superhelixes) make up a brigade (fibril) and brigades – a division (fibres). Divisions make an army. The army is a vast formation. It needs artillery, trains and supply lines. Therefore, collagen fibres overgrow with other proteins and covalent bonding, and the army becomes rigid and not very mobile. From the development stage at the level of fibrils, collagen may not be hydrated (it is insoluble). This slight but important difference between a superhelix and a fibril gives rise to further numerous misunderstandings regarding Natural Collagen. There are a few individuals, possessing some knowledge on the biochemistry of proteins, who have heard or read that fibres or even their fibril components, are insoluble. They understand neither the fact that pure collagen may take the form of hydrated gel, nor the fact that (unlike the hydrolysates of mammal collagen) they are the only ones known to date, that are practical for extraction. This gel is not a bundle of peptides shred for the purposes of dilution, but living trihelical tertiary collagen.

Here are some reminders, just for clarity:

- primary collagen – a polypeptide
- secondary collagen – a complex polypeptide, leaving the mother cell as a helix
- tertiary collagen – a triple helix of three polypeptide chains
- quaternary collagen – a fibril
- pentanary collagen – a fibre



Let us also specify what Polish fish collagen is, and also what this thing is which, has been known to the field of cosmetology and implantology for years as “bovine collagen”.

Natural Collagen is a protein present in the skin of freshwater fish which has passed, in its intact and isolated form, and through a process of extraction and hydration, from its maternal organism to a water solution which preserves its tertiary triple helix structure.

It takes the form of a natural gel of high cosmetic value. Thanks to its conformation, fish collagen displays biological activity. While penetrating the extracellular space of the epidermis, it has an indisputably confirmed influence on the activity of keratinocytes and fibroblasts and brings about a full range of cosmetic and dermatological effects.

So called “bovine collagen” is a fibre of pentanary insoluble collagen cut from the nape of young cattle. This protein has such a large molecular mass, that it really has no chance to penetrate the barrier of the stratum corneum other than by invasive sclerotherapy, for which fragments of these fibres are used. The implants of bovine collagen fibres integrate in approximately 92% of patients and degrade after approximately seven months. In a highly shredded form bovine fibres are also used to produce protein hydrolysates. This is a biologically inactive emulsifier because it is only a pulp of fragments cut from peptide chains and used as a filler in diet supplements or as a component in cosmetics. It creates a water retaining “film” on the surface of the skin. This “collagen” has been used for many years in cosmetology. Aside from this it is only a small part of a natural sequence of almost three thousand amino acids.

The above information should have been detailed a long time ago. This might have prevented numerous misunderstandings in which the parties did not fully realize what they were arguing about.





There have been many myths, of a more or less believable nature, created around Polish fish collagen which, while advertising it, have also infantilized it to its detriment.

It is understandable that each time you make a solid presentation - full of biochemical details – the result could be something the average listener would neither stand nor understand. Therefore, for the common good, almost every presenter announcing the wonders of fish collagen must simplify the subject. In some cases however, out of all good intentions, slips of the tongue or “nonsense” appears.

Here are some of them:

- fish collagen is identical to human collagen,

yes it is in the sense that in practice, it consists of the same amino acids and up to the level of tertiary connections, a chemist would find no significant differences (slight differences in the structure of chain  $\alpha 2$ ). But a biologist who knows that collagen in the human skin is a pentanary fabric and that it takes the form of a triple helix only temporarily, for a short time, at the production stage, when it leaves the cell, would definitely protest such a statement.

- collagen in gel form penetrates the skin and reaches even the organs,

this sounds wonderful but... a doctor, a biologist or even a cosmetologist would not only protest such a statement, but declare it nonsense. That's because this is a gross simplification; it is not collagen, but the products of its decomposition, not through the skin but through the epidermis, and not directly, but as a result of stimulation of protein growth cells in our organism.

- by applying collagen we provide our skin from the outside, what it lacks from the inside,

this is also a fantastic marketing slogan, but it is nonsense biochemically. A biologist would be convinced upon hearing something like; “...by rubbing on this gel, you make amino acids from the product's decomposition, creating an environment favourable for keratynocytes to increase the number of cytokines, which in turn stimulate the proliferation of fibroblasts and the production of collagen depending on the condition of the skin.”

The above stated facts may sooner or later be confirmed through clinical trials – they may be, but do not necessarily have to be.

Fortunately, Natural Collagen has been registered as a cosmetic, not a medicine, and neither EU nor Polish legislation requires us to present evidence more advanced than current scientific developments. It is worth noting that no one, absolutely no one in the world today; no laboratory has access to the unlimited, practicable amount of tropocollagen (tertiary collagen) which has been isolated from a vertebrate organism into a water solution – necessary to carry out such tests. Therefore there are no such tests.

Therefore, no one has the right to negate a phenomenon which they know nothing about, especially if these phenomena are supported by a group of hundreds of thousands of people who literally tested it on their own skin. All opinions stating collagen's impermeability to the skin, do not refer to us because they were formulated based on the observations of products shredding the fibre of bovine collagen, not superhelices of fish collagen. In Poland in particular, no one has the right to remark on the properties of tertiary collagen, isolated in its intact structure because...no one has ever tested it.

Testing tertiary collagen, isolated from the organism, is not as simple as all those who are “demanding tests” would like to imagine. This protein is so highly unstable that exhaustive testing on it can only be carried out by a few laboratories in the world. And they demand a fortune to do it.

Collagen is an intracellular protein, functioning in an excellently protected hermetic environment, right up until the three-helix stage of its development. Once it leaves the cells it becomes completely unprotected. It's thought to be a miracle that Polish hydration technology has, at precisely this moment, allowed it to pass into a water solution and even seal it in a jar without it losing its spiral triplet structure. Because this is the moment where the superhelix gets destroyed by everything: temperature, pH change, the smallest dose of enzymes, collagenases that might be present in fungus, mites and even in the air in bacteria. Most of all it can be destroyed by even the smallest dose of RTG or any other radiation, used in analysis.

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The high molecular mass of tropocollagen does not facilitate mass spectrum or chromatographic analysis.

To be honest ... there is nowhere in Poland to order such studies. Only tests of qualitative-quantitative composition, applying electrophoresis and HPCL methods are available here.

By means of strong connections (and at high cost) we managed to obtain some analyses, including densitometric tests which were supervised by none other than Eng. Maria Sadowska PhD, indisputably the most recognized scientific authority in the world, with regard to collagen derived from fish skin.

At present, protein biochemistry as a science generally seems to be in a state of, if not stagnation, than perhaps slower development than for example in, genetics or nonorganic chemistry. The so called "scientific evidence of efficacy" for substances destined for pharmacies and cosmetology, is almost exclusively gained from tests made in corporate laboratories or in institutions sponsored by them, strictly upon order and with assumptions made beforehand.

A cosmo pharmaceutical dermatological preparation, launched successfully on the market in such conditions and without this type of support, must be more than good, it must be a real biochemical sensation. And Natural Collagen really is!

***The Editors***

## A WIDE FRAGMENT OF WHAT COULD BE BREAKTHROUGH RESEARCH...

... the research was carried out in the period between 12.03 – 15.04 2006...

It aimed at specifying the influence of extracorporeal collagen derived in the form of hydrated gel (hydrate) from the skin of the pisciculture fish *Hypophthalmichthys molitrix*... on the human organism using in vitro cultures, as well as investigation by a team of researchers of the mechanisms of permeability (transdermality) of the studied substance after application onto the skin...

...the peptide particles and amino acids which are the products of decomposition of extracorporeal collagen helixes, applied on skin in the form of fish collagen hydrate, indicate an astonishing ability to relocate - so called migration...

The assistants observing indicated a similarity of this migration to the movement of peptides along the fibroglia in the process of cerebral cortex tissue growth...

The research focused on discovering the molecular mechanism guiding the amino acids (the products of decomposition of extracorporeal collagen) to the area of extracellular matrix (ECM) and creating a collagen scaffold supporting the structure of the tissue...

The receptors for the extracellular matrix proteins are integrins – integral proteins of the keratinocyte cell membrane which participate in the adhesive processes of extracorporeal amino acids to the proteins of the ECM (extracellular matrix) and in adhesion of the type: cell - extracorporeal protein. From among the ECM proteins, which are natural ligands for integrins, laminin indicates the strongest induction properties in the adhesive process.

...research with the use of the immunofluorescent method has indicated that, for example, laminin is present along the paths where the products of the fish collagen's decomposition permeate into the organism. A positive reaction of the keratinocyte receptor was observed for the developing NGF gradient – a new growth factor, when the extending axons of skin neurons were accompanied by peptides of extracorporeal collagen... For example, the protein kinase was observed suggesting a stage of adhesion of amino acid aggregation to the neuro cell membrane.

If the process of high share of collagen proteins in the development of the nervous system is already known, resulting from their profuse presence along the paths of moving neurons, (in vivo studies of migration of neuron crest cells to the area of the neuron tube ideally along homogeneous, ordered collagen fibres) then no one has ever described reversed symbiosis, especially in relation to extracorporeal collagen, whose transdermal properties were considered impossible...

It was observed in the in vitro study that after six hours, the human fibroblast culture with a sample of extracorporeal collagen peptide chains produced by fibroblasts, was extended in comparison to the culture without extracorporeal collagen and in comparison with the fibroblast culture blocked by serum "anti-human fibronectin". After six hours the average extension increased over two times ( $0.168 \pm 0.019$  versus  $0.441 \pm 0.038$  and  $0.188 \pm 0.018$  versus  $0.441 \pm 0.038$ ) in correlation with the amount of amino acids (only the amount of hydroxiprolin was studied) whose quantitative growth produced incomparable results.

...in the case of the fibroblast culture blocked by serum anti FN, no differences of statistical significance were found in comparison to the culture without extraporeal collagen.

Modification of the base, by blocking fibronectin with serum, eliminated factors that might influence anisotropy of the base...

...the chains alpha 1 and alpha 2 most probably coded by separate genes (COL alpha 1 and COL alpha 2) identical to human collagen type I, were well noticeable during decomposition in the extraporeal collagen observed in the in vitro tissue culture.

...as an experiment, independent from observations made with a Jenawert metallurgical microscope with Nikon UFX – DX C and D micrography attachments and an OLYMPUS IMT 2 inverted microscope; a Pharmanex Biophotonic scanner was used and so far applied solely for determining the level of carotenoids in the skin (Skin Carotenoid Score – SCS). A separate protocol was made.

...the second part of the research was aimed at estimating the possibility of immunotropic interaction in extracorporeal collagen derived from fish skin. Microcultures of mononuclear blood cells of healthy donors (PBMC) were used.

100 µg/ml of hydrated collagen was added to the liquid culture (RPM/1640) and this emulsifier was used to start the culture.

15 experiments were carried out comparing the results of the cultures in the control liquid and in liquid with the addition of collagen hydrate on the platelets nuncoln microplate. The sets of immunological cells and vitality of PBMC cells was tested in this way.

15% of previously audiologicaly inactivated serum was added to each culture.

Particular triplets of the culture were stimulated with phytohemagglutinin ((PHAHA 16. 0.5 µg/culture) and then left without stimulation and incubated through three full days and nights in the incubation chamber at a temperature of 36°C and an air humidity of over 60%.

Once the culture was finished, the level of inbuilt 3HTdR was measured withan ERNO 1900 T scintillator , which in the amount of 0.5 µC/culture was used to mark the isolated fragments...

A statistically significant lowering of suppressing activity on the part of T lymphocytes (the SAT index in the control cultures and in the cultures containing collagen  $30.41 \pm 14.05$  versus  $16.18 \pm 15.1$ ) characterised the cultures containing the addition of extracorporeal collagen.

A statistically significant lowering of LM index dependent on the ratio  $IL - 1\beta / IL - 1ra$  was also observed. The LM parameter for control cultures in comparison with cultures containing collagen was  $13.18 \pm 8.3$  versus  $8.9 \pm 6.8$ .

Statistically speaking a highly significant lowering of receptor  $IL - 1\beta$  production (in control cultures and containing extraporeal collagen  $684 \pm 238$  versus  $396 \pm 174$  pq/ml) was also discovered.

Changes of statistically lower significance were observed in the marking of cytokines with concentration of opposing receptor  $IL - 1ra$  (in control cultures in comparison with cultures containing extraporeal collagen  $2307 \pm 630$  versus  $2812 \pm 329$  pq/ml)

### Final conclusions [excerpts]

- 2) ...collagen derived from fish skin taking the form of hydrated gel shows... high hygroscopy... has a tertiary structure which in the process of permeating to the extracellular matrix of the epidermis decomposes to components of primary structure... without breaking peptide bonds at this stage, which is rather a research sensation.
- 4) The products of decomposition of extraporeal collagen protein have the capacity to migrate within the extracellular space of the epidermis...
- 7) Glycine and hydroxyproline produced after decomposition of extracorporeal protein may influence the amount of cytokines produced by the keratinocytes of the stratum basale...
- 8) The fibroblasts of the human tissue culture treated with stimulation by the extracorporeal collagen peptides... indicate increased activity displayed by the increase of component amino acids in the produced peptide chains...
- 9) The extraporeal collagen derived from fish may lead to functional changes in the cell which is proven by lowering the immunocontrol properties of T lymphocytes and lowering immunogenic activity of monocytes. The immunosuppressant effect of extraporeal collagen – subjected to in vitro study - allows the presumption that it possesses, at the least, anti-inflammatory properties.
- 10) None of the research carried out allows any conclusions to be drawn as per the influence of extraporeal collagen on the human organism in vivo.
- 11) Research on the influence of the fish-derived extraporeal collagen meets difficulties in the form of a complete lack

of any literature on the subject. This is in fact, pioneering research which requires:

- a) obtaining permits to carry out a full range of in vivo tests
- b) access to the most modern testing apparatus
- c) clinical conditions, including representative groups of probands
- d) high financial expenditure...

## EDITOR'S COMMENTS

*We kindly request that you refer back to the title of the chapter. This research could really have been a breakthrough. It would be our overwhelming desire to be able to publish it in its full, 11-page length, while writing with pride, pleno titule, the name of the Polish research institute, and at the end of the text, printing the names and seals of the luminaries of Polish science who carried out these tests .*

*Were it not for the fact that it gives neither direct evidence that fish collagen has transepidermal properties, nor does it prove scientifically any of the practical effects stated by the purchasers of over half a million packages - this might have been breakthrough research.*

*It indisputably proves the migration capabilities of our collagen in the extracellular space of the skin, which in practice means a transdermal capability – a fact so often negated by “specialists” that you might encounter. It shows an unexplained but noticeable influence of fish collagen application on the activity of the cells, which produce our natural human collagen and as a result keep US IN OUR PRIME.*

*These studies legitimise the small, at present, field of medical collagen and its direct effects. They scientifically prove only (and at the same time as much) that fish collagen has the capacity to reduce inflammatory states at the cellular level. This may not mean much, e.g. only satisfactory symptomatic treatment. But this may yet mean a lot.*

*Enough to take the breath away.*

*Unfortunately, we are not publishing this research in full. Why? Because we are not authorised and we do not have the right to do so.*

*If you don't know what's going on...its usually about money.*

*So we have excellent Polish research on collagen and at the same time we do not have it. Just like in an American courtroom, where clear evidence is given, but the judge tells the jury: you cannot take it into account.*

*The costs of this research grew dramatically while the analytical needs necessitated by it were getting noticed.*

*We continued with the ever growing payments required by this research for many months, while feeling all along that they should not burden the balance sheet of COLWAY Unlimited.*

*We have some resentment towards the orderer and investor of this research, for not concluding a clear contract preventing the research institute from raising prices, while pushing costs to the ceiling.*

*We also have general reservations about whether it should be the distribution organization striving for research affirmation of the products it distributes, as this is the basis of marketing activity. On the other hand, we understand the orderer's desire to withdraw from paying a final bill which exceeded by several times, the cost calculation and the terms and conditions of the preliminary contract.*

*The researchers escalated their financial claims once they noticed the significance of the research results. They calculated not current, but future profits that might be obtained, once Natural Collagen is registered as a medicine.*

*Several months of negotiations failed at reaching a compromise, despite numerous attempts. As a result, we still do not have authorized research proving the transdermal properties and efficacy of Natural Collagen.*

---

*One may look at it from another perspective. None of our Polish competitors – producers of fish collagen - even attempted to affirm their activity scientifically. We were the only ones. Dr. Przybylski, a PhD in chemistry who advertises fish collagen as a panacea for cancer, osteoporosis and multiple sclerosis, does not care to provide (despite the passing of quite some time) any clinical proof of these claims.*

*One may also hear opinions that, the indisputable scientific evidence confirming that which has been shown in life, might paradoxically be harmful to the consumer, and the network (where the overwhelming part of Natural Collagen trade takes place). This would attract immediate interest in the product from pharmaceutical wholesalers or other entities willing to invest capital in an advertising campaign for collagen.*

*COLWAY is not interested in doing what, might in perspective, damage the network's monopoly, which is truly the apple of the Management Board's eye.*

*Let us then accept that this research is an unofficial "leak" and that we may only use it while arguing with doctors, cosmetologists and biochemists questioning the sensations connected with collagen - based on their out of date knowledge. Let us enjoy what we have. Any specialist reading the fragments cited from this research - shall have discovered a true sensation. . .*

## EXPERT TESTIMONY – 2

I know how to successfully apply Natural Collagen to the daily work of my beauty parlour.

But a year ago I had many questions, doubts and even reservations.

While observing systematically the effects of this biologically active “living” protein, I realized how exceptional the cosmetic appeared. Its effectiveness was surprising and even shocking:

- the speed of healing scrapes, burns and scratches (even those of cat’s claws) is incredible,
- the improvements shown in juvenile acne or acne rosacea are much faster than cosmetics used so far,
- after applying Natural Collagen, frostbitten skin from a cryotherapy cabin stopped burning, after repeated application, within 24 hours, the condition of the skin allowed the continuation of the cryotherapy.

I will add some “collagen surprises”:

- Natural Collagen applied to rejuvenate complexion, likewise caused significant and constant regrowth of the eyebrow hair of my 72 year-old client,
- Natural Collagen applied to rejuvenate the skin of a person with a thyroplasty scar, also made the scar elastic and smooth; the skin around the scar was less discolored and creased and the scar was less visible
- Natural Collagen applied to a 28 year-old woman having problems with the effects of seborrhoeic skin, not only – as promised – sped up healing of her chronic herpetic eczema, but after four months of application, removed the herpetic changes. They did not return even after sunbathing in strong sun,
- Natural Collagen used for swollen legs due to vein failure, caused complete absorption (disappearance) of the blood extravasations around the ankles.

In all described cases Natural Collagen was applied as a systematic, home based, therapy over several weeks. Permanent shallowing of wrinkles resulted from the thickening of the epidermis, normalization of the work of the sebaceous and sweat glands and increasing elasticity and permeability of blood vessels resulted from the influence of Natural Collagen on the amount and quality of the collagen produced by the fibroblasts of the person undergoing therapy.

One needs time to raise the value of the supporting structure of the skin (elasticity, resilience and density) and successfully slow down the aging processes, and this is not served by a long series of treatments.

The first problem connected with the application of Natural Collagen in the beauty parlour was the time necessary to reach the presumed effects, equal to those obtained through a several week-long therapy at home

The second problem was the optimal activity of Natural Collagen at a temperature from 41°F– 78°F (5°C to 26°C ).

After using Natural Collagen for one year only in the beauty parlour, and monitoring the effects of the home therapy I had recommended, I found a solution to the aforementioned problems:

- the best results of therapy with Natural Collagen can be obtained through treatment carried out in the beauty parlour together with application at home,
- it is necessary to exclude from treatment carried out in the beauty parlour, activities increasing the body temperature to the values exceeding the tolerance of Natural Collagen (a reminder that human body temperature does not prevent the application of Natural Collagen),

- while carrying out treatment in the parlour, Natural Collagen should be applied before or after activities raising the body temperature.

Please find below a description of practical solutions illustrating the conclusions discussed.

General principles:

- Natural Collagen cannot be used as a lubricating gel, i.e. we don't do massages with it,
- Natural Collagen cannot be applied as contact gel (it is not applied under an ultrasound handset),
- Natural Collagen should be applied directly onto the skin after removing make-up, after cleaning and peeling and on skin hydrated with mineral water (do not use alcohol tonic),
- A thin layer of Natural Collagen applied on the skin requires a minimum of 10 minutes to be absorbed. The peptides of the fish protein, remnants of the helix structures contained in the gel of transdermal properties, are then absorbed into the dermis.

Further activities depend on the objective of the treatment.

These are some examples:

1. **Regenerative treatment for "face-neck-cleavage" (lifting-moisturising).** After collagen is absorbed we perform a massage using a properly selected cosmetic, the best is non-warming lymphatic drainage. We apply an appropriate serum-concentrate (e.g. with vitamin E) and a mask of algae or collagen sheet, i.e. phycollagen or microcollagen. Then we proceed on as in the last phase of all parlour treatments. Applying Natural Collagen after lifting the masks used at the beginning of the treatment also brings very good effects. This usually cause increased congestion. The Natural Collagen here, plays the role of a high-protein concentrate, while at the same time it has soothing and calming effect.
2. **Calming and desensitizing treatment** (for allergic skin with vasomotor neurosis and telangiectasias, neurodermatitis and atopic dermatitis). In these treatments the effects are exceptional! They calm, sooth and relieve itching and burning. They also reduce sensitivity and immunization, increasing tolerance for other cosmetics, i.e. cleansing and protective cosmetics and even medicines necessary to prevent this type skin of condition from constantly deteriorating. It is necessary to take a series of 6 to 12 treatments, once or twice a week. By as soon as after the third or fourth treatments, preparations previously not well tolerated, can be introduced.
3. **All-body treatment** (skin preparations – the same as for face).
 

Massages: a) rejuvenating (after sunbathing or overuse of ultraviolet light)

b) anti-cellulite and firming while slimming, i.e. lymphatic drainage, isometric massage (not warming).

Caution: do not use any warming preparations, e.g., creams with cinnamon.
4. **Treatment for striae.** I advise starting these with an exfoliation of fruit acid or a dermabrasion. After Natural Collagen is absorbed, rub in the chosen cosmetic, at best a "cold" one.
5. **Hand treatment** – rejuvenating, depigmenting and desensitizing. Very good results can be obtained by applying Natural Collagen after paraffin - on already cooled skin of course – and rubbing in vitamin A+E at the end.
6. Natural Collagen should be used to achieve immediate effects as well, owing to its bactericidal, anti-inflammatory, styptic and soothing properties. Natural Collagen should be applied after:
  - a) closing vessels,
  - b) cleaning complexion,



- c) wax depilation (mainly small areas – face, bikini),
- d) peeling with acids, herbs, after dermabrasion).

After two years of experience I think that applying Natural Collagen in a beauty parlour requires additional knowledge and observation of proven procedures, which in practice very often means changing old habits. This is decidedly worth doing due to the effects brought about by the application of Natural Collagen. The active fish protein directly minimizes the sources of skin aging. Its effects are not limited to beautifying skin: it works as a medicine influencing the structure and functioning of tissues (cosmo pharmaceutical).

Finally let me present information of fundamental significance in the treatment of acne rosacea and juvenile acne. Home therapy with Natural Collagen should be done only after previous partial curing of acne in a beauty parlour with the methods used to date. In such cases one may resign from the use of antibiotics, hormones and acid preparations which, if applied over a long time can bring about side effects.

It needs to be added that using Natural Collagen in beauty parlours was made more convenient once Natural Collagen of higher temperature resistance was launched on the market. Natural Collagen Q 5 – 26 is a preparation working optimally in temperatures up to 78°F (26°C) and not as previously, in 2004-2005, at temperatures up to 73°F (23°C).

It is for the above reasons that Natural Collagen outclasses the competition's preparations.

**Aleksandra Izykowska**  
**Certified Beautician**  
**Warsaw, October 2006**



CATHEDRAL

THE GDANSK UNIVERSITY OF TECHNOLOGY

CHEMICAL FACULTY • DEPARTMENT OF FOOD CHEMISTRY, TECHNOLOGY and BIOTECHNOLOGY

**Colway Unlimited**

ul. Hippična 19

84-207 Koleczkowo

PG — KCTB /01/01/2007

Gdansk, 23.02. 2007

We are forwarding the results of the densitometric and chemical testing on two collagen specimens delivered on 24.01.2007

Specimen 1 – source: African Catfish 33%, Silver Carp 67%

Specimen 2 – source: Silver Carp 100%

Executed according to the scope of research included in order no. 01/01/2007

Contract lead by

Dr hab.inż. Maria Sadowska

Enclosure

Set of test results

**GDANSK UNIVERSITY OF TECHNOLOGY**

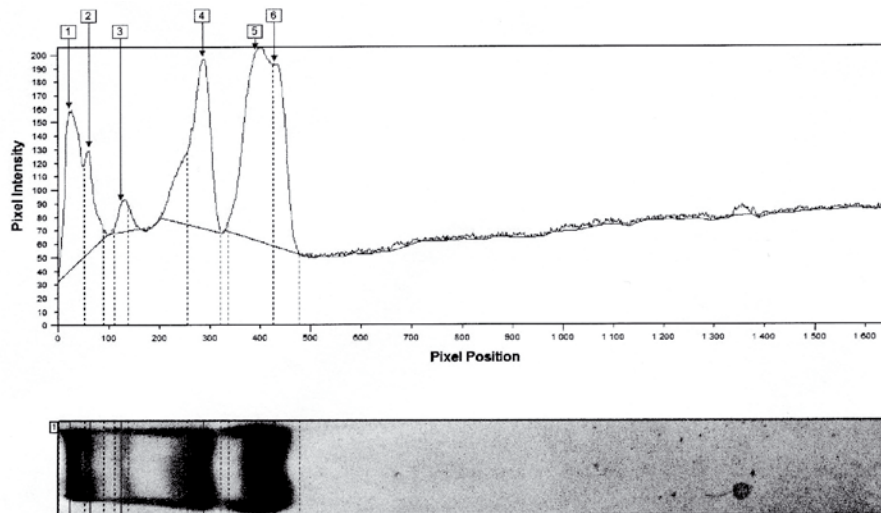
Chemical Faculty • Department of Food Chemistry, Technology and Biotechnology

ul. Narutowicza 11f12, 80-952 Gdańsk, Poland +48 58 / 347-12- 46, fax +48 58/347-26-94

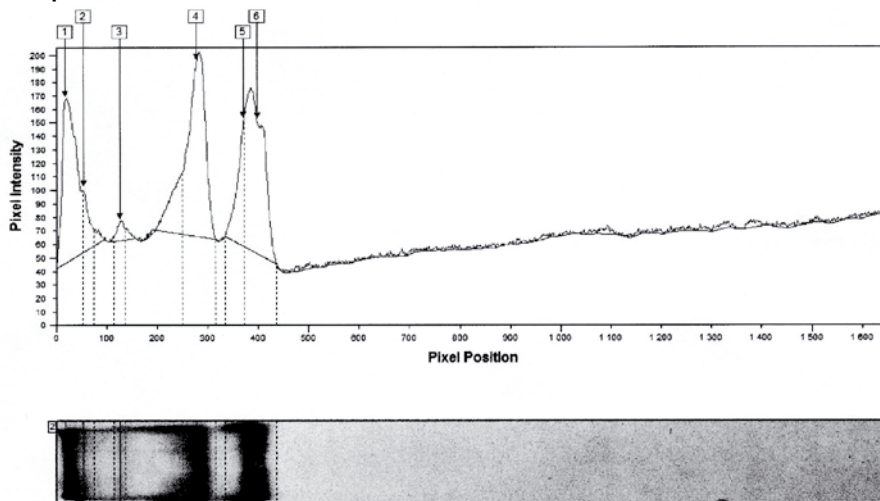


## 1. Densitometric Analysis of collagen preparation

### a) Sum from Silver Carp



### b) Silver Carp





CATHEDRAL

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CHEMICAL FACULTY • DEPARTMENT OF FOOD CHEMISTRY, TECHNOLOGY and BIOTECHNOLOGY

Lp.	Subunit of Collagen	Sum from Silver Carp		Silver Carp	
		V [px]	Percentage distribution [%]	V [px]	Percentage distribution [%]
1	γ	783922	17.43	675404	24.35
2		305390	6.79	102803	3.71
3		83663	1.86	37722	1.36
4	β	951501	21.15	869317	31.34
5	α1	1607290	35.73	222710	8.03
6	α2	766369	17.04	865727	31.21

## 2. Chemical Analysis

	Sum from Silver Carp	Silver Carp
Hydroxyproline [%]	0.301 ± 0,0083	0.190 ± 0.0059
Conversion factor	10.7	10.0
Collagen [%]	3.22 ± 0.089	1.9 ± 0.059
Raw protein [%]	3.4 ± 0.21	2,0 ± 0.10
Dry mass [%]	4.20 ± 0.015	2.28 ± 0.059
Molecular mass [kDa]	358 – 378	
α1 chain [kDa]	127 – 130	
α2 chain [kDa]	104 – 118	
pH	3.3	3.6

## COMMENTS ON GDANSK RESEARCH

In our opinion this research is sensational for several reasons:

- Firstly, due to the very person authorising it. Eng. Maria Sadowska PhD is indisputably the “Number One” Pole in the field of protein biochemistry. While within the field of collagen from fish skin she is most probably “Number One” in the whole world!

Of those persons who have contributed to the creation of Polish fish collagen in its present form, her contributions have been the greatest. Even though we are not yet publishing a description of these analyses and the full conclusions which may be drawn from them (they are our jealously kept secret) any sceptics may now phone the Chemical Faculty of the Gdansk University of Technology and ask if this is true.

But only a year ago COLWAY could merely dream of research on collagen gel under the auspices of the very Dr. Maria Sadowska.

- Secondly, because the research correlates ideally to findings formulated in significant laboratories around the world.
- Thirdly, due to their findings. The densitometric and chemical analyses show clearly that our product is the purest tropocollagen, as if taken directly from a vertebrate organism.

This triple-helix protein which for the first time ever, has had its conformation preserved and has allowed humans to close it in a bottle, like a “genie”, is now being released to make dreams come true.

- Fourthly, because optically, graphically, spectrally and proportionally the analyses indicate an alpha1-helix, whose peptide chains determine the transepidermal properties resulting in the effect the product has. Many of the giants from the cosmetic and medicine markets would strongly wish to join in documentation offering such a spectral range.
- Finally, because these analyses concluded in the first success in biotechnological experiments, several months in length, and were undertaken solely at the behest of COLWAY. For some time they were an “open secret” which we may now, officially, be proud of.

Sample 1 – is a very well known collagen from Silver Carp which is at present manufactured by three Polish facilities.

Sample 2 – is collagen which is at the disposal of our Network. It was worked out biotechnologically via hundreds of trials on various fish living throughout the world...

Now then, here are some people who had their hand in the history of Polish collagen; Mieczysław Skrodzki (hydration), Józef Przybylski (filtration), Leszek Kalecki (temperature resistance) and now we have also added our hand...

Even a complete laymen, after reading this script, would know that the quality of collagen gel is easiest to measure by the quantity of its hydroxyproline. And likewise, that the more “collagen in the collagen” the more dry matter remains after complete sublimation of H<sub>2</sub>O.

Our dear readers should also realise that lower pH, and most of all, percentage share of alpha1-helix in the spiral triplet, are properties most desired in collagen gel.

Have a look at the spectral graphs and at the tables, pH better by three points, two-thirds more hydroxyproline (the most precious amino acid), collagen and dry matter in general!

Alpha1 helices over four times “fatter”!

Therefore we consider this research sensational.

**We continue to leave competition behind !**

# CONCLUSIONS RELATED TO THE CHOICE OF RAW MATERIALS OBTAINING INDUSTRIAL COLLAGEN FOR THE COSMETICS INDUSTRY

## BASED ON RESEARCH CARRIED OUT IN POLAND – WITH SPECIAL CONSIDERATION GIVEN TO COLLAGEN DERIVED FROM SILVER CARP

*(Extensive fragments from the work of Mariusz Gromnicki, PhD - reporting for Glavin Associates)*

...the method of deriving and modifying collagen from fish skin was achieved in the 1980s by scientists from Gdańsk – Eng. Maria Sadowska PhD and Eng. Ilona Kolodziejska PhD. They described two ways of obtaining collagen from the waste of the fish processing industry.

The first one is a precipitation of all other components from the waste material apart from collagen i.e. fibres of other proteins, muscles, lipids, pigments and fats.

The second is an extraction of collagen from skin pulp done directly with the use of acid polysaccharide of vegetable origin.

This invention was somehow medially appropriated by Prof. Jozef Przybylski, former Director of the Institute of Chemistry at Gdańsk University where, with the significant participation of Przybylski, a method of collagen filtration was worked out. The collagen extracted with methods by Sadowska and Kolodziejska (with modifications introduced by Skrodzki and Kowalska-Gwardys) through silk fibroids, allowed for maintaining of the triploid helix structure in the protein constituting the basis for emulgates that might be used in cosmetology.

...collagen of fresh water fish feeding in European and west Asian waters differs substantially physicochemically from other animal proteins and from the collagen of other fish [comparative studies carried out by E. Krajewski on the example of cod]

...already within the scope of photodegradation and especially photochemical reactions taking place in vitro and in vivo, in the case of biopolymers, these differences are very substantial. Collagen derived from the skin of Balkan Carp [A. Anikowska 2003] preserves substantial resistance to UVB rays (290-320 nm) and UVA (320-400 nm). Modifications of its biopolymers taking place during irradiation  $\lambda < 300$  nm are incomparably smaller than in other proteins subject to comparative studies. Silver Carp seems to be exceptional in this respect. Apart from being the source of exceptionally valuable collagen, it is also the source of precious amino acids and polypeptides, while the oil derived from it does not yield to the resistance properties of preparations from shark liver, so widely advertised at present. It is a very good carrier for enzymes and antiseptics.

...Balkan Carp is easy to breed in relatively high environmental temperatures which adds to the usable properties of the collagen derived from it.

For example, while the use for production was collagen hydrate, the raw material derived from Salmonidae or Cyprinidae bred in Poland or in Slovakia, it was necessary to improve its usable properties, and especially to raise the temperature of denaturation by introducing it to cosmetically produced collagen gels with intramolecular and intermolecular cross-linking bonds. This was done chemically with the use of tannin or preservatives (EUXYL 600) or enzymatically with the use of transglutaminase .

Breeding Balkan Carp in fresh water gives, despite its substantial costs, the possibility of obtaining in a completely natural manner, the raw material being the basis for collagen cosmetic hydrates which do not undergo denaturation even in temperatures exceeding 25°C.

...the use of collagen derived from Balkan Carp was influenced by laboratory competition between manufacturers of collagen cosmetic gels in Poland. They were forced to find fish collagen of the highest possible storage temperature.

In the case of collagen, denaturation means the passing from helix structure to a static bundle of proteins, i.e. it comes about from irreversible changes in its secondary and tertiary structure or changes in its hypermolecular structure taking place without breaking the peptide bonds, preserving the primary structure of collagen that, however, excludes it from any biological activity.

In specific cases of collagen derived from fresh water fish, denaturation temperature was a serious problem for the cosmetic

manufacturers, because it determined the market demand for their preparation. The decisive line seemed to be the room temperature 68° F – 73° F (20°C – 23°C ) below which denaturing collagen gel had no chance for commercial success.

The temperature of collagen denaturation depends on three factors: the natural temperature of the feeding grounds from which the raw material was taken, the content of water in collagen and on the degree of its cross-linking. For these three reasons the skin of carp feeding in the breeding water areas of southern Europe is, of course, a raw material more valuable than the skin of Salmonidae or other Cyprinidae.

In the case of fish collagen there is no such issue as collagen diseases attacking the tissue – enzymes of high peculiarity for collagen and in the collagen derived from Silver Carp were practically free of problems of decomposition by non-specific protease and the depolymerisation process of extracellular breaking of structures.

Collagen from Silver Carp also displays other glycosylation of hydroxylized residues.

The Biotechnological Plant in Zulawka also carries out research, by means of chromatographics, on changes taking place in the primary structure of collagen and on comparative examples of collagen from bovine calf skin used for skin implants and collagen from Silver Carp. A great difference in the level of UVC irradiation causing destruction of aromatic amino acids for both research materials was shown.

In the aforementioned facility, a team of researchers emphasized the exceptional “legibility” of superhelix chains observed in the collagen of Silver Carp, i.e. a clear separation of three chains of procollagen joining in the shape of three-strand rope, which is stabilized by producing a disulfide bonding. They play a more significant role than in human collagen, which is stabilized mostly by Van Der Waals bonding.

...a very high note for collagen derived in this way from fish skin was given by Prof. Anna Podhajska PhD, from the Biotechnology Department of Gdansk University and Prof. Henryk Szarmach PhD, from the Polish Dermatological Association.

In the cosmetics produced in Poland and having great market success, collagen derived from Silver Carp has begun to dominate as a raw material but its value seems to be growing the further south in Europe the fish is bred.

It is an important fact that penetration of the extracellular space of the skin by the hydrated collagen from the Silver Carp is at present indisputable. Objective evidence for this is in the measures of the hydroxyproline in the biopsy of particular tissues before and after application of collagen preparations on the epidermis.

Collagen derived from Silver Carp in the form of gel emulgate with elastin and an addition of distillate or osmotic water, a 1% - 2% admixture of lactic acid and 1% admixture of preservative (EUXYL 600) or (ethylomethyloprophyloparaben) has started a sensation in Poland (and slowly in Europe) as a dermatological cosmetic with a wide range of applications and a very effective anti-wrinkle preparation. These preparations are enthusiastically received on the Polish market, despite having no current advertising campaign.

I did not collect any well argued negative opinions.

I confirm all findings presented in the preliminary report.

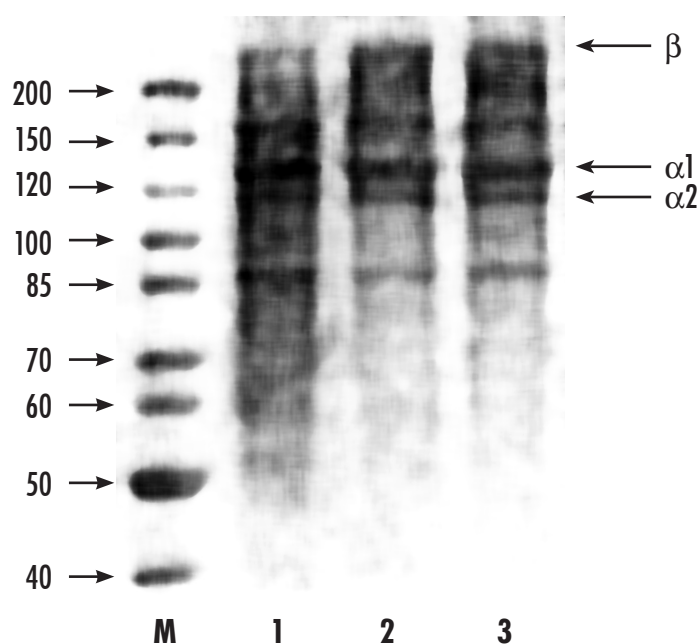
Among the other specialists who acquainted themselves with this subject – positive or highly positive opinions were given by: Michal Pelzer PhD, Artur Markowski PhD, Anna Niewieczeral PhD, Joanna Dobrzynska PhD, Wanda Brajczewska-Fischer PhD, Joanna Kolinek PhD, Halina Pawlak PhD, Henryk Krezel PhD and Prof. Danuta Kusewicz PhD. Portions of their opinions are attached as supplementary material.

# RESEARCH FROM THE CANADIAN-AMERICAN FIRM GLAVIN ASSOCIATES 02/23/2007

## POLISH FISH COLLAGEN PREPARATION NATURAL COLLAGEN Q 5-26

Preparation containing collagen extracted from fish skins is homogeneous, viscous gel, without mechanical impurities. It is transparent and not colored. Water content varies from 95.0 to 97.5% The protein content in dry weight is estimated to 96,2%. Total nitrogen is 14.3%. Content of hydroxyproline is 10.8% and percentage of elastin is up to 8,7%, pH of the preparation is 3.59. Dominating aminoacids are glycine (30,4%). proline (11,1%), alanine (11,0%), hydroxyproline (10,8%), hydroxylysine (6,1%), glutamine (6,1%), and arginine (4,6%). Apart of protein fraction there is a trace amounts of free amino acids, peptides, lipids, carbohydrates, amines, etc. which stabilizes triple-helical structure of collagen.

Fish collagen under investigation is essentially composed of  $\alpha 1$  (120 kDa) and  $\alpha 2$  (130 kDa) chains that are present in a ratio of approximately 2:1 and  $\beta$  (220 kDa) component (crosslinked dimer of  $\alpha$  chains). Percentage contribution was adequately. The collagen therefore is the main protein in the preparation with average of 85% of total proteins content. The remaining 15% of protein fraction are smaller fragments that appear under the  $\alpha 1$  and  $\alpha 2$  bands corresponding to molecular masses ranging from 70 - 150 kDa, including clearly shown elastin band (8%) and the rest unknown proteins (7%).



SDS-PAGE electropherogram pattern of protein from the gel under investigation.  
M – molecular weights protein marker (kDa)  
1 – extracted with glycolic acid  
2 – after one filtration  
3 – after many filtrations steps

The point of the denaturation is observed at 27°C. Denaturation point for pure fish collagen is up to 18°C. Obtained collagen in studied preparation possess higher temperature resistant than pure fish collagen. This most probably stabilized by non-collagenous compounds.



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## COMMENTS ON THE ANALYSIS OF THE CANADIAN ELECTROFEROGRAM

Glavin Associates tried to undertake some typical industrial espionage. On their orders two men – Mariusz Gromnicki PhD and Eng. Andrzej Trela “poked their noses” around the subject of fish collagen for several months. They managed to learn a lot, but not enough to steal the method for making the product.

It is said that that Glavin managed to extract collagen from the skin of Silver Carp but neither managed to transfer the solution into a spiral conformation nor to get close to the room temperatures in denaturation limits.

By curious coincidence we managed to carry out some counter-espionage (which proves only that MLM networks can take you virtually anywhere).

We have not heard from Trela, Gromnicki or Glavin for a year.

We publish these materials as a historical anecdote rather than, but also because, it is a kind of success.

## EXCERPT FROM THE BOOK COLLAGEN – THE ELIXIR OF BEAUTY AND HEALTH

S.A. BATIECZKA and T.A. ANDRYIUK

“...the basis of the intercellular matrix and so the connective tissue is collagen... securing mechanisms of elasticity and firmness of the organism is tissues. ...collagen differs from other proteins by the high content in its amino acids of: proline and hydroxyproline, which are conditions for its endurance.”

“...collagen is considered the most important substance in determining the youthful look of the skin: firmness, smoothness and elasticity.”

“...the primary alpha structure of the collagen chains is incredible; each third amino acid in the polypeptide chain is represented by glycine and approximately 1/4 of amino acid residue is proline or 4-hydroxyproline, approximately 11% is alanine... and an incredible amino acid - hydroxylysine”

“...the collagen fibres get swollen with each inflammatory state and then they are partially decomposed ... There is a close connection between the condition of the collagen in the organism and the majority of diseases ...”

“... an organism suffering from collagen deficiency starts to feed on its tissues... When the nitric balance is disturbed, decomposition of proteins exceeds their synthesis which accelerates the aging of the organism and brings about many premature diseases...”

“...clinical research carried out on an immense group of over 32 thousand of elderly subjects... had indicated that 62% of them... do not consume the necessary amount of protein for their natural demands.”

We would most willingly copy about three fourths of the book cited above for this text. Yet, we respect Dr. Siergiej Baticzka and his copyrights. This scientific dissertation, which in some places almost reads as fiction, can be purchased in COLWAY. We encourage you to buy it. Every Distributor of Natural Collagen should have it. In it you will find much more information on connective tissue, proteins and especially collagen. The chapters on the skin and its functions as well as hits and myths in cosmetology are excellent. There are a lot of clear and educational drawings. This is a very good handbook on the proteins of biochemistry and a great dietary manual.

There is another sensational chapter from this book whose action takes place off its pages. Doctor Baticzko had known for many years that stomach acids do not decompose the peptide alpha – chains of collagen in the digestive system. He was working in Ukraine, where his name recognition ensures that no one would dare to attack him, when he started enthusiastically recommending consumption .... of a cosmetic from Poland!

A year and a half after starting these experiments it turned out that Dr. Siergiej Baticzka was right. He was spectacularly successful in using Natural Collagen, applied both externally and internally, in the treatment of such diseases as:

- lumbalgia and intervertebral disc hernia
- degenerative joint disease
- rheumatoid arthritis
- periartthritis
- dermatitis, covering with blackheads
- cataracts
- macular degeneration in the retina
- diverticulosis of the intestine
- thrombophlebitis



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- bronchiectasia
  - emphysema
  - periostitis
  - cirrhosis
  - cystitis
  - stomach ulcers
  - duodenal ulcers
  - mastopathy
  - styne
  - angina

We have enumerated only those diseases in which inhibition or partial curing with collagen therapy is still some kind of novelty.

Dr. Baticzka also recommends collagen gel for problems which have been alleviated with the use of collagen for a very long time in Poland or in the Czech Republic. Here we refer to:

- burns
- varicose veins
- haemorrhoids
- psoriasis
- dermatosis
- striae
- alopecia areata
- paradontosis
- chafing, frost bite and bedsores
- herpes
- warts
- scars and keloids
- restoration of hair colour

...and many others.

If not you, then some of your relatives, friends or neighbours suffer from the above mentioned health problems. Nevertheless, when at the end of 2005, a doctor from Ukraine started to prescribe for them not only external skin application, but also the frequent swallowing, of a preparation registered in Poland as a cosmetic – even we found ourselves lacking in faith. It is hard to say now whether it was the collagen, or Dr. Baticzka, we didn't believe in. Collagen did not fail and Dr. Baticzka; a fellow at the Chernihiv Academy, a clinician in Kiev and Odessa, an intern in Germany, Switzerland, the USA, Japan and China, a lecturer in Great Britain, a member and authority of numerous organizations, prize-winner and doctor of medicine in two specializations – turned out to be great! He had not only knowledge, but also courage. Today the door is open. Swallowing fish collagen for curative purposes, not in gel but in lyophilizate form in capsules, will not surprise anyone. Why and how to do it? Read the book by Dr. Sergey Baticzka and Tatiana Andryiuk. It complements our text perfectly .

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## LYOPHILIZED COLLAGEN – BUSINESS PERSPECTIVES

Lyophilization, as we all know, is a water solution of collagen and relies, as a frozen hydrate, on the possibility of the highest density below 1040 F (400C) and the vacuum (approx. 1 Pa) necessary to start the sublimation of water. Further on, heat is provided in a precise and controlled manner, maintaining sublimation, while the water vapour produced, is removed by freezing.

This is a difficult and expensive process, carried out to a satisfying degree of quality in only two centres in Poland - yet its effects are astonishing.

Collagen transfigured in this manner to dry matter is characterised by its excellent solubility and high hygroscopicity.

Lyophilizate contains less than 2% water and almost 98% pure collagen! It may take the form of sheets, a powder or granulated mass. It is incredibly light, with a high caloric content, it may be stored for several dozen years without preservatives. Once provided a dry environment, it shall not lose its properties for half a century!

It is also possible to produce lyophilizate in synthesis with previously prepared solutions, e.g. herb extracts, plant extracts, fruit acids, vitamin "cocktails" etc.

Collagen lyophilizate offers various possibilities. It may be added to creams, masks, balms, beauty masks and collagen sheets. It gives sensational effects in the dressing of burns and haemorrhages (also in dentistry), it is outstanding in accelerating the effects of scarification after external injuries. It has an anti-inflammatory effect. As an absorbable suture and vascular prostheses it allows compresses of the highest quality to be produced. If it were not for the price of deriving it – you could easily imagine purchasing a lunch, packed in collagen wrapping, which you would then eat at great benefit to your organism.

Unfortunately, the price of fish collagen lyophilizate is still exorbitant. It reaches €160.00 per gram which, despite its considerable advantage, hinders competition with bovine proteins and their dry matter.

We should be aware however, that we have access to a semi-finished product unique in the world. We never know who we'll meet in life or what contracts we can conclude. There are thousands of factories in the world, whose people do not know that they need this product, because they do not know that it exists.

A tiny portion of lyophilizate, weighing very little, can provide a soldier, Himalayan climber or even an astronaut with a protein of full value for many days. Sportspeople (mostly in the muscle tissue building phase), hard-working people, convalescents, people allergic to animal proteins - they all constitute a potential market.

COLWAY has already undertaken some activities towards the practical use of dry collagen matter.

In spring 2007, the network will receive the precursors to nutricosmetic capsules, which will be the world's latest generation of diet supplement and protein nutrient all in one.

There are some products (let's roll off a few of the recent hits: Evelle, Imedeen and Innéov) which can storm the marketplace. They are the avant-garde of the wellness trend. And they cannot even compare with our preparation!

You will get a product with a striking portion of peptides, mainly absorbed through the intestines, which cannot be allergenic, and which replace deliciously necessary (but fattening) foods like pork knuckles, giblets, chicken skin and gelatine. Within a month after taking collagen capsules it thickens the skin, improves the structure of the connective tissue and stimulates not only fibroblasts but also chondrocytes.

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## How does it work?

- Well, chains of human collagen are built of 20 amino acids, both these produced by the organism itself and supplied from food. Our human collagen is a specific amino acid store and similar to other vertebrates, it decides whether the organism maintains it or lacks it.

Parts of these proteins are naturally decomposed by the stomach's acids, while some parts undergo hydroxylation or decompose into other acids.

Our protein is type I collagen. When consumed it is only partially absorbed from the alimentary canal. It is resistant to proteolytic enzymes and the low pH of gastric juices, therefore it is a revelation for peristalsis and indigestion disorders. One should remember that collagen is also a bone matrix. We can improve the condition of the skeleton primarily through internal supplementation. When consumed collagen is an excellent carrier of antiseptics. The doses of active substances included in the capsules have been maintained in rigorous frames specified by diet supplement engineers and specialists. Its overall effect embraces the face, the skin of the whole body and the connective tissue almost totally.. It stimulates hair growth tissue, where the exfoliation of collagen as a result of oral application can be observed the most clearly.

We don't often come across foods supplying our organism with, for example, monomers of glyconjugates (particles building tissues). Crab soup, stewed mushrooms, pork knuckles and dessert gelatine are their typical sources. Most importantly they contain easily absorbed hydroxyamino acids and amino saccharides.

No diet supplements possessing these such properties exist as of yet. You will be getting the first ones. The ability of the skin to retain water after systematic internal supplementation with collagen is growing in an incredible way. Some enthusiasts even talk about a periodic doubling of this ability as well as about increased density and elasticity.

We will say and write a lot about collagen in capsules. This area is developing.

If you want to know more how collagen works from the inside – we advise ordering Dr. Sergeiej Baticzka's book without delay. Because, when it comes to this field - this Ukrainian researcher is a man ahead of his time.

***The Editors***

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## CONCLUSION

Collagen is a subject which can turn into a passion.

People who dig deeper into this subject, very often get a feeling of enchantment similar to that of peering up into the night sky at the mass of stars, and realizing how immense the earth is, and how tiny and insignificant we are as humans.

Well, the microcosm is equally fascinating. The only life in the Universe known to us is based on the protein particle... created by a chain of amino acids.

Have you ever considered how fragile life really is then - in a biochemical sense?

How little it takes to stop it in one moment?

Well a trifle would be enough. For example by raising the temperature of our body by only 420F (5.5 0C). Then the bonding stabilizing our protein helices breaks down and the whole complex structure is destroyed.

I heartily encourage everyone fascinated with collagen as a cosmo pharmaceutical, a medicine and a money maker... to expand their knowledge on it.

It will help you to get some distance on the phenomenon of life and to hold Mother Nature in respect. It will broaden your horizons and help you better understand your own organism, and for sure, facilitate your making money in our business.

*Jaroslav Zych*

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