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NEW SKIN

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NEW SKIN

NOVI SAD, 2000

About the book:

NEW SKIN is a monograph that is a result of many years work, personal experience, clinical observations made by the author, as well as systematic follow up of all the available literature, and scientific accomplishments of the modern medicine.

Mr. Belic, MD and co-authors have taken this problem seriously, and made all the potential readers familiar with all the necessary facts competently, expertly, and with great deal of knowledge and responsibility.

Some of the most distinguished researchers in their fields, have taken part in this study and published their knowledge, opinions and their viewpoints.

Authors tried to express their personal experiences and compare them with results obtained and published in contemporary literature.

Miroslava Dokmanović-Đorđević, PhD

In his work, a surgeon opens, dissects up to the tiniest detail, repairs, moves, removes and finally closes. In this entirely special study authors went even further. Somewhere in the text authors say: "we tried to reach the greatest depths…". They go up to the very cell itself. That far, but it is still not the end. They also reveal certain physiologic processes that occur at the level of cellular organels. From that level they are constructing a ladder going toward the top where the NEW SKIN can be found.

Surgeon patiently combined atoms and molecules of oxygen with many others. Transforming energy from one form to another he found something new.

The exactness of electron microscopy is an invisible world, which we were able to come to know through photography and which electron microscopy made clear and natural to us. Biochemical and physiologic occurrences helped us to connect well-known facts in an unbreakable chain. At the end of this chain is NEW SKIN.

He managed in a very subtle way, not to be boring while expressing many scientific detailes. At the same time he enabled everybody who would be interested, to repeat the whole process. Financial situation could not be avoided even in this study, but it was a kind of impulse making him work harder.

Authors were up-to-date throughout the paper. Sometimes they were even a step in front of their time. They incorporated the marketing study in health and health management.

The detailed analysis and clearness of conclusions point to a high degree of expertise. Using a multidisciplinary approach for a subject that was kept a side for such a long time made it look even more interesting and more luminous, special and clear in it's uniqueness.

Dr sci. Sava Perović, PhD

CONTENTS:

PREFACE 1

My first encounter with amniotic membranes was at The Clinical Center in Zemun.

Back than, I was making first steps in, than new medical branch, known as Hyperbaric Medicine. That is the field in which I have done my thesis.

Amniotic membranes were then considered, as it is known in medical jargon as "incidental finding". Some would call it faith; some would call it coincidence. I consider it as both, since it has become like an infection for me. It penetrates deep, very deep in reflections and leaves an indelible scar. Maybe that is why, later, a form of our treatment was a struggle against scars, but on the skin.

Many ideas born during the gynecologic practice are now materialized. I could see them (amniotic membranes), even touch them.

"Incidental finding" had to be put to a sidetrack, but it was never forgotten.

My thesis is defended long ago.

By the turn of events, that have only one, real director, whose name is life, the sidetrack ceased being it. Many trains will pass on it: nice and ugly, pleasant and unpleasant, and all of them have found their way to this book.

As I have wrote in my thesis, I repeat here that I owe an immense gratitude to my teacher – Deklev, MD. The famous "Uncle Nikoja", for introducing me to this field of medicine. He showed me many dark places of this field that, he thought, were worth exploring.

One of the authors

PREFACE 2

A team, that worked very hard, on problems regarding this field, is made from people specializing in different medical branches. Most of us are doctorate holders in Medical Sciences. Some of us have two specialties.

We must say, without the unnecessary modesty, that we are the most distinguished experts each in its field of work and interest.

In order to have the same level of expertise in our book it is logical that we had to seek help from others, qualified as well. Some of them were, and still are, true geniuses.

Now, we express gratitude for all the time spent with us. For their patients, all the energy invested; physical, mental, electric, and any other. For the use of the equipment, and particularly for constructing devices especially for us.

We apologize if we bothered them.

We thank anyone who gave us any kind of support. Finally we thank all those who underestimated us and who disturbed us. They gave us the most powerful stimulus to persevere.

All the authors

INTRODUCTION

The end of fertilization represents the beginning of life. Many think that from biological as well as philosophical point of view, life is not yet fully defined.

On the other hand, definition of fertilization is clear: "Fertilization represents union of male and female gamete". In human population this union occurs in the ampullar part of the Fallopian tube.

Why there are so many spermatocytes and only one will fertilize the ovum?

We waited quite a long time to get the answer. Modern molecular biology and biochemistry gave us, as it is considered, the right answer, based on experimental findings beyond any doubt.

Spermatocytes move, without having anything or anyone "pulling" or "leading" them towards the ovum. The "rendez-vous" is by pure chance, as many things are, indeed. In science it often happened that a right path and a right direction are determined by the very same chance. Measuring the size of the ovum, at the level of angstrem, it is observed that an ovum is very big, and regarding spermatocytes, which are small, their number is more than considerable. The nature provided a chance with some help, in form of their number. That is why fertilization happens. That is why life exists.

We may say with certainty, that great many chemical factors, referred to as – **mediators**, represent an important ground favoring the fertilization. A number of studies were devoted to the determination of the substances necessary in this, relatively short, but immensely important encounter. On one hand we have **fertilizin**. Chemically it is between mucopolysaccharides and glycoproteins, with molecular weight of 10000. It is proved that it is present in greatest number on the surface of spermatocytes. Just before fertilization there is a reaction **fertilizin – antifertilizin**, with a purpose of momentary reduction in the number of spermatocytes, around the ovum, and facilitating union of an ovum and a spermatocyte, i.e. an entrance of only one male gamete in the ovum. Simple penetration of the spermatocyte in the ovum does not represent fertilization. The one that has succeeded in penetration, and has all the necessary qualities, undergoes further changes that precede fertilization. The first one is known as -

capacitation, and comprises biological and biochemical preparation of male gamete for fertilization.

Biological preparation understands preservation of all the sexual qualities that are genetically determined. Biochemical changes are structurally more evident. While preparing for fertilization the spermatocyte loses a glycoprotein part of its membrane. After this, all conditions, necessary for an **acrosomic reaction**, are met. All following changes have a common purpose of enabling the penetration of spermatocyte in the ovum. Acrosome contains several enzymes that help the penetration:

1. Hyaluronidase
2. Two enzymes very similar to Trypsin
3. Lyzin

In dissolving the **zona pellucida**, most important is lyzin. Hyaluronidase and mucous membrane of the Fallopian tube enable the spermatocyte to penetrate the barrier that a corona radiata represents. Modern technology, especially some of it's branches (electron microscope and newer biochemical procedures) provided the authors (1), (9) with some data that before, one could only fantasize about, and which point to the adrenalin and noradrenalin as most important in activating and directing the acrosomal reaction. Advantages of those procedures provide us with even more data and more precise details. We are now able to comprehend the continuity and chronology in the process of fertilization (12). Three events are clearly demarcated, mainly in regard of the timing, although still related. Only as such they represent an entirety necessary for successful fertilization and development of a new life. At the same time, with respect to the chronology, we must insist on the anatomical and physiological changes characteristic for every phase.

First phase:

The penetration of spermatocyte through corona radiata

Second phase:

Penetration of the spermatocyte through the zona pellucida

Third phase:

Union of the membranes of the spermatocyte and the ovum

The beginning of this phase is designated by deliberation of hyaluronidase. It happens in the acrosome. After deliberation this enzyme starts to demonstrate it's main effect. It is shown in active, microscopically clearly evident separation of the cells of the corona

radiata. Hyaluronidase is very effective in destroying the preexisting compactness. In that way one of the hurdles is overcome, and a passage is made for the spermatocyte. It is a step forward, but the journey is by no means over. In chain of related events, there is another barrier, whose removal, by the absence of a single factor, may be stopped. Some studies of this subject (11) point that, in the process of cell separation of the corona radiata, enzymes, secreted by the mucous membrane of the Fallopian tube help hyaluronidase. This separation and the passage of the spermatocyte, is only one third of the required path. An assignment, set by nature, continues, and enters a second phase.

Penetration of the spermatocyte through the zona pellucida.

This zone represents another hurdle on the path toward fertilization. It is neither less, nor more important than the others, but it has it's specificity. It is considered that if a spermatocyte penetrates this zone it is inside the ovum. Detailed biochemical analysis at this level (3), point to a protein whose role is to represent a specific receptor for a spermatocyte. The process of penetration through zona pellucida is associated with certain biochemical reactions, again enabling the fertilization to occur. Proceeding of these reactions is chronologically precisely determined. They are directed by an adequate number of catalysts. The importance of certain chemicals in this process was long discussed over. However, newer studies (1) show that one is, nevertheless, more important than the others. That is **acrosin**. When all the conditions are met, the penetration of the spermatocyte is relatively short. At the same time, on the outer surface of the membrane, changes occur that go in the opposite direction. All available mechanisms, that the spermatocyte has, are activated to prevent any other spermatocyte from penetrating the ovum. Mercilessness and punctuality of the process is confirmed by the fact that only one spermatocyte can fertilize the ovum. On the other hand it is a confirmation of a theory that mobility, as great as it is, by itself is not enough without the chemical mediators. But what happens if two spermatocytes fertilize an ovum? That is something very rare but not unknown of. There are so many precautions either physical or chemical, but it can still happen. However, nature has its answer even in this case. Fertilization by two spermatocytes or more, leads to such a chromosomal combination that is incompatible with the survival of the embryo, and in exceptional cases when a birth occurs such a newborn is condemn to death in a

matter of hours. That is why we may say that a normal fertilization is one of the prerequisites for life.

Union of the membranes of the ovum and the spermatocyte.

After the acrosomal reaction, only the inner acrosomal membrane covers spermatocyte, at some time. In such a situation the union of membranes occurs. This process may seem, somewhat, slow, but from physiologic point of view it is striking. **For the first time, throughout the process of fertilization, two parts, two cells, unite and make a new quality.**

In the part lying just behind the acrosome, cytoplasmic membrane of the spermatocyte makes a contact with microvillous processes of the ovum.

In human population, after a penetration of the spermatocytes following reactions occur in the ovum:

1. Cortical reaction
2. Zonal reaction
3. Continuation of the second meiotic division
4. Metabolic activation of the ovum

Everything that follows has an only purpose of preventing any other spermatocyte from penetrating into the ovum. How this is important is evident when we consider the number of spermatocytes and their mobility.

Cortical reaction

Cortical part of the ovum secretes special granules, which make the membrane of the ovum impenetrable for any other spermatocyte.

Zonal reaction

It has been mentioned that some parts of the cellular membrane of the ovum contain specific receptors for spermatocytes. This reaction removes those receptors in their entirety.

Continuation of the second meiotic division

Continuation and completing of the second meiotic division results in definite mature ovum. One of its most important characteristics is a haploid number of chromosomes.

Metabolic activation of the ovum

From modern point of view, it is considered that a spermatocyte carries a factor, that is an initiator of the reactions in the ovum (3). First thing that happens is an increase in oxidative reactions. **Micro-measurements show that oxygen consumption increases by six**

hundred times! Isn't it impressive? It is very difficult to be indifferent. From now on, oxygen consideration does not stop. "Vaccination" is just a beginning but immunity is permanent.

Changes on the electrolyte level are also important. That is a concentration of certain electrolytes, in the ovum, as well as in its surroundings, changes. These changes influence the shape of the ovum. It's volume decreases and it shrinks. Most important are changes in potassium ion concentration inside the ovum, and calcium ion concentration around the ovum. An increase in the number of ribosomes implies an increase in the protein synthesis. All this looks like a real, complex, fight to make a new life, which lasts till the end.

Following the proverb – Si quid agis confice – we continue in the same direction.

Chronology of the events after the union of male and female gamete.

Upon union of the two gametes, in an adequate environment of healthy and fully developed female body, a beginning of a new life starts. New life has been created. This new organism will carry, in its further growth and development, the characteristics of both gametes it has been formed from. One of those characteristics is a **diploid number of chromosomes.**

The next step in the development is known as - mitotic division.

Its characteristics are formation of two cells, than four, and so on, till a new organism is formed. The first cells that are formed are called **blastomeres**. With continual divisions, growing number of cells group into formation that looks like a mulberry fruit, and because of it, it is called **morula** (Fig. 1). From the moment of fertilization, through new cell formation, and their grouping to form morula, it takes, in human population, three days. Morula is now, as a form of a new life, in its initial position, at the entrance into uterus. From this phase divisions continue. Further increase in the number of the cells, leads to regrouping, position changing, and as will later be more evident certain separations. One group of cells will position themselves more toward the inside, forming the **inner cell mass**. The other group of cell will position themselves more toward the outside, forming the **outer cell mass.** Newly formed and regrouped cells are still surrounded by a layer of zona pellucida. Inner cell mass will form the embryo. Outer cell mass will at first form – **trophoblast,** and with further development it will make part of the placenta. Newer procedures in investigation of the

changes after fertilization enlightened an interesting phenomenon (7). With every following division, cells tend to be smaller, so that morula with further divisions does not change its size. But is does not stay still. It travels on. It enters the uterus. Once in uterus it starts changing again. Most prominent changes occur on zona pellucida, resulting in loosening of its cells, allowing the surrounding fluid to enter. Fluid influx causes a cavity formation inside morula, which is soon filled with it. **Blastocyst** is formed. Cells in the inner cell mass regroup and create a formative mass, that will be, by the pressure exerted by the fluid, propelled to the embryonic pole of the blastocyst. A new structure is thereby formed, characterized by the cavity filled with fluid, and a group of cells at its margin. It is called **blastula**.

To differentiate it further some authors prefer to call it at this stage **early blastula** (2) (Fig. 2). At this stadium blastula is bounded by a number of cells with diploid number of chromosomes, called **trophoblast**. Another characteristic of early blastula is disappearance of zona pellucida. Its disappearance makes more favorable ground for action of enzymes secreted by cells of the trophoblast. For the following reaction, endometrial cells will help these enzymes. If all the conditions are physiologic, around the sixth day after fertilization, cells of the trophoblast start engulfing the uterine mucosa. At this stage the blastula is referred to as **late blastocela** (Fig. 3). This very important step in the survival and development of the zygote is known as – **nidation.** Analysis of the available data shows that up to thirty percent of the fertilized ova do not survive beyond this stage. In such cases, all is over in the first week, with a sort of abortion, which is macroscopically unnoticed. Causes of this abortion may vary: from an inappropriate penetration of spermatocyte to the inadequate implantation. In the second week, thanks to the invasiveness of trophoblastic cells, a more intimate relation develops between mother and her embryo. From the moment when a blastula enters and gradually becomes part of the endometrium, trophoblast starts differentiating in two layers: an outer one, called **syncytial trophoblast,** and an inner one called **cytotrophoblast.** In the future, syncytial trophoblast will be responsible for the nutrition of the embryo. At the same time, at the embryonic pole, a cavity starts to form between the formative mass and trophoblast. It is to become an **amniotic space**, bounded by precursors of **amniotic cells**. This is our first official encounter with these cells. This is where our delight begins. An occupation begins, that is to

become a preoccupation, and is; also, about to mark a life path that may last even beyond the life itself. Someone will continue to work on it, maybe many will? "Preoccupation" is a right word, since it implies that "something", never to be abandoned, has been reached, and it is going to make us experiment, treat, record, write and think. This may sound like Raskoljnikov...but never mind. As for the end of the story, I know not much about it. I think many ends it will have to endure.

With further development, formative mass will differentiate in two layers. One, made of high columnar cells will form **epiblast**. Somewhat lower in position, layer made of cuboidal cells, will form **hypoblast**. These two layers, although made of different cells, make a **germ disc**. With further cell differentiation, further implantation of the blastula, deeper into the endometrium, occurs as well. Around the tenth day after fertilization, a defect in the endometrium, made by the blastula implantating, becomes covered with fibrous tissue. Inside the syncytial trophoblast, at the embryonic pole, **lacunes** appear. They are soon filled with maternal blood. Blood comes from the invaded endometrial capillaries, and will serve as a new source of nourishment. The exchange is, mostly, done by diffusion. Due to their importance, regarding nutrition, lacunes promptly enlarge. By observing this stadium, one can see that it is dominated by lacunes and is consecutively referred to as lacunar stage (Fig. 4).

L – conspicuous increase in number of lacunes
AC – for its increase, amniotic space is now considered as **amniotic cavity**
YS – the first appearance of the yolk sac

We may conclude, by chronological observation that the earliest period of life is filled with very important changes. Several new characteristics develop every half a day. From what we now, it is enough to lose just forty-eight hours of development to be faced with a huge, insurmountable gap. New layers and new cavities are being formed; all of them representing a precursor of something that is bound to develop some time or another.

A global picture gives us a following situation: development of fertilized ovum is very intensive. After nidation it is under the endometrium and covered by a blood coagulum, and fibrous tissue. Intensity of this process is reflected in the promptness of the forming cover over the implanted blastocyst with newly formed and clearly differentiated epithelium, as soon as on the eleventh day. At the same time, changes at the embryonic pole continue. Lacunes are enlarging. It

is positive, but less important then their union, resulting in a substantial increase in volume. (Fig. 5).

EL – enlarged lacunes with substantially greater volumes
AC – amniotic cavity
YS – yolk sac

The growing lacunar spaces become confluent and form an initial - **intervillous space.** Endometrial capillaries enlarge and are engorged with blood, and become sinusoidal in shape. At this stage, cells of the syncytial trophoblast, once again, demonstrate their aggressive nature, this time towards the capillaries, which are, as we already mentioned, enlarged and sinusoidal. By violating the integrity of their walls, maternal blood may fill the lacunar spaces. There is now, for the first time a possibility for development of placental circulation. Regarding the germ disc, some new cells begin to appear. Results of microscopic studies imply that these cells, most closely, resemble mesenchymal cells. Intensity of the cell development, as well as the appearance of polymorphism, continue. Diversity of the cells, as well as new formations, becomes more and more prominent.

Chronologically, we are in the second week. Cell divisions continue, as well as cell regrouping into tissues, and than eventually organs. Embryo lives and develops.

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ELECTRON MICROSCOPY AND HISTOPHYSIOLOGY

In our desire to register and follow the very inception of the amniotic cells, we started from the very beginning, indeed. From fertilization. Fertilization itself has been registered through the milieu of the changes enabling it. We have already pointed out that a single missing link will make the whole process stop, and end in a most unfavorable way. We mentioned the first appearances of any new structure. Following the chronology of events, we've come to the second gestational week. It seems logical to continue in the same way. That is exactly what we did, but now our attention was focused to a specific group of cells: amniotic cells.

This part of our study was characterized by the time it consumed. We often felt as if the whole eternity has passed, and we are still unable to find the missing link. There were times when everything came easily to us, but it was rare, very rare. That's why we called this part of the study third phase. We simply couldn't get an aborted embryo of a specific gestational age. Following them "grow up" (amniotic cells), we wanted to get to now them. To see their "back" as well. We believed that the understanding of their architecture and "visible" physiologic changes would help us explain the reactions that will be later discussed in more detail. We tried to get to the very "heart" of those tiny and tender amniotic membranes. A fact that, maybe, we will have to face parts of these cells, organelles, did not scare us. On the contrary, it was an even greater stimulus for us. To be able to do this, we had to turn to the **electron microscopy.**

A light microscope was discovered at the end of the 16th century. The first to use two lenses was Zacharias Jannsen. A Dutchman, Lewenhoch Antun (1632-1723), constructed the best simple microscope in a series of 500 pieces. Giovanni Faber named it in 1625 (micros – small; skopeo – observe).

Ernest Ruska made the first version of an electron microscope in 1931, as a student graduate assignment, and in 1932 with his professor M. Knul he made an electron microscope with a magnification of 17x. At the same time, by the same constructors, a first scanning microscope with a magnification of 10x was made.

Understanding of electron's behavior at the speed close to the speed of light ($3x10^8$ m/s) it is possible to determine the wavelength, by the Broly's equation:

$$\lambda=\frac{h}{mv}$$

h – Plank's constant; m – electron mass; v – electron speed

As electron has negative charge, it is accelerated in the electric field, where the wavelength depends on the positive voltage U:

$$\lambda=\sqrt{\frac{150}{U}}$$

Electron deviation is done in the electromagnetic field.

That means that compared to the light microscope, electron microscope has, instead of light, a beam of electrons, and instead of lenses made of glass, magnetic lenses (electromagnetic field).

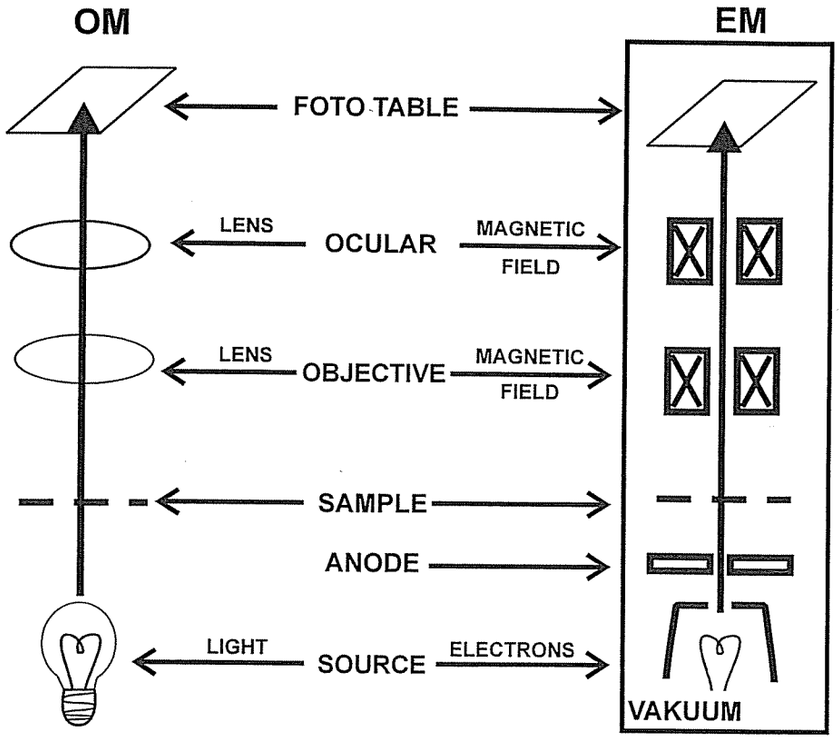
Besides the magnification, very important is a power of resolution d. It is defined as the smallest distance between two points (or two lines) that can still be differentiated as two. The power of resolution can be calculated according to the following formula:

$$d=\frac{0,61\lambda}{n\sin\alpha}$$ for a light microscope, and

$$d=\frac{\lambda}{\sin\alpha}$$ for an electron microscope

where n – is an index of refraction of the lenses, and angle at which you see is d.

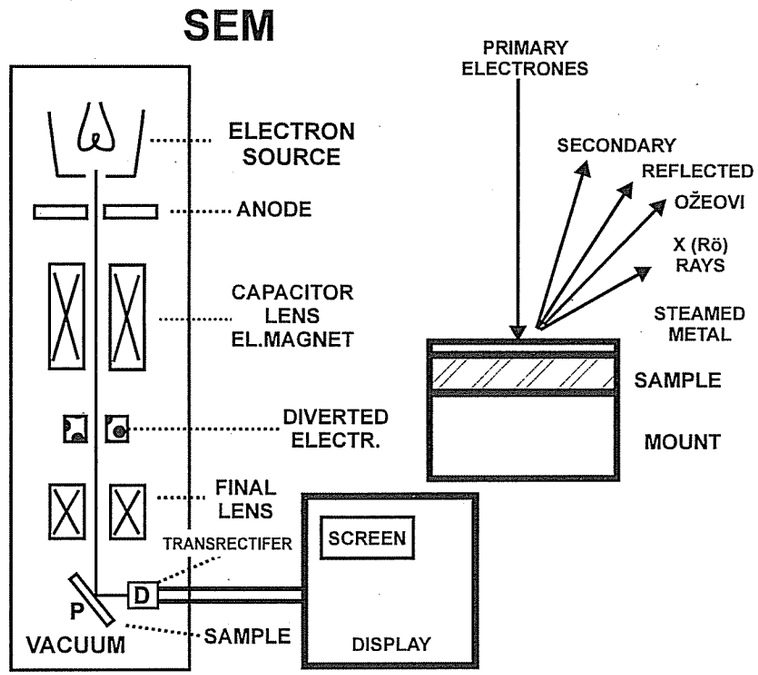
THE SCECH OF OPTIC AND ELECTRONIC MICROSCOPE.



SCANNING ELECTRON MICROSCOPE

Scanning electron microscope (SEM) forms a picture at the screen of a cathode tube, which receives a signal from the sample, scanned by the narrow electron beam. The position of this beam is synchronized with an electronic beam on the cathode tube. In such a way one gets point of the sample identical with a point on the screen. Variations in the intensity of the signal are used to form intensity of the beam that is responsible for the formation of the image on the screen.

Electron beam (primary electrons PE) reaches the sample, from which secondary electrons (SE) are expelled, and then pulled towards detector bars (positively charged). Photoelectrons are multiplied in a detector, and a signal made in such a way is introduced in the cathode tube.



In a crash of a PE beam and a solid sample, that occurs in the vacuum, one gets different sorts of electrons and electro-magnetic radiation: reflected electrons (RE), secondary electrons (SE), Oge electrons, continual x – rays and characteristic x – rays. In order to get more secondary electrons, biologic sample is steamed in vacuum with atoms of gold (Au), platinum (Pt) or palladium (Pd).

A sample is first fixated in glutaraldehyde (2,5% in a phosphate buffer 0,1mol.) for 30 minutes, flushed with a buffer, and than fixed in 1% osmium-tetroxide (OsO_4) for another 30 minutes, and again flushed with a buffer. Dehydration is done by ethyl-alcohol solutions in water of increasing concentration: 10%, 30%, 70%, 96% and 100% for 20

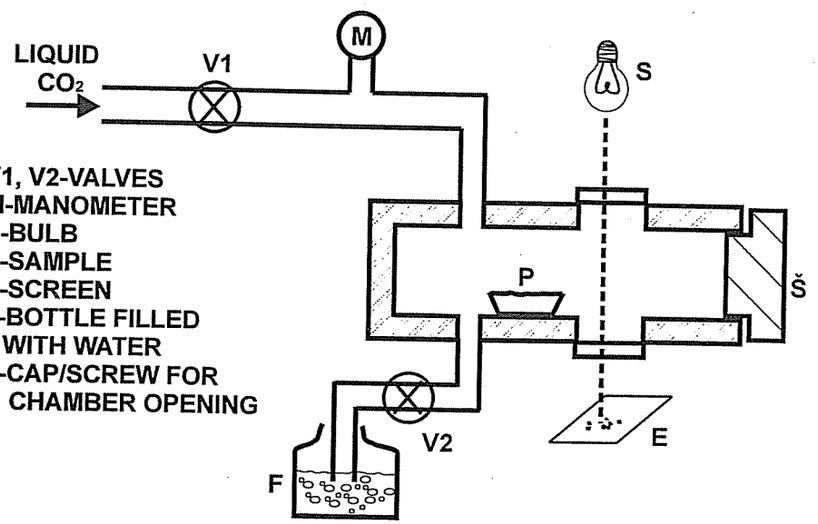
minutes in each. Then, it is put into acetone and ethyl alcohol solution of, again, increasing concentration: 30%, 50% and 100%.

Drying is done at the critical point of CO_2 according to Anderson.

An acetone dish, with a sample, is put in a chamber and tightly closed. Liquid carbon dioxide is slowly introduced in the chamber (by opening the valve (V1)). While passing from the steal container into a chamber with a manometer (M) a pressure of approximately sixty atmospheres is registered. After this, the chamber is heated, and a pressure is increased up to 105 atmospheres. A liquid stage of carbon dioxide is lost in qa process of heating, and transformed into a gas. This gas is than removed from the chamber, by simple opening of the valve (V2). The exit of the gas is registered by the appearance of bubbles in a bottle. To get a sample, a cap should be opened (C).

An obtained sample is than metalized. It is done in a special steamer, in which a sample is unceasingly rotating. To metalize a sample it is possible to use platinum or gold, although certain alloys of these metals can be used as well.

The sheme of a chabmer used
to dry a sample for SEM over the critical point.



After detailed analysis of a certain material, we have decided to divide a whole process and present it in several, not many, stages, still following the chronology as we did before. The stages are following:

First trimester:

Every beggining is difficult. We are convinced that for the first time appears something that should be registered, and later on recognized as present or absent. When it comes to terminology, we tried not to stick out, but to be in accordance with what was generally accepted

Second trimester:

A golden mean always stays golden. The experience is now greater, so any new moment does not present a problem.

Third trimester:

For us the most important was the end of this trimester. We found it easier in that way to establish continuity, and underline certain parts of a following phase.

Results of the amniotic membrane analysis after the delivery:

We don't know if we may say that this part is the most important one, but we have to emphasize its importance. We had to determine exactly what we were to "conserve". Whether obtained membranes were worth processing by the mentioned technology and would justify enabling them with a "second life".

First trimester: (Fig. 6, 7, 8)

Magnification: 1000, 2000, 5000

Amniotic cells are uneven and irregular (Fig. 6). Some are more oval, some are polygonal. It is due to their immaturity. It is confirmed by following characteristics: their boundaries are not clear, intercellular spaces as well (Fig. 7). At this stage of cellular differentiation, the nuclei are most prominent. They are swollen, big, and filled with chromatin material. These facts point to the phase of cellular maturation. Some of the nuclei occupy most of the cell. Microvillous processes are present. They are registered as uneven, whitish bands. They are unevenly arranged over cellular surface, as if it was done

randomly. Their number is greatest at the side of the cell where a nucleus makes a convexity (Fig. 8). With a magnification of five thousand times, inter-cellular junctions may be seen in intercellular spaces. They are darker than the surrounding area, and are clearly demarcated. In their proximity, the number of microvillous processes is lower. (Arrows)

Second trimester: (Fig. 9, 10)

Magnification: 2000 and 5000

Amniotic cells are more clearly demarcated, and intercellular spaces are more differentiated, forming shallow creases (Fig. 9). Cells of polygonal shape are dominant. (Fig. 11) Intercellular channels are now clearly visible. They are usually positioned at the cellular angles. Their configuration and function are, according to our study, at their best around the eighteenth gestational week. Secretory granules appear, at this stage of pregnancy. They are round, white, clearly demarcated, and filled with secretory material. They are found close to the cellular membrane, and are connected with microvillous processes. Such an appearance of secretory granules points to the very prominent secretory activity of amniotic cells.

Microvillous processes now gather around the channels, going through the intercellular spaces, surrounding them and giving them the appearance of craters, of different depths. Openness of these channels suggests a good absorptive function of amniotic cells.

Third trimester:

Magnification 2000 and 5000 (Fig. 11, 12)

As the pregnancy comes to an end, amniotic cells, in their elementary appearance, undergo further changes. The base narrows, they elongate and become narrower and higher, with a shape similar to the parallelogram (13). The presence of intercellular channels is now permanent. What is new, is that some of them coalesce and make bigger openings. (Fig. 12) They are seen as black, irregular areas. In some parts they can occupy the entire width of an intercellular space. (Fig. 12) Villous processes are also present. Compared to the earlier stages, their arrangement is far more regular. (Fig. 12) Secretory granules are not missing, either. Everything we mentioned so far points to a substantial secretory activity. Following the logic, we may conclude, due to an increase in intercellular channels, that the absorptive activity is improved as well.

Results of the analysis of amniotic membranes after delivery: Magnification 2000 and 5000 (Fig. 13, 14)

A net of microvillous processes is evident on the cellular surface. Secretory granules are also present with all their characteristics: round shaped, clearly demarcated and filled with secretory material. (Fig. 13) Some of them are smaller, suggesting that they are still developing. Intercellular channels are present. Some of them are combined, with a gaping appearance. (Fig. 13) All these facts suggest that we have normal amniotic cells, with all the characteristics of their maturity and appropriate function. We had every reason to be content. Maybe more than that, since we proved the existence of something very important, although we could not explain why was it so.

It is very important for the amniotic cells to keep all their characteristics regarding histomorphologic structure and physiologic functions. In that way, every condition is made for us to enable them a "second" life in which they will keep all the above mentioned characteristics.

What we did not know then was going to reappear now in form of different questions, with a same meaning. How come the amniotic cells were still active and alive, even after fulfilling their role in a creation of the most beautiful thing on this planet, that is life? Were they predetermined to play yet another role, provided they are given the possibility? We have seen that for fertilization, and everything that follows, an entire chain of events is necessary. Maybe we will not find the right answer. That is why we would do everything in our power to discover and direct any possibility that might lead to their "second" life, about which was so little known.

At the end, to see what was this architecture based on; we damaged the amniotic membranes. This disruption enabled us to cast more light on those tiny, tender amniotic membranes. These disruptions led us to a gibro-elastic layer. (Fig. 15-16) It is represented as whitish, thinner or thicker cylindrical fibers. Some were continuous, some discontinuous. (Fig. 16) Their remarkable strength, and resistance to trauma, speaks of their content and name – fibro-elastic fibers.

Magnification 5000 and 10000 (Fig. 15, 16).

ABOUT AMNIOTIC FLUID

The first interest in this field appeared during the medical studies. To be precise, at the time when we were at the Gynecology Clinic. At that time we were in contact with deliveries day and night. For two weeks we would sleep at the Clinic, eat there, but most of the time we would spend on the delivery ward. We would observe, help, even do something ourselves, but most importantly we learned a great deal. Taking part in a delivery of twins was a pleasure and an honor. Placenta, newborn babies, amniotic fluid and the whole atmosphere of the delivery ward, seemed to fascinate every one of us. Surgery, which than was pretty mystic, and many would probably like it, but only few would be fascinated, due to a lack of contact with it. Only few of us had the honor to really participate in any of the operations.

Back then, we read about the amniotic fluid. It helped us to expand our knowledge, but there was something about it that did not leave us at ease. So many deliveries, and all the newborns without any sign of infection. Attention was more and more pointed towards amniotic fluid. How was it produced? What was its role? How would it provide such a protection? There were many questions. Answers were different. Some were insufficient, some, even from the literature, suspicious. By trying to find the answers it was inevitable to get to the amniotic part of the placenta.

Some parts of this study, especially the one in which we studied the architecture of these cells by electron microscope, confirmed the theory that amniotic cells are responsible for the production of the amniotic fluid. Amniotic fluid in this study was, we may say, only mentioned. There is another chapter in which it is given the due attention.

Amniotic fluid is usually of a pale yellow color (the color changes during the pregnancy), specific weight of 1005 to 1000. Biochemicaly it is most like diluted plasma. Logically, its major constituent is water, up to 98%. It is found to contain all-important minerals, as well as organic substances (1). At some time it was believed that it has a constant quality and that its only purpose is mechanical protection of the embryo. Later it was proved to be quite the opposite. It has been determined that it was not a passive product of the pregnancy, but a very dynamic, circulating fluid, full of metabolic

changes. It is now a well-known fact that kidneys of the embryo at some stage have an active role in excretion of some metabolic products and that urine is excreted via amniotic fluid. Urea, uric acid and creatinin have the same concentration in amniotic fluid as in plasma, up to the twentieth gestational week, and later their concentration increases compared to that in plasma (4). It is proved that the embryo swallows amniotic fluid and then part of it excretes through the kidney (3). Thanks to the cell membrane structure, water exchange is done by diffusion. As of organic substances, amniotic fluid contains most of the protein fractions, prostaglandins, hormones, enzymes and glucose.

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ABOUT OXYGEN, OTHER GASES AND FLUIDS

Every story about a gas starts somewhat like this: "Oxygen is a gas without color..." Oxygen is always at the first place in our study. It is the very essence of our journey, and our search for answers. Thus, later we will say: "Following the oxygen..." Using what we knew, and what others, who knew more on the subject, advised, we believe we have at least added a single stone to the pyramid of understanding something so incomprehensive to us before. Many worked on this pyramid even more then we did. It would be pretentious to state that we said everything, since here, as in construction business, with time more and more of a new material will be added.

At the beginning we remembered some of our school days. Back then, we were thought what oxygen is, who discovered it, and how important it is for life. It can all be said in a sentence: if there are roles assigned in a cell, oxygen, undoubtedly, has a leading role.

Oxygen is the constituent of air, which contains other gases as well, that we did not omit without mentioning their most important characteristics. Air, as a gas, and especially some of its constituents are permanently connected with the blood. It is a fluid with which gases come in close contact, therefore requiring a further explanation. Air is everywhere around us. We breathe it, for the organism to extract oxygen from it, transport it by blood, to every cell, and transform it into carbon dioxide that is removed from the cell.

From the time of ancient Greece there was certain knowledge about the air. Even then was known that the air was a material substance. It was known that it couldn't be felt by touching, but that it could be felt as wind, by breathing.

Later, Galileo discovered that air has its weight. His student, Toricceli, as it often happens went even further. He performed a famous experiment, we all now have to learn: he filled a tube, made of glass, 1 meter long and closed at one end, with mercury. He than closed it at the other end as well, and in a vertical position submerged it into a container filled with mercury. Then he opened the lower end of the tube. Mercury started to flow out, but only until the mercury in the tube was 760 mm above the level of the mercury in the container. Mercury

could not flow out any further because of the atmospheric pressure exerted on the mercury in the container. This way, the atmospheric pressure was proved. It is the pressure that air exerts on the earth. The space in the tube, that appeared after the flow out of mercury is known as the "Toricceli's vacuum" It was the first example of vacuum, made by a man.

Forces that act between the molecules of gases are not too strong, enabling them greater mobility compared to the fluid and solid substances. It is one of the reasons why the pressure, applied to a gas, is transmitted equally in all directions. That is also why the atmospheric pressure acts on every inch of the earth, and every human body. A man does not feel the pressure that air is exerting, either as pressure or as weight, because within each man the pressure is exactly the same so they nullify each other.

Every external pressure exerted on the fluid is transmitted in all directions. For that reason it is independent of the direction in which it is applied. This pressure is known as hydrostatic pressure and the gravitational force does not cause it.

When we measure a pressure of a gas, we encounter following terms:

1. Atmospheric pressure;
   It is the pressure of the air. Its value is:
   P_A=1 atm=1,0134 * 10^5 Pa=1,033 kp/ cm^2
2. Barometric pressure
   It represents a value of the atmospheric pressure depending on the actual atmospheric conditions. It is expressed in mm of mercury.
3. Relative pressure
   It is a difference between a measured pressure and the atmospheric pressure.
   P_a=P-P_A
4. Absolute pressure
   It is equal to the sum of the relative and atmospheric pressure. It represents the total pressure in a container.
   P= P_a+ P_A

Force acting on an object submerged in a fluid is called – **thrust**. Thanks to the existence of this force, objects do float. It acts in a same way as the gravitational force, but in an opposite direction. Thrust of a certain fluid depends on its density. Gases have their density too. Relative density of a gas is determined in a relation to the density of air.

It is a value without the unit, which relates the density of a gas to the density of air at the temperature of 15° C, and pressure of 1 atmosphere. The behavior of gases in mutual relations and in relation with fluids has been studied quite a lot, and acquired knowledge is formulated as a law. At different temperatures and at different pressures, gases behave in a different manner. It is explained by the kinetic theory of gases. Its principles are based on following facts:

a) Molecules of a gas collide and after the collision they move in a rectilinear way, at a constant speed. During the collision with another molecule of a gas or a molecule of a container in which a gas is, there is energy exchange as well as a change in a direction of its motion.
b) A moving molecule of a gas moves in all directions. Colliding among themselves, gas molecules move in a chaotic way around the container in which a gas is. Every moment there are a number of gas molecules colliding with a container. Summated, this action is known as pressure of a gas exerting on the walls of a container, as well as any surface in contact with a gas. This pressure depends on a kinetic energy of every molecule and a number of these collisions in a unit of time.

A molecule mass and the speed of its movement determine kinetic energy of a gas. A molecule mass is different for every particular gas, and its speed is dependent on a temperature. A condition of a gas is, therefore, determined by following measurable parameters: pressure, volume and temperature. Change of one of these parameters leads to a change of others.

Boyl's law – At a constant temperature the product of a pressure and a volume of a gas is constant. With every increase of a pressure, a volume decreases.

Sharl's law – At relatively low pressure and temperature of a gas, the product of the pressure and volume, divided by the temperature has a constant value.

Dalton's law – Pressure of a gas mixture is equal to the sum of partial pressures of every particular gas. Any gas in a mixture acts as if it is the only gas in a container.

Henry's law – A quantity of a gas that will dilute in a fluid is proportional to the partial pressure of a gas.

Upon a contact of a gas and a fluid, due to a partial pressure of a gas, a number of its molecules will enter the fluid. From that moment on, there is a solution of a gas and a fluid. The quantity of a gas in a fluid is expressed in units of pressure. The difference between the partial pressure of a gas above the fluid and its pressure in the fluid is called a gradient of pressure. Gradient of a pressure represents a quantity of a gas trying to enter or exit the fluid. If the gradient is high (high partial pressure of a gas above, and low pressure of a gas in the fluid) more gas is going to be dissolved. The entrance of an increasing number of gas molecules will cause the pressure of a gas in a fluid to increase as well, until it reaches the value of a partial pressure above the fluid. Once this value is reached, we say that the fluid is saturated with a gas and a gradient pressure equals zero. If the temperature is constant, a number of gas molecules entering the fluid is equal to the number of gas molecules exiting it. Temperature has a great influence on a solubility of a gas. As the temperature is lower, gas solubility is greater. While heating the water, gas bubbles appear even before the boiling temperature is reached. With an increase in temperature of a fluid, gas solubility decreases, and it exits the fluid in a form of the bubbles. These facts revolutionized surgery. An idea was born, that was soon carried out, and performance of a surgical intervention in hypothermia became a common practice. The main reason for it is better solubility of oxygen in blood (physical and chemical) at lower temperatures. Presence of oxygen in blood in higher concentration enables the cells, especially the ones sensitive to a lack of oxygen, to endure for a longer time. On the other hand, a cell metabolism, which is directly dependent on oxygen, is lower at lower temperatures.

During our study, particularly at this stage, one could hear, as a joke, following sentence: "A man lives to breathe oxygen". A fact is that human body is made, and functions in such a way to enable oxygen to reach every cell. Respiratory system, blood, circulatory system, and at the end cells have a same prupose. The nobility and particularity of a cell is determined by the time that can pass, without any irreversible cell damage, in conditions of decreased or even absent oxygen.

Wherever one goes, he finds oxygen. It is present everywhere in different forms. It is one of the reasons why we decided to follow oxygen, to its greatest and most hidden depths, in our search for answers regarding amniotic cells and their exceptional qualities. Now,

they are not as hidden as they were. Our role might be minor, but is notable.

Accumulation of knowledge about the life of a cell and of an organism inspired a man to find new ways to harm other human beings. Suffocation, poisoning, hanging, burning, cutting, stabbing. These, rather inhuman, forms of behavior have an only purpose of denying organism oxygen at any level.

We breathe oxygen from the air that surrounds us. In air, even when as pure as it can be, oxygen isn't the only gas. At rather constant concentrations one can find nitrogen, carbon dioxide, hydrogen, helium, and some other gases.

OXYGEN

Oxygen is a gas without the color or smell. It is somewhat heavier than the air. It can be diluted in water, but only to a certain degree. In normal conditions (temperature, pressure), in a hundred liters of water, four liters of oxygen can be diluted. It is the most widespread element on the earth. Its atoms make up to 60% of all the atoms.

Its discovery is related to Joseph Prestley, and a year 1774. As this, very important gas can be complicated; its discovery could not be quite simple. Prestley, namely, discovered that in the presence of oxygen the flame of a candle is stronger.

Antoan Loran Lavoasier worked with this gas, as well. He is significant for naming it as OXYGEN. However, we must also mention Karl Wilhelm Schale, who was actually the first to study oxygen, but he published his study after the others.

Oxygen is mostly found in the nature as a two-valence molecule. It can be transformed into a fluid or a solid substance, but at substantially lower temperatures. Its most common compounds with other substances are called oxides, and reaction in which oxide is formed is called oxidation.

The name of Christian Schönbein is also related to this gas. He discovered in 1870 the existence of oxygen in yet another form – OZONE. Its characteristics are pale blue color, greater density than oxygen (it has three atoms) and lower stability. In higher layers of atmosphere its presence is very valuable, because it absorbs the ultraviolet radiation. Its deficiency or rarefaction is very up-to-date. Its

great reactivity is used in industry as well. For its bactericidal activity it is used for water purification. It is also used as an air refresher.

For its spread, it's chemical activity, and necessity of oxidative processes , oxygen is either directly or indirectly related to every life form on the earth.

NITROGEN

Nitrogen is a gas without color, smell or taste. Its molecules are constituents of all living creatures, and in contrast to oxygen it is not necessary for life. On the other hand, again in contrast to oxygen it is a rather inert gas.

CARBON DIOXIDE

It is a gas without color. In lower concentrations it is without the smell or taste as well. In higher concentrations it has a sour smell and taste. It is chemically active. It is a product of animal cell metabolism. In a clear air, its concentration is constant.

HELIUM

Helium is a gas without the color, smell or taste. It is present in an atomic form. It is so inert; it does not even form molecules with itself. It is rather insoluble in water. It is considered as a rare element. In air it is found only in traces.

HYDROGEN

It is a gas without color, smell or taste. It is chemically very active, so it is rarely found in atomic form. It is found in greater concentration at higher altitudes. If it is mixed with air at concentration greater then 5,3%, the mixture is highly explosive.

BIOCHEMISTRY AND PHYSIOLOGY

(Oxygen in a cell life)

Interruption of an umbilical cord leads to an interruption in the oxygen delivery to a newborn baby. In order to live, baby must start breathing. At the very beginning of life one is asked: "to be or not to be". Who does not breathe, does not live. A newborn must start using oxygen to live and to develop. The way might be different now, but the purpose has remained the same – life preservation.

(Fig. 17) The way of O_2 to RBC

If we go back a little, we will remember that blastocyst was the first form in which fluid appears. With further development, this fluid will make blood and other liquids in an organism: lymph, aqueous humor in the eye, cerebrospinal fluid, endo, and perylymph of an ear. There are certain laws regarding the substance exchange between the blood and other fluids and, of course, cells. It has been determined that there are two directions, quite clearly explained. One, bringing the necessary substances for the growth and development of a cell, and the other, removing the substances not needed by the cell, usually those produced in a process of a metabolism. Hydrostatic pressure at the arterial capillary end is 3,3 mm Hg. Osmotic pressure in the same part of the circulatory system is 2,6mmHg. Permeability of endothelial cells is such, that with a difference in pressures, all molecules with smaller molecular weight than proteins, can pass through it. At the venous capillary end hydrostatic pressure drops to a value of 1,2 mm Hg, therefore, fluids now move in an opposite direction. A specific pigment is necessary for transport of oxygen to the cells. In human population this pigment is hemoglobin.

The main phenomenon in this transport is known as diffusion. It enables the movement of a substance from one space to another, providing there is a difference in concentration. Diffusion is powerful, but it is not almighty. Its limits can be explained by Fick's constant. Capacity of substance diffusing at one time is proportional to the area through which it occurs. It means that every diluted substance has a different diffusion range. This range is called diffusion constant, or,

according to the author – Krogh's constant. Mathematically represented, it is clear that diffusion by itself is far from being an adequate means of transport for any metabolite, let alone oxygen. Circulation in an organism mainly increases the transport of the necessary substances by diffusion (4). Its permanence is one of the important prerequisites for a normal function of the organism as a whole.

By using Fick's and Krogh's constant we can define - range of diffusion. It represents a quantity of a gas capable of diffusing through the area of one square centimeter, in one minute, providing there is a pressure difference of one atmosphere. We can measure the diffusion range for oxygen in a same way. For oxygen, a quantity of 0,000014 ml can diffuse in a minute through one-centimeter square at a pressure difference of one atmosphere; therefore the diffusion range for oxygen is 0,000014.

A process of oxigenation plays a very important role in a cell metabolism. It is a source of energy required by the organism. Without this basic cellular energy one cannot imagine survival of a single cell. And globally considered, the same thing can be said for the whole organism. Renewal of cell structures, as well as any biologic process occurring at the cellular level, could not happen without this energy. Oxygen has a remarkable role in all these processes. Without it, there would be no life, at least not in a way we know it. Regarding the biochemical reactions, occurring at the cellular level, the core of the explanation was reached after the discovery of the **redox – system**. Basically, everything that is essential can be understood with a help of a few basic reactions.

Oxidase reactions

These reactions are catalyzed by ferments found in the inner part of a mitochondrial membrane. These reactions are known as **mitochondrial oxygenation**. In this unique system of cellular energy production, in which oxygen represents a primary and indispensable component.

Oxygenase reactions

They are related to the electron – transport system of an endoplasmatic reticulum, and are referred to as **microsomal oxidation**.

Reactions of unsaturated fatty acid peroxidation

This type of reactions occurs in parts of a cell membrane full of lipoproteins. As there are no membranes without unsaturated fatty acids, although their number may vary, we may say that this type of

reaction is present everywhere and in any time, only differing in its intensity.

Oxidative reactions

These reactions are related to the intracellular structures, and result in hydrogen – peroxide production.

The whole importance of oxygen wouldn't be complete, if there wasn't yet another side, of these, for life very sensitive phenomenon. That is, any oxygen reaction in biologic relations must be controlled. Namely, while these reactions occur, there are moments when oxygen, or its atoms, attack crucial elements for a cell life. The possibility of oxygen attacking is demonstrated through its following three characteristics:

1. **Redox – potential**
2. **Acido – base constant of reduced oxygen**
3. **Complex of ion formation between oxygen and metals**

Energy that changes by the unit change of oxidative state, is referred to as redox – potential, it and represents the free energy formed by oxidation.

Oxidative power has two types of reactions with one electron. Redox free energy changes between 1 O_2 + H_2O and is dependent of the reaction (H+): "O_2 +2e+2H→ H_2O, and if compared, it is opposite to the reaction: O_2+e→O^-_2 where it is all about a single electron transfer. Oxidative potential of oxygen ranges from +0,8V to +1,3V.

Reaction of oxygenation, regarding thermodynamics, may be represented as:

PH(OH) +Fe(O) → PhOH + Fe(O^-), and basic mechanisms are:

$MO^+ + X^-$	→ $MO + X^{\bullet}$	electron transfer
$MO^{-2} + XH$	→ $MOH + X^{\bullet}$	hybrid transfer – hydrogen atom
	→ $MOH + X^+$	
$MO^+ + X^-$	→ $M^+ + XO^-$	hydroxile transfer – hydrogen atom
$MO^+ + X^-$	→ $M^+ + XO_2$	dioxygen transfer

Molecules of adenosin triphosphate (ATP) are made by nature in order to represent true source of cellular energy. It can be found in a form of enzyme, and is made from inorganic phosphate and adenosine diphosphate (ADP). This enzyme, again, can be made from adenosine monophosphate (AMP) and inorganic phosphate. Molecules of adenosine triphosphate release the energy in a process of phosphate cleavage. This energy is used for the cell survival and its regeneration, or more precisely for a cell metabolism. Since the source of this energy is ATP, we may say that it is a cellular energy reservoir. This energy is constantly regenerated through the process of cellular oxidation. The main "victim" of cellular oxydation is glucose. During the process of glucose oxidation, carbon dioxide and hydrogen atoms are produced. Hydrogen, than undergoes further reactions. These reactions seem to change a level of energy, starting from its lowest value, but further production necessitates more complex reactions, in which oxygen reduction occurs and hydrogen serves as electron recipient.

Analysis of the end results of glucose oxudation, may seem strange at the beginning, especially for inexperienced persons. But only at the beginning, when even cardinal things may be overlooked. With measurement, that is by no means mistaken, one may conclude that after the oxydation is over there is less energy than before. Thus, it could be said that in a process of oxidation energy is consumed, rather than produced. It is strange. But it can be easily explained when one takes into account the reservoirs, in which produced energy is stored. We would not dare to proclaim any form of energy as more important than the others, but we may say that this stored energy is most important for the survival of the amniotic membranes, in a specific conditions we are able to provide. We are aware that without the energy regeneration there would not be any cell metabolism, as well as many other processes. But we are happiest when all the sheep are in a pen.

Nature, as the greatest artist, a man had the opportunity of studiyng, provided energy with a specific kind of adaptability. This artist provided energy with several types of reservoirs. Our knowledge points to two of them. Adenosine triphosphate may serve as a reservoir in two forms: as great and small units. Small units are specialized for so called – prompt actions. In extrodinary, urgent circumstances, when energy is urgently needed, small units are promptly cleaved providing the necessary energy.

During the process known as ***oxidative phosphorilation***, following reactions in cell metabolism occur: electrons from the organic substances that are been oxidated (glucose in this particular case) transform electron recepient, known as ***acceptor***, (molecular oxygen in this case). ((Fig. 21), Oxidativ phosphorilation). It is necessary, before the oxidation that glucose passes through the cell membrane and enters into the cell. In order for the adenosine triphosphate synthesis to occur in a regular way it is necessary that a specific enzyme, referred to as adenosine triphosphatase takes an active part in this process. The necessary reactants for ATP synthesis are adenosine diphosphate and inorganic phosphorus. Many reactions occur in a process of cell metabolism, some of them are explained in detail, some of them are just mentioned, and some of them go without mentioning.

Process of electron expulsion through cell mebrane is enabled by a difference in proton electrochemical activity on the innner and outer surface of cell membrane. Mitchel's theory confirms that this energy is a result of an electrochemical gradient, for whose creation and existence is mostly responsible adenosine triphosphatase. All these reactions take place in cell organels, to be precise in mitochondrias. They represent the most imortant organels as far as oxygen is concerned. In accordance with Mitchel's theory we consider that energy transformation occurs in mitochondria providing a specific condition is met – a trigger is present. It is enabled by their ability to use energy formed in a process of electron transfer occurring in a respiratory chain. The basic prerequisite, however, is the existence of electrochemical gradient on both sides of mitochondrial membrane.

Comprehending all the above mentioned facts regarding oxygen importance in mitochondrial reactions, the way of its transformation, role of certain mediators, as well as the exact place where these reactions occur were our most important aims.

We were also in a position to determine what is important and, later properly inerpret it:

1. In amniotic cells we determined that adenosine triphosphate served as an energy reservoir. It is specific, however, that in amniotic cells only great units could be found. Small units of this energy reservoir weren't detected.
2. As "special forces for prompt action" , as we called them, are not present, we may conclude that emergency release and use of energy does not take place at all.
3. For basic functions amniotic cells use only about 8-10% of total energy. That is why they do not "grow old", and keep all of their characteristics providing they have been offered an opportunity of a "second life".

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ALLERGIC AND IMMUNOLOGIC REACTIONS

We can not say that we were convinced, but we believed, deep inside, that these reactions would not happen. Even slightest theoretical possibility, of them appearing, made us prove experimentally that they wouldn't.

Sticking to certain basic rules regarding experimental study, we had to adapt to our capabilities. In a first place we mean our financial capabilities, that made us improvise, but without violating the basic principles. A foundation had to be present; meaning that anyone interested in this subject could repeat the whole process.

In many good quality studies available to us, especially from certain countries, the use of rhesus monkey is advised in these or similar experiments. We know why. In a rhesus monkey, one may register changes that are most alike to those occurring in humans.

- Respiratory changes
- Changes in blood (eosinophilia)
- ECG changes
- Systemic reactions
- Skin changes

However, we had to decide among following experimental animals:

- Rabbit (Oryctolagus cuniculus)
- Rat (Mill-Hill-Hooded)
- Mice (Mus musculus)

If there was any pleasure, it is represented in a fact that this choice of experimental animals had its advantages. First of all it can fit into our financial resources, and on the other hand, presence of more than one animal species gives a greater opportunity to any of side effects to appear.

Capability of mammals to recognize a foreign substance in their organism, and produce an immune response, either humoral or cellular, is under genetic supervision. Genes for this type of reaction can be found in a major histocompatibility complex. In mice, this complex is known as – H-2 (2). It is fond on the seventeenth chromosome. In

human population, this complex is found in sixteenth chromosome. Allergy or increased sensitivity (hypersensitivity) is a phenomenon produced in several ways. Because of that it is manifested in different ways, and on different organs: as respiratory distress, skin changes, autoimmunity. The last one mentioned (autoimmunity) might produce effects anywhere in, or on the organism. What is common for all these phenomenons is an immunologic basis, an interaction between the antigen (either endogenous or egzogenous) and specific antibody or lymphocyte (2).

There are several classifications of these reactions, but the one most widely accepted is that according to Gell and Coombs. Based on the immunology they concluded that all the reactions might be classified in four groups:

Type 1 hypersensitivity

In this type of immediate hypersensitivity antigens of IgE class play the most prominent role.

Type 2 hypersensitivity

Reactions belonging to this type of hypersensitivity are characterized by cytotoxic reactions initiated by IgG or IgM, with cell bound antigen.

Type 3 hypersensitivity

For this type of immune response is characteristic presence of soluble complex of antigen – antibody with fixation of complement

Type 4 hypersensitivity (4)

This type is known as delayed cellular hypersensitivity. It appears when there is an interreaction between lymphocytes.

Type 1 hypersensitivity

This type of reaction may have different manifestations, as a result of common underlying processes. The most often are:

1. Sudden bronchospastic or even asthmatic attack
2. Allergic rhinitis
3. Urticaria and angioedema
4. Food or drug allergy
5. Hypersensitivity to insect's venom
6. Systemic anaphylaxis

Basic processes in this type of hypersensitivity are:

a) vasodilatation
b) increased capillary permeability
c) smooth muscle contractions

Results of these processes are:

1) urticaria
2) angioedema
3) hypotension
4) bronchospasm
5) spasm of gastrointestinal smooth musculature

Specific symptoms in gastrointestinal tract will depend of the exact localization of these reactions.

Type 2 hypersensitivity

Reactions that belong to this type of hypersensitivity are characterized by their **cytotoxic** or **cytolytic** quality. This type of hypersensitivity may look rather similar to the reactions of the type 4 hypersensitivity that is also characterized by cytotoxic effects, but the mechanism is different. Typical reaction for this type is demonstrated in reaction of antibody with the antigen on the cell surface. Antigen may be part of the cell structure, the cell may absorb it or a cell itself may have some antigenic qualities. Complement is usually, but not always, necessary for the cell destruction.

Type 3 hypersensitivity

This type of reaction is known as **toxic reaction complex** or **soluble complex of hypersensitivity**. In experimental studies, and in clinical studies as well, it has been determined that this type of reaction occurs in form of:

a) Arthus reaction
b) Serum sickness
c) Some allergic reactions to drugs

What is characteristic for these reactions is that antigen is present in considerable quantities. Consecutively, antibodies are also formed in greater quantities, forming the soluble antigen – antibody complex. This immunocomplex fixates complement in a classical or any other way, causing migration of the polymorphonuclear leucocytes and release of factors influencing increased capillary permeability. As a

result of all these processes, acute inflammatory reaction occurs, usually accompanied by vasculitis.

Type 4 hypersensitivity

This type of reaction is known as – **delayed hypersensitivity** or **cellular hypersensitivity**. This name is given to it due to the fact that symptoms appear 24 to 72 hours after the encounter with the antigen. This prolonged allergic reaction is caused by the attack of leukocytes towards the antigen. Following disorders are caused by this type of reaction:

a) Contact dermatitis
b) Tuberculin hypersensitivity
c) Graft rejection (7)

Considering the possibility of appearing any of the above-mentioned reactions (although deep inside we disbelieved it) we regularly observed our experimental animals. Observation would begin just after the application of "antigen". Day and night a qualified person would be with the animals, providing us with constant supervision.

We think we followed the professional logic, when we, considering our method, concluded that it was a form of a transplantation. On injured skin of the experimental animal we would apply something that would stay fixed in its place. We were on our own on several grounds. First of all, we could not find any literature about this subject or anything like it. We were left with classical, established principals regarding experimental studies.

All animals were given anesthesia before the procedure. We always used standard, already accepted methods of laboratory work with experimental animals. They would be put on a special support adapted to their size. They would be positioned on their left side, with all four extremities fixed. Than we would remove all the hairs from the presented right side of the abdomen and then make lesions. In all the animals lesions were made in two ways. One was application of boiling water and formation of burns. The other way was surgical skin removal. Now the most time consuming part of the study came. Amniotic membranes were diluted in a physiologic solution. We would start from the lowest concentration, and end with amniotic membrane themselves. For each concentration a series of at least six animals was used, every animal species, with each of the two types of lesion. In cases that were considered as suspicious in any regard, we would repeat

the whole procedure with the same concentration of amniotic membranes. The area of our focus would be protected with as little bandages as possible. The animals would spend their time in their cages. Standard laboratory methods were used regarding the nutrition and hygiene.

In accordance with the theoretical knowledge, the observation period was limited to ten days. Nevertheless, fear of a delayed reaction existed even beyond this period.

All animals that would survive, would be further observed, although not so closely, in order to exclude any kind of reaction.
In cases of graft rejection, there are certain changes in an organism that precede some serious side effects. At first an increased activity of accessory T – cells is noticed. They immediately start producing and secreting IL-2 that is necessary for activation of CD8 T – cells and B – cells. As a result of exposure to an antigen, and interleukin, there is a clonic reaction of proliferation and maturation of the aloantigenic cells. Result of these reactions is formation of "true" effector T – cells that are distributed by blood throughout the body (5). In such a way they reach the transplant itself. Lesions appear on transplant on the sites of antigen presence. Antibodies are formed that immediately initiate the antigen – antibody reaction. Besides IL-2 and JFNY, T – cells form some other lymphokines: growth and differentiation factors for B – cells. They play an important role in antibody formation. Lesions on the transplant are the result of direct activation of the complement, but they may also be formed by the accumulation of the active cells, characterized by a strong cytotoxic effect. Accumulating cells include monocytes, macrophages and in smaller number polymorhonuclear neutrophils. Although several cell types are present, according to the most recent studies, most responsible for graft rejection are T – cells. To verify these reactions, there are numerous laboratory tests. For known reasons we have chosen the following ones:

1. electrophoresis and
2. agglutination

During the transplantation area inspection, we mostly feared that we might find softening and swelling of what we applied.
We will make a digression here which we consider proper for several reasons. We would like to mention a phenomenon related to the area of

the lesion that made us worry. We were just making our first successful steps.

During the treatment of skin defects, we noticed hypersecretion on the bandages. It was most prominent 2-3 days after the application of the amniotic material. Secretion was of a greenish color, with a foul smell. The general condition of the animals was good. We soon realized, to our relief, that the wounds were contaminated, a fact that corresponded with the findings on the bandages. The secretion could be found until the wound was clean, a time necessary for this process depended on the degree of contamination. Once the wound is clean those secretions would not reappear. Epithelium formation would follow, and all the processes that fitted our, still experimental, experience.

The other reason for a digression is that we wanted to point out the following fact: in any pioneer study, all kinds of surprises are possible even if one conforms to all the principles and rules regarding the experimental work.

Side effects (softening and swelling) at the area of amniotic material application did not appear in our experimental animals.
Regarding the systemic effects, that we feared as well, we observed the appetite of the animals. In cases of graft rejection, there was from the beginning a conspicuous lack of appetite. Signs of general malaise would appear. Animal becomes less mobile, than quiet, with substantial muscle weakness.

Nobody was happier than we were when everything was over and none of the above-mentioned symptoms appeared.

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TRIGGER

In previous chapters, especially in the last one, we explained in detail the "address" where energy is stored in amniotic cells. This storage place is characteristic for other cells as well but some differences exist. We now know in what form energy is stored and we know its transformational possibilities.

In this part, we would like to further explain conditions that must be met in order for the transformation to occur. Trigger is one of the prerequisites for this transformation. We proved it, both theoretically and practically, long time ago. This trigger has several names with the identical meaning:

- unintact skin
- lesion(s) on the skin
- skin wound

Trying to include all kinds of skin lesions we treated, we classified them in groups according to the mechanism of injury:

a) Burns
b) Injuries of different etiology
c) Circulatory disturbances
d) Inadequate surgical wound treatment

Regardless of the macroscopic appearance of the wound, i.e. presence or absence of any sign of infection, the trigger is present. Treatment with amniotic membranes could be done, but its duration would depend on following factors:

a) Wound depth
b) Wound width
c) Wound localization
d) Circulatory conditions
e) Degree of wound pollution

However the end result would be the same. It would always end with the new skin formation. Its quality was different, depending on the quality of the trigger. We consider factor "d" as the one influencing most, both of the qualities. Therefore we would expect that children would have the skin of the best quality.
As it is.

More details about the newly formed skin and its quality will be mentioned in one of the following chapters.

We had it all, but we couldn't prove it. We needed something that could be registered. What anyone else, who is interested, could repeat. The measurement of the bioelectric activity. However, either it's intensity of frequency, did not interest us. We wanted to know if they existed or not.

If amniotic membrane is applied on an injured skin, the trigger initiating the energy transformation is induced. It is visually manifested in the disappearance and expenditure of the amniotic material. The necessity of a trigger is known in certain diseases as well. In these cases this energy transformation is as unfavorable as it could be. In these diseases, the symptoms would not appear until the trigger initiates the whole process.

If we look at this problem from another angle, we may expect that if we apply amniotic membranes on an intact skin the trigger would be missed. It would mean that the bioelectric activity would be absent. We had everything we needed. We only had to do it. Following already determined direction. **Dictum factum**

The scheme of the apparatus for the measurement of the bioelectric activity.

In this part of the experimental study we used the same experimental animals:

Rat – Mill-Hill-Hooded
Rabbit – Oryctolagus
Mice – Mus musculus

The beginning of the procedure was similar as in previous part of the experimental study. The animals would be given anesthesia before the procedure. They would be put on a special kind of support, adapted to their size, on their left side. Their extremities would be fixed, but the right back extremity was fixed to a specific, smaller and softer support in full extension. The animal was then shaved to facilitate electrode application and enable good quality of bioelectric activity measurements. On the right thigh we would apply amniotic membranes on shaved and intact skin. Over it the electrode would be applied. (Fig. 18) It was a more demanding job then we ever considered in theory.

Apparatus did not register any bioelectrical activity, (Fig. 23) No bioelectric activity. (Fig. 19) At least not in most of the experimental animals. Only once, in a rabbit a slight bioelectric activity

was registered. It was week, but nevertheless present. After detailed inspection we noticed that during the process of shaving slight lacerations were made. The skin continuity was damaged, even though minimally, it was enough for a trigger to activate and produce the bioelectric activity we registered. A repeated experiment with more careful shaving, showed that we were right. No bioelectric activity was registered.

As this first part of the scenario was a complete success, we dared to rely on logic and assume that the other part would be successful too.

We did not lose time.

The procedure was the same up to the very application of the membranes. They should be put on damaged skin, and it was done in one of the two following ways: by surgical skin removal, or by the application of boiling water and formation of the burns. Amniotic membrane would be applied in such a way to cover the entire damaged area, and a part of the intact skin, all around the lesion. If the wound is uneven, the amniotic membranes should be in direct contact with all the recesses and bulges. Electrode would be applied in the usual way. The apparatus were not still now. (Fig. 20) Bioelectric activity was present. Just as we expected. Everything fitted our mosaic. Cell energy transformation was initiated provided there was a trigger. Now we were able to register it.

It is clear that amniotic membranes in their "second life", provided all the necessary conditions are met (including the damaged skin), completely disappear. It is achieved by energy transformation, and it does not happen immediately, but they do disappear and a new quality appears. At the next treatment, amniotic membranes, or more precisely amniotic cell cannot be registered not even microscopically. But we can register something new: aboundant epithelization starting from the periphery and spreading towards the center. The presence of amniotic membranes on the intact skin is visible by the naked eye. They are changed, dry, but present. (Fig. 21)

SKIN

By carrying out our idea, adequately using the trigger and activating the process of energy transformation, we managed to create skin on body parts that for whatever reason did not have it. A great deal was already achieved, but we thought that something was missing. Everything had to be undoubted. It had to undergo professional technical processing and interpretation. Those were the pieces missing in our mosaic.

We solved innumerous cases of **ulcus cruris**. Skin changes in this disorder are caused by inadequate vascular supply, of whatever etiology. Malnourished area, especially if an injury is interposed, represents an ideal ground for the development of this disease. We managed to create new skin even in these conditions. But of what quality? The same quality as the trigger; if not diseased, than changed. The most common etiologic factor for these changes was **diabetes mellitus**. The youngest patient was forty-five. In pediatric population, even though it is not an uncommon disease in childhood, we have never seen such a skin change as ulcus cruris.

One patient was particularly interesting. A female patient, sixty-seven years old, had burns on both lower legs and feet. (Fig. 22) She spilled boiling water on them. After the treatment, that lasted relatively short (less than three weeks), the result was excellent. The patient kept showing her legs to everybody, repeating that at the site of the burns she now had new, soft, fine skin, and the kind of skin she had when she was twenty. (Fig. 23)

But this wasn't the kind of pathology we wanted to treat. We decided to try treating gastroschisis. Omphaloceles we avoided, because we knew what kind of pathology it is. We knew what our limits are, we did not want to mess with the peritoneum. Besides we wanted to avoid any kind of charlatanry. Time will show that it was a right choice.

We have chosen the above-mentioned congenital anomaly (gastroschisis), because it comprises a complete defect of a part of the abdominal wall. Whatever it's size, the defect is complete. On the other hand, the age of the "old" and "new" skin was the same. Not identical but approximately the same.

Let us start from the beginning.

During the intrauterine development, the defect appears on the abdominal wall around the umbilicus for unknown reasons. This anomaly may be associated with other congenital anomalies. There is no separation between the abdominal cavity and the environment. The intraabdominal organs communicate with the amniotic fluid during the intrauterine life. Those organs are usually small bowels; sometimes mobile parts of the large bowel and in extreme cases even parenchimatous organs. An incomplete abdominal cavity does not develop properly. At birth, it is of smaller volume, then it normally is.

Such a child is transferred, immediately after birth to the Pediatric Surgery Clinic. Our task is to put everything in its place: to replace intraabdominal organs in the abdominal cavity and to close the communication with the outside. But as it is a case with every good intention, we have certain problems to solve first. In this case, the problem is serious. We are supposed to put something of a substantial volume into a cavity that cannot fit it. There are cases when the abdominal cavity can accommodate all the intraabdominal organs even without dehiscence (partial or complete). But there are still problems to solve. The intraabdominal pressure is exerted on the vascular system, therefore impeding the circulation, as well as on the diaphragm and it is well known that newborns get most of the oxygen through ***diaphragmatic breathing***. All together it leads to a general aggravation of the newborn's general condition.

Our aim was to heal the child. We tried, in accordance with the anesthesiologist, to return the intraabdominal organs in the abdominal cavity without jeopardizing normal circulation and breathing. As soon as these functions would be impeded, the anesthesiologist would alert us to stop. Remaining bowels we would cover with the "Liodure" by forming ventral hernia. "Liodure" would be submerged in physiologic solution an hour prior to the application, in order for it to be elastic and easily modeled. An adequate configuration of the ventral hernia (Fig. 24) would be made; it's contents being the bowels that if put into the abdominal cavity would exert a fatal increase of the intraabdominal pressure.

In time "Liodure" would dry, shrink and decrease in size. (Fig. 25) During this process, we believe that it would represent a biologic pressure. This pressure was lower than the normal, but it was still enough to slowly replace the bowels left outside into the abdominal cavity. Over this period a newborn would be normally fed, it would

have regular stool; it would grow and develop. This biologic pressure lasted two to three weeks; the age discrepancy between the "old" and "new" skin partly depended on the success of the treatment. As the hernia would decrease in size we would remove from the periphery a part of a "Liodure" and apply amniotic membranes. (Fig. 26) After a certain period of time that would depend on the size of the defect, it would be completely closed. (Fig. 27) From that time on, the old and new skin would grow and develop together. However, even after complete closure of the skin defect, there was still a ventral hernia due to muscle deficiency.

Once the defect was closed we could only wait. Hernia reconstruction is usually done at he age of four to five years. Until then we would have to go together through the common childhood diseases, respiratory infections, and anything else that time would bring. All this time we feared that, who knows what, might happen. We suggested postponing of the regular vaccination, asked for these children not to spend too much time with sick children. Here, we must emphasize a good cooperation and understanding from anyone who was in contact with these kids.

Most important fact was that the old and the new skin were under same internal and external influences. External being clothing, water, soap, heat, cold...

This new skin reacted in a same way to the pox. The skin changes would appear and disappear at the same time as on the other parts of the body. Macroscopically, they would not differ from the changes on the other sites. This new skin behaved "like" it was part of the organism. Pathologic expertise would make "like" unnecessary.
Once the adequate age was reached, it was time to reconstruct ventral hernia.

After the usual preoperative preparation, we approached surgery. Following tasks were ahead of us: we were to separate muscles from the skin, suture them, close the abdominal cavity and reconstruct the umbilicus. The separation was the slowest process, but without any problems. Bleeding was minimal so we did not have to use a unit of blood we preoperatively reserved. Umbilicus reconstruction wasn't something we do very often, but we were satisfied with the results.

Once it was over, we noticed that a part of a new skin was excised. We decided to send it at the Institute of Pathology for histologic examination. On the accompanying form we fulfilled the

general data. On the question:"What is sent?" we wrote: "excised skin of the abdominal wall". But we did not dare to write the clinical diagnosis. Maybe it was an unfriendly gesture. But this unfriendly gesture can be explained by our desire to avoid any suggestion at all to the pathologist.

Again we had to wait. We were sure again, but... We had goose pimples. What eye can see is one, what if an expert under microscope sees something else? There is always a but. It is always hardest the first time. Fortunately it was a wait of only a few days. Finally, a pathologist called. We expected a hoarse male voice (I'll never know why) that would start with innumerous unpleasant questions. But on the contrary, there was a pleasant female twitter (we later found out that the owner of the voice was even more agreeable than the voice) apologizing for the disturbance, and inquiring about the reason why we sent her a sample of normal skin. It was music for the ears and for the soul. The first part of the report stated:

"Three fragments of the skin, the greatest diameter 4x3x1 cm, were received. Without any sign of pathologic changes". Signature.
(Fig. 1 skin histologic appearance)
The other part of the same pathologic report stated:

"In the macroscopically described sample we found morphologic elements of the skin, without histologic changes. Epidermis is of normal architecture, underlying dermis containing usual skin adnexae: hair follicles at different stages of maturation, sweat, sebaceous and apocrine glands." Signature. (Fig. 28)

That was, thus, our new skin. But to obtain full documentation, the above-mentioned beauty, the pathologist made the necessary photographs. She insisted on specific staining of the samples, by Mallory and Gomori stains, because it would provide the best results. That is what all the pathologists think. We could do nothing, but agree. It is a field we knew so little about. (Fig. 29)

Clearly visible skin epidermis. Normal architecture. Several follicles of different maturity are visible in dermis. **(Mallory 6,3x10)** (Fig. 30)

Longitudinal section of the hair follicle with accompanying sebaceous gland **(Mallory 6,3x10)** (Fig. 31)

Hair follicles of different maturity, embeded in dermis **(Mallory 6,3x10)** (Fig. 32)

A hair follicle with a hair, sebaceous and sweat gland **(Mallory 6,3x25)** (Fig. 33)

Focus on this photograph is on skin adnexae. It is one of the proofs that it is really skin. We have never heard that any substance; very similar to the skin contained skin adnexae. In this case: sweat glands in the reticular dermis **(Mallory 6,3x25)** (Fig. 34–35)

So, our long journey from mitochondria to the skin was over. It was a thorny path, on many occasions even a winding one. There were many obstacles we had to conquer. The most difficult ones were those made by people. Not nature. Nature was helping us, directing us giving us strength. It was a great help. As colleagues are concerned, obstacles made by them were not a rarity. It really hurt us. But our belief was stronger. Our results removed all the obstacles by themselves.

There are still some that are trying to prove the opposite. It is more a sign of protest than anything else. But we will forget it all. After all, they are our colleagues.

All that is left for us is to patent the invention, and than work in peace.

We expect never again to be said:

FERRO ET IGNI,

Because we are convinced that:

SIC ITUR AD ASTRA.

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HEALTH MARKETING STUDY

With application of management in the process of health insurance (with special regard to the treatment with amniotic membranes) we consider that a multi-disciplinary approach to the problem solving was respected.

For a successful implementation of the marketing concept in a health institution it is necessary to have a specific information exchange between the patient and an institution. A study of a specific, health marketing, in such cases may be defined as a mean used by those providing the health services, to make them as similar as possible to those required by the patient. In order to perform a true marketing study, it had to be a systematic process that would include gathering, analysis and interpretation of the relevant data. All these processes must be present in order to make the best decision. The aims of a marketing study are the following:

- Decrease of the uncertainty of a decision making process regarding general marketing activity, as well as specific aspects of marketing;
- Help control carrying out of the marketing activities.

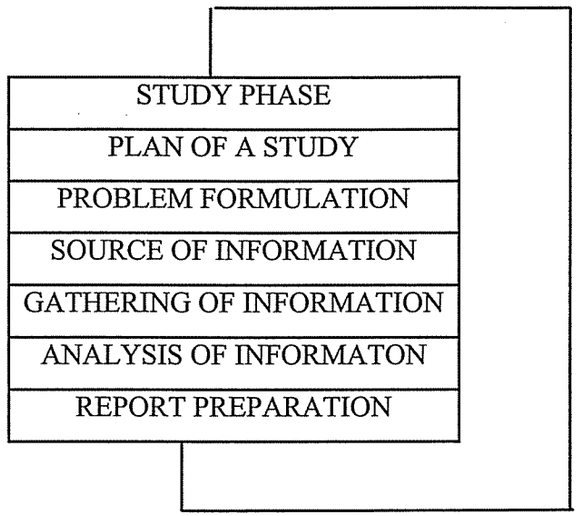
Any kind of marketing, including health marketing, must be accepted as an entirety, made of following components:

- Information system,
- Internal accounting system,
- Analytic marketing system
- Advertising.

A marketing study should always be included. The final aim is to properly understand a certain marketing problem, which is always present. According to the majority of the authors, in order to make a marketing study successful; it must comprise following phases:

1. Definition of the problem and aims of the study,
2. Development of a source of information,
3. Gathering of the information,
4. Analysis of the information,
5. Presentation of the results.

In such a context, any problem consideration must face with following seven steps, and, as it is often case, a feedback.
A process of marketing study in health – especially adapted to the treatment with amniotic membranes:



This has a task of answering following questions:
Is there an interest for this kind of treatment?
If there is, how to develop it?
Is it's quality and price comparable with the treatment of competitive institutions?

But in this particular case a study had to be somewhat modified. We had to rely on the discussions we had with our colleagues, during numerous meetings on the congresses and seminars. All the impressions were the same; there practically was no competition at all. A completely new moment. We were confused at first. On one hand, we were out of the above-mentioned scheme, but on the other hand we were building something new with a sensation of relief equal to that of concern.

The already mentioned importance in the continuity of the process of a marketing study relates to the changes of the two very important dynamics: economical and political.

The mandatory follow up of the technology is imperative to our field, as a part of medicine. It wouldn't let us slow down, because a slight omission might allow the competition to reach our goal by another method we were unaware of, and overtake us. Thus, the role of a manager is to follow all the data whose analysis would help reach proper good quality decisions. The execution of the decisions that have been made is also going to be based on the data analysis, done by the qualified people. The main information necessary for the process of decision making may be gathered in a following ways:

Desk research – based on the information gathered from the official publications and statistics. This form of data collecting is known as – secondary investigation.

Original investigation – compared to the previous type represents a primary investigation and requires a great deal of investments. It is done as a sampled survey, whose aim is gathering of new information.

Investigation done by the direct observation of the patient – in this case the main aim is to understand as well as possible the patient, and to realize what are the patient's desires. Through this investigation we must find, and at the same time eliminate, any mistake we might have done, and make sure we do not repeat the same mistake.

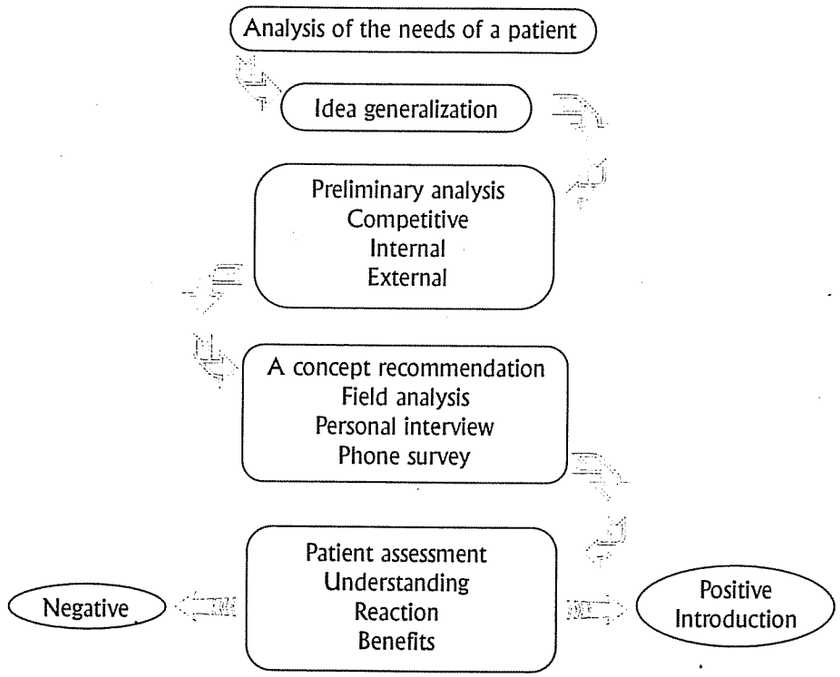
SPECIFICITY OF A MARKETING STUDY REGARDING HEALTH SERVICES

At the first glance, one might say that there is a similarity of marketing investigation regarding health services and patient's needs. The main differences relate to following facts:

- Ethics – certain health institutions still consider use of marketing investigation unethical;
- Size – certain health institutions are small and of local importance, so they couldn't justify such a research;
- Economics – identical institutions do not have the same financial resources;

- Monopolistic institutions – institutions that have a monopoly on specific medical service, and are still prepared to invest in this kind of investigation are rare;
- Manager – if there is a person that might be called a manager in a health institutions, it is certain that he doesn't have a proper education and that investments in marketing studies are deliberately avoided.

All these problems, regarding a marketing study of health services, are even more complicated when a new form of a treatment appears. There are many directions where our study should go, meaning noticing, ranking and analyzing of the particular elements of a new form of treatment. In these cases a specific kind of a marketing study was developed. Our case is such an example. The following scheme illustrates it clearly:



It is clear that this model is made of several phases. The beginning has a common foundation. Idea regarding a new form of treatment starts at the same time as the analysis of the needs of a patient, and if possible even the patient's desires. In such a way a foundation is made that should be acceptable for both sides: the one that is treating and the other that is being treated. During further investigation the idea undergoes a preliminary analysis, whose task is to consider, up to a slightest detail, competition, if it exists. The idea is then analyzed internally. That way a subjective opinion is obtained regarding the work with the patient. External analysis comprises an opinion from the patient. Positive results lead to a marketing test or immediate application of a new form of treatment.

There is a process with which we may test a new kind of treatment to make sure there is a full understanding by a patient of a procedure to be carried out:

Does the patient understand the value of a procedure compared to the others?

Does the patient realize that this procedure is a definitive one? That the treated lesions will not reappear at the site of a treatment?

An understanding that procedure is a unique one, is also very useful to the patient that does not need to go from one doctor to another, and from one hospital to another. All these tests must be well chosen and properly applied, especially if there is a similar procedure, even if far less successful than the one we are studying.

Marketing studies of health services in general and especially of new kinds of treatment were neglected for a long time. Now-days, there is a great interest in investing in the marketing investigation. Amounts, which are in present financial situation quite considerable, are being invested in marketing studies. With such investments, new forms of treatment may be presented to future patients in form of photographs, slides, films...

HEALTH MANAGEMENT

Management is as old as human population is. Its development followed the development of human society and productive relations in these societies. It is a fact that today it is a universal and complex process, that is, naturally, always in a dynamic balance with the environment. Its development while going forward, some times slower, some times faster, resulted in a following situation: a concentration of capital, work power, and work machinery in this, last century of this millennium. Thus, even in health institutions a need has arisen for a competent management, that would provide safety for everyone, and at the same time enable further development and growth. Technologic accomplishments enabled medicine to develop itself. Medicine is institutionalized, and market is included in the health care. Models of health care moved from regulatory to the competitive forms. All these particular factors, analyzed together, made the directors become capable managers not only administrative leaders, as it was case before. Management education in health care is relatively new form of training for health care directors, but it has shown how necessary it is for the health services to continue improving in these very difficult times. Modern management in health care is more complex and considerably different than management in other productive branches. The basic concepts, as well as the role of a director are the same. But health management, either in theory or practice, has certain particularities. As Peach said: "Health management is a process that makes sure that the health services use all the available resources in a best possible way to obtain a goal of health improvement and preservation in a society"

Specificity of management in health institutions

Many relations between the components of management in health institutions may not be foreseen, so the goal attaining is more or less uncertain. A manager, in a process of decision making, should keep in mind the urgency and uniqueness of conditions regarding health services, as well as the time period between the decision making and its

results. It is impossible, and very dangerous to apply results and investigation from one situation to another.

A basic product in health institutions is a service that will depend on the disease, so it is rather difficult to assess its quality. The end-result of such a service is health, which is very difficult to measure due to a huge number of assessment criteria.

Personnel selection is very sensitive, because there is a need for work coordination of different experts, with different views on a problem solving possibilities. Situation is even more complex due to a social status of health workers, as well as the autonomy of clinical practice. Any form of management in health institution is, regardless of the quality of the services it provides, under a constant public supervision. The pressure from the government as well as different political and social groups is further imposed. The pressure relates to preservation and improvement of a quality of provided health services, even if the investments are stagnating or even decreasing.

For a good management, a good communication inside the organization is very important as well as a good communication between health institutions among themselves and sectors not related to health care. The most often cause of a conflict is a lack of communication between the director and employees, that clearly affects health services and their effectiveness.

It is considered that basic concepts, principles and managerial skills of industrial and other institutions may apply to health institutions, with respect to their social role and specificity of internal environment. Theory agrees that there are several basic roles of a health institutions in any society:

- It is part of a country's national politics;
- It employs a great number of people;
- It provides health protection;
- It performs different kinds of investigations;
- It is carrying out a continual education;
- It has an important role as a factor of country's social stability.

All the above-mentioned facts greatly influence characteristics of health management. Health institutions are known in management theory as complex institutions with consecutively very complex management. A wide differentiation and specialization of tasks is evident, and persons of different degree of education, work experience and functions perform them. At the top of this complex is a modern

hospital. A structure of management in a health institution is divided in four parts, also referred to as four powers:

- Administrative committee
- Administration
- Doctors
- Director

Administrative committee is legally responsible for the whole institution, for health service providing, communication with the public, and help in material and equipment acquisition. It includes representatives of different social groups, and it should represent the society in which a health institution works.

Doctors – as a dominant profession they are included in three different managerial processes:

- Patient management,
- Management of a team of doctors,
- Health institution management.

Their role is permanent with all signs of domination, since they are responsible for a basic activity of the institution – health service providing. Potentially they should be best managers, but there is a paradox. Doctors have, due to a nature of their work, great power and authority, but they do not have a responsibility regarding financial risks. A considerable number of doctors are unaware of the society they work in, due to their concentration to the benefit of their patients and their personal improvement. It unavoidably leads to a discrepancy of personal interest and those of the institution. A concept of effective management in health organizations is impossible to separate from the clinical autonomy, because only their balance leads to an effective functioning of the institution.

Administration – one of the centers of power in a health institution is made of director, heads of department, and chiefs of auxiliary services. It is responsible for the operative management, but its authority is also a direct work with the patients. Director's task is to make decisions, plan, coordinate and control the activities of employees, all in order to make work with patients effective and successful. In many health institutions directors are the doctors from the same institution, but they are now increasingly replaced with professional managers.

When analyzing a management of a health institution, following responsibilities must be considered:

- Responsibility toward the patient,
- Responsibility toward the employees,
- Responsibility toward investors,
- Responsibility toward the society,
- Responsibility toward self.

Health management has general and specific aims. This classification is generally accepted and is a result of positive experiences. General aims are:

Existence and development of the institution – lately there is a growing pressure to decrease the amount of money intended for the function of health institutions. As a result many health institutions have difficulties in their work, and health protection rights decrease and limit to certain procedures. Managers try to keep their institutions working and develop by introducing new diagnostic and therapeutic procedures, all in a complex of competitive market.

Decrease of expenditures – by decreasing the price of health services and increasing the number of provided services, a decrease in a cost of functioning of the health institutions, hospitals at the first place, is conveyed. In modern management, a basic economic principle must be respected – to obtain maximal results with minimal financial investment.

Competition at this pseudomarket – becomes less important and requires smaller continual investments – if a small number of institutions, or none may provide the service at all. Amniotic membranes are such a service.

Specific aims:

Health improvement (as a part of strategy – "Health for all in a year 2000");

Improvement of the quality of the provided services (regardless the cost, doctors, and other health workers are directly responsible for the quality of the services they provide, as well as the end-results of the treatment). That is why the standards of the quality of health services are being formed and changed;

Involvement of doctors in a process of management (in order for health management to preserve and improve its quality, and its justification, it is considered necessary to include doctors in a process of management);

Coordination of management and autonomy in clinical practice (autonomy in clinical work understands a right of a free practice

outside the hierarchical management, a right to admit or not a particular patient, responsibility for organizing and supervising the activities of associates and a right for a privilege due to a great medical knowledge);

Patient satisfaction (lately, a patient's satisfaction is more and more taken as an instrument of quality control of health services, and a need for inclusion of users of health services in a process of decision making is emphasized);

Health management is relatively new discipline. Its importance is growing, as well as its appreciation. The validity of certain theories is determined as well as any reason for its rejection. Health management develops in two directions:

System analysis – is done through analysis of a concept and comparative analysis.

Operational approach – is occupied by investigation of the optimal managerial models, using in this process: investigations based on surveys, and investigation based on data collecting from routine statistical and medical documentation. These investigations are useful for more reliable assessment of changes in health system, and possibly management. With this type of investigation main problems are lack of reliable, up-to-date and objective data as well as difficulties with the application of the investigation in practice.

Specificity of management in health institutions as well as characteristics of a time we live in, suggest, according to the several authors, that health management will in future develop through:

1. process of participative management and
2. Decentralization.

Patients, as users of health services – either routine, everyday services or special, unique in the world services, as our procedure is – may be under different influences when deciding whether to undergo a particular procedure or not. A factor that is considered as very important is the so-called service ambient. It is considered necessary to investigate all the elements in order to design this ambient as adequately as possible, so one may consider "atmosphere" as important moment in marketing. The atmosphere that is created may considerably influence the behavior of the patient, so following details, fitted in the whole environment should be used for an atmosphere creation:

Attention attracting mechanisms – color, noise, silence;

Message forming mechanisms – communication with certain, other doctors and patients, creation of a higher level of concern for patient (it is important that patient feels it is so);
Effect creating mechanisms – combination of light and sound effects in order to create feeling of security and confidence in a staff.

Atmosphere and sensations

Created atmosphere in an ambient	**Reactions in form feelings**
Elegant	Status
Professional	Security and trust
Cordial	Happiness and pleasure
Dark	Depression and disconsolation
Threatening	Worry
Warm	Comfort
Interesting	Attraction

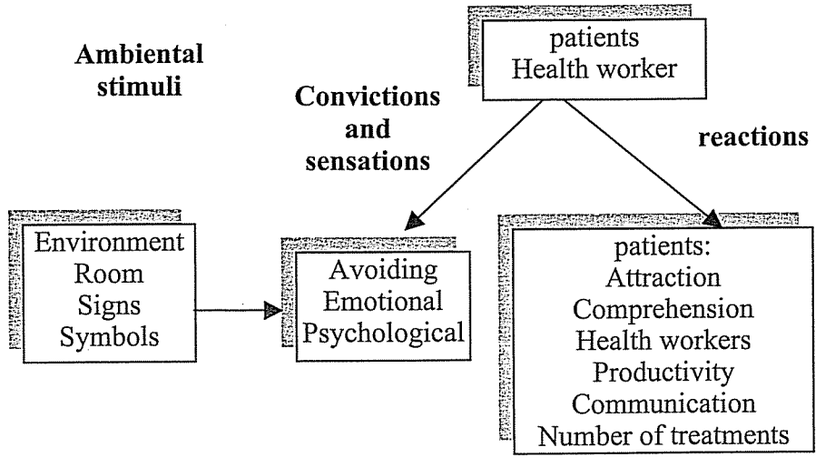
In these situations it is desirable to consider ambiental stimuli:

- Environment – temperature, air quality, noise, music, smell;
- Space – room, equipment, furniture;
- Signs, symbols.

As far as convictions and sensations are concerned, they can be:

- Comprehension – conviction;
- Emotional – mood, sympathy, attitude, lack of sympathy;
- Avoiding – avoids interaction, wishes to leave and does not return.

The influence of ambient on a behavior of health workers and patients is demonstrated in following scheme:



Design and ambient creation are very important and they should be a result of careful consideration. It is believed, especially in large institutions, that it should be done by a specialized service organization. Ambient includes physical and nonphysical contact, i.e. the environment where the treatment takes place, and where health workers and patients meet. A capacity of a health institution significantly influences the performance and type of communication. That is why symmetry, rhythm, colors and other fundaments should be taken into account, combined and developed. They should produce in a patient cognitive and emotional reaction that would influence visual perception and the appreciation of a treatment quality.

Furniture might create domestic atmosphere, or create an insurmountable barrier. It may be a part of a combination that includes computer, viewing-box, and necessary instruments. It may act as a protection for more sensitive equipment. All of it together speaks of a status of the health institution. Space, equipment, furniture and decorations are mutually dependant elements that characterize a way of life. They improve a quality of life. Regarding ambient in which health services are provided (treatment), all the elements of an ambient should be used and adapted. Light, heating, ventilation, height and shape of the chairs, kind of furniture and a quality of equipment, all may serve to create either domestic or hi-tech atmosphere. When designing such

ambient factors such as daylight, colors, activities performed in a room as well as activities that take place in close proximity should all be considered.

Daylight and its influence on temperature, glow, visibility, penetration and color perception are very important. The direction and intensity of light significantly influences a color perception. What is required from the ambient must be clearly defined. The level of visibility will depend on the type of activities performed in a particular space, and the age of the patients. For example, if the precision of a procedure is to be emphasized, intensive light is required. Creative use of light may create an agreeable environment even if in that vary environment an unpleasant, or even painful procedure is to be done. Interaction of light and shadow may have multiple positive effects. Something might be accentuated to attract attention and influence decision making of the patient.

Color as an element of the interior has its own language. Like music, it may provoke emotions like excitement, tranquility, or quite the opposite, sorrow. Color consists of three elements:

1. Shade – blue, marine, carmine...
2. Quality – darker, paler
3. Cleanliness of a color

There are numerous theories about the effects of colors, but, a color cannot be understood if we disregard the effects of lighting, differentiation of similar shades of a color, i.e. the different perception of a shade depending on a surrounding color as well as environmental and emotional effects of a color. If lighting in the environment is inadequate, the creative use of color will not have any effects. It is known that lower level of light, together with worm colors will create an atmosphere of a friendly, intimate ambient, which relaxes and refreshes. Worm colors are orange, red and yellow. Their positive effect on a patient, and health workers is much clearer if the lighting is positioned somewhat lower than usual. On the other hand, blue and green are more effective if the lights are positioned higher. With use of a "white light" these colors may create an agreeable work environment. Color must be considered in a complex of other colors, and it cannot be isolated. It should be considered with regard to lighting and neighboring colors.

Regarding their optical value and creation of emotions in most people, colors are classified as warm and cold. Worm colors include

red, orange, yellow and their shades. Green, blue and violet are considered as cold colors as well as their shades. It is one of the reasons why a choice of colors in above-mentioned combinations should be used for modification of the perception, i.e. as one of the functional approaches in designing an environment. The purpose of this design is to make health workers and patients as satisfied as they can be. Color, as a mode for increase of efficiency at work place, with simultaneous creation of a relaxed and pleasant atmosphere, must not be neglected.

RESULTS

Many factors were involved, so it seemed much more complicated than one should expect. Not even today, after so many tries, an international certificate for a patent of amniotic membrane use hasn't been obtained. It comprises detailed description of the procedure, as well as contents of any of the three fluids that make an entirety. It describes amniotic membranes with all of their noble characteristics. We are going to present here only a sketch of an entire protocol.

We obtained the placentas from the Clinic of Gynecology. They were obtained from women who were healthy and whose pregnancy was regularly controlled. In such a way we wanted to make sure that by helping on one hand we would not harm in any other way. The Ethical committees of both Institutes approved it.

The first step would be to put a placenta in a fluid number one. It would be left there for twenty-four hours. (Fig. 35) After that, thanks to the fluid the amniotic membrane would be easily separated from the rest of the placenta. The next step is cleaning of the amniotic membrane in a fluid number two. This fluid should further separate amniotic membranes from any unwanted parts that might interfere with further functions of amniotic membranes. In jargon, we say that this fluid removes the dirt from the amniotic membranes. This "dirt" includes coagulated blood and yellow gelatinous substance whose purpose is to keep the amniotic and chorionic part of the placenta together. Amniotic membranes stay in fluid number two for seventy-two hours. After that, they are of a mother-of-pearl color, with a characteristic glow. (Fig. 2) Practically they are ready for use. There are no obstacles for them to, in a presence of a trigger, express their characteristics.

There is one, last step. Fluid number three. This fluid substance, although it comes third, is by no means less important. It keeps amniotic membranes alive, for now up to two months, in a refrigerator, at a temperature of +4° C. (Fig. 37)

This time period, in which amniotic membrane could be kept, with preservation of all of their characteristics is our great achievement. We can work in a great comfort, since we do not have to prepare them every 2-3 days as we used to do.

Every beginning is tough, and not every firstborn is loved by everyone. This treatment with amniotic membranes was greeted rather coldly. Many were suspicious, but not regarding the treatment and its benefits. They weren't interested in a type of treatment, duration of treatment etc. As it is often case in similar situations the worst characteristics come to the surface: Why him? We know him well. It is not possible.

The greatest opponents were those who knew nothing of our procedure, and did not even wish to find out something about it. There had to some public mockery. Even a godfather was found who named the amniotic membranes – slime. It was accepted by many, but never all. I mean colleagues.

In the name of the truth, we must say that the first positive reactions were from a nurse who said: "There is something". They see everything. This one even articulated what she saw. And, slowly everything started to change. Friends, relatives started to bring their children, and once a mother-in-law was treated, a new moment appeared. Mockery was soon over. The considerations were different.

My grandmother used to say: "They will believe once the fairies come in front of their eyes". She was a very old and even wiser woman.

We wanted to present at least one case of treatment for each kind of pathology and ethyology.

Regarding the age of the patients, we will present some that are not interesting for pediatric surgeons. We wanted to emphasize the universality of our procedure regarding human population. Our youngest patient was six hours old, and the oldest was seventy-five.

Let us start with older patients.

At the end of the World war II, during the last fights for the liberation of the country, when the enemies were retreating, our patient, NN, then a twenty-year-old, was wounded in a lower leg, just above the ankle. According to the data from the anamnesis, this wound has never fully restored. In years local situation got worse. According to the patient, this wound was open throughout thirty years. He was never treated for thrombophlebitis or wound infection. Oh my God. That is impossible. That was the usual reaction whenever this case has been presented at a congress abroad. (Fig. 38) The surrounding tissues were changed. A disturbance in lymph and blood circulation that lasted for years led to a condition very similar to elephantiasis. The dominant

lesion was a wound approximately 1,5 cm in diameter, and 1 cm deep. A challenge was so great it could not be rejected.

We will not talk about the duration of the treatment; it will be described in detail in another publication. As the length of a treatment is influenced by several factors:

a) presence of a disease that influences the state of circulatory system (Diabetes mellitus)
b) The time that passed from the appearance of the lesion,
c) The size and depth of a lesion,
d) The localization of it, we think we would be too wordy, without a real need.

Step, by step, we succeeded (Fig. 39). Patient was satisfied.

The following case is also an interesting one. It is a case of burns caused by hot milk spilled on dorsal forearm, hand and fingers. (Fig. 40) Anyone can conclude what intensity of burns it was. We must point out the number of hairs on the skin in the area of burns. On Fig. 41, the recovery of a burned skin is evident. And at last, on Fig. 42 it is clear that hairs are present on the skin around the area of burns, as well as on previously burned skin, and that there is no difference in color between those two areas.

The next case is interested for several reasons. One of the reasons is understanding of the challenges that are yet to appear, and questions that are arising and that, for now stay unanswered.

Our patient, a boy NN was having a non-lymphocitic leukemia. During the course of disease a mechanical ileus developed as a complication. Postoperatively, on the abdominal wall there was a complete dehiscence of a surgical wound. (Fig. 43) The abdominal musculature could be seen at some places. The size of a defect, as well as a possibility of even deeper dehiscence was disturbing. A surgical reoperation would be necessary for God knows how many times. That is why we decided to try to close the defect with amniotic membranes. Considering the age of the patient, results were, as we expected, excellent (Fig. 44, 45, 46).

A therapy of a primary disease continued. This aggressive therapy not only affected the diseased cell, but also, relatively new cells in a postoperative scar. There was dehiscence of peritoneum and muscles. Up to the newly formed cells by amniotic membranes, they resisted it. A ventral hernia developed that was covered by skin only. (Fig. 47) This situation was new to us and we did not know why the

therapy did not affect new cells. We believe that this problem requires a multidisciplinary approach. Our task was done, the wound was closed, peristaltics regular. We prescribed an elastic belt. If the primary disease allows it, one-day ventral hernia will be closed.

Unwanted child usually takes a road that those who do not want it would chose. Our next patient, a little girl sure had angels looking after her, and we may rightly consider us as one of them. Mother was hiding a pregnancy with a corset, and she gave birth to a little girl in total secrecy. After that baby was thrown on to a garbage. The end of the December and the temperature typical for this month were meant to put an end to her life. Soon local abandoned dogs visited the site. Situation is even more serious. What was left of the gluteal and anal region may be seen on. (Fig. 48) A newborn girl was brought to the Pediatric Surgery Clinic in a rather poor general state. She was hypothermic with all the signs of the shock. Our treatment began the third day when the signs of septic shock subsided, although there were macroscopic signs of local purulent infection. After detailed cleaning of the injured area, simultaneously with other forms of therapy we started with the application of the amniotic membranes. We filled every recess and then covered the entire region with them. After ten days there were no signs of septic shock, and local finding was considerably improved (Fig. 49). This was our first case in which an accompanying problem was solved simultaneously with the application of the amniotic membranes. Although it was the first such case it was by no means the last.

In septic states, among other changes, especially in children is a significant trombocytopenia, with tendency toward further decrease of their number. Before our treatment began, adequate therapy was introduces, but thrombocytes were still falling. Second day after we began application of amniotic membranes, this fall stopped, and then gradually a number of thrombocytes was increasing. After ten days their number was within normal range, and till the end of the treatment they would not decrease any further. Why? Another question without the answer.

We might even forget about this, but it repeated in many other patients. Maybe one day somebody else will solve this mystery.

An unsolved problem. He probably requires another approach in solving. Who knows? We continued with the treatment. After six weeks, there was obvious local as well as general improvement (Fig. 50). Treatment ended with the best possible results as always. Visible

rectal prolapse was later surgically corrected. (Fig. 51) This girl is today ten years old.

Next case is another patient with burns, but because of its particularity, we believe it deserves to be presented.

A four-month old girl was put into her crib. It was approximately five years ago. It was time of energy restrictions. Mother put a candle next to a baby's crib. A sudden draft knocked it over. A pillow caught fire. A little girl suffered serious burns on her head, mostly occipital region and face. Baby's head and face looked mummified. (Fig. 52) At one place on a scalp bone was clearly visible. Cheeks, just below the eyes suffered the worst injuries. Mouth was intact. Child was relatively promptly transferred from a smaller city to our Clinic. Baby was in such a general state, that there was no reason for optimism. She was put on assisted ventilation. During the preoperative preparations, there were some serious heart complications. Septic shock has already damaged the heart. Both contractile and cells. Cardiologist was immediately consulted, but the baby was in such a state that nobody could guarantee that she would survive anesthesia. And? Who else could help, but us? We did not need anesthesia. Our procedure is not a painful one. Besides amniotic membranes are capable of performing a "silent" necrectomy. All the necrotic parts are at the following bandaging removed with routine toilette. This procedure is repeated several times, until only health tissue is left. Regarding certain form of analgesia during the application of amniotic membranes the investigations are not completed. We will talk more about it, some other time.

We started with the treatment while the baby was in the Intensive Care Unit. After a week the general state of a little girl started to improve. She was breathing spontaneously. We go on. After two weeks, little girl is a nurse's lap. The local finding on her face is improving. She is normally fed.

Treatment with amniotic membranes continues, but it was not all milk and honey. We were having problems with the above-mentioned lesion where bone was visible was posing problems. (Fig. 53) Although, there were no signs of infection, and the surrounding tissues were healthy (and epithelization starts from the periphery towards the center), the development of the new skin was very slow. We had to be, like many times before, very patient.

One would say: "Zucker kommt zu letzt", others would say: "the evening crowns the day", and we will selfishly conclude: "Pleasure is ours". (Fig. 54)

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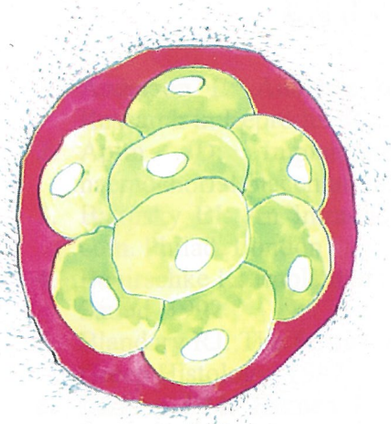
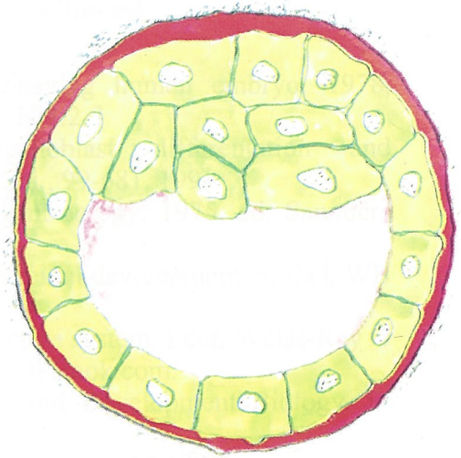
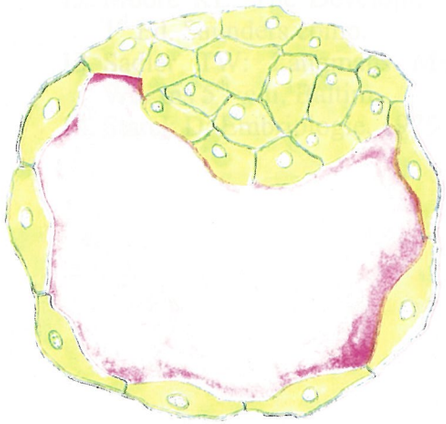


SLIKA 1 FIGURE 1



SLIKA 2 FIGURE 2



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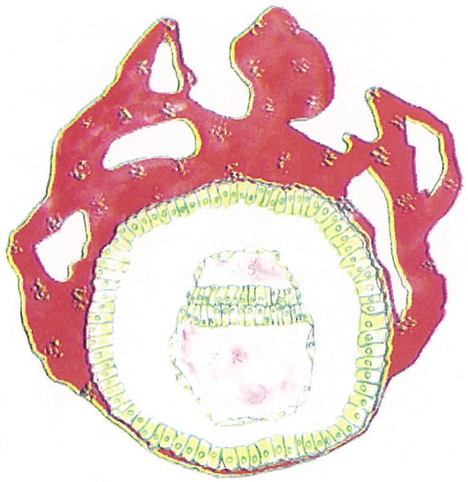

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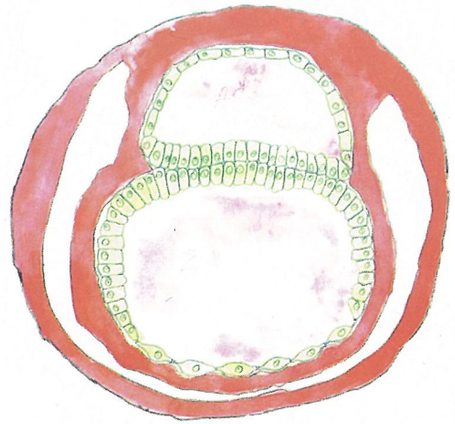
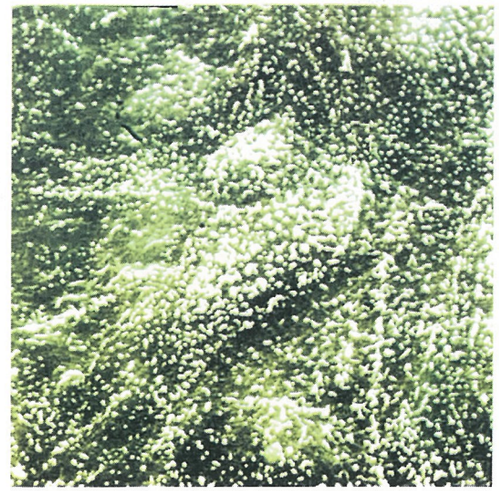
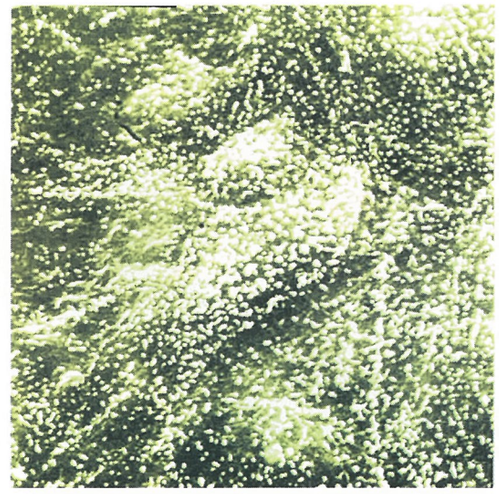


SLIKA 5 FIGURE 5

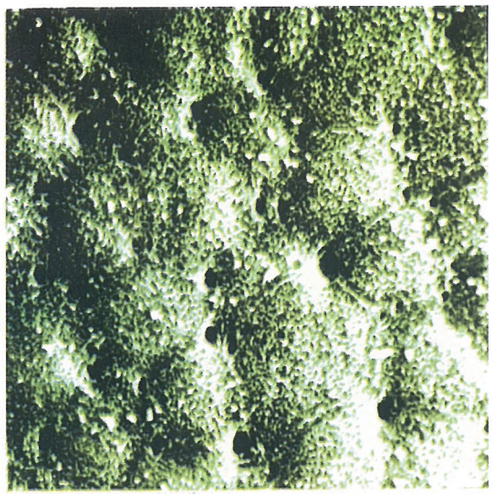


SLIKA 6. Prvi trimestar: uvećanje 1000
FIGURE 6. First trimester: magnification 1000

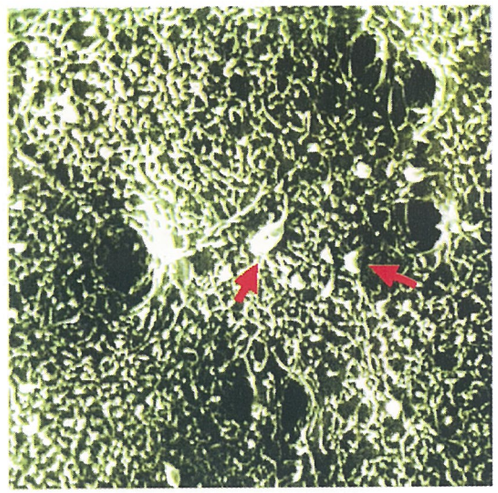


SLIKA 7. Prvi trimestar: uvećanje 2000
FIGURE 7. First trimester: magnification 2000

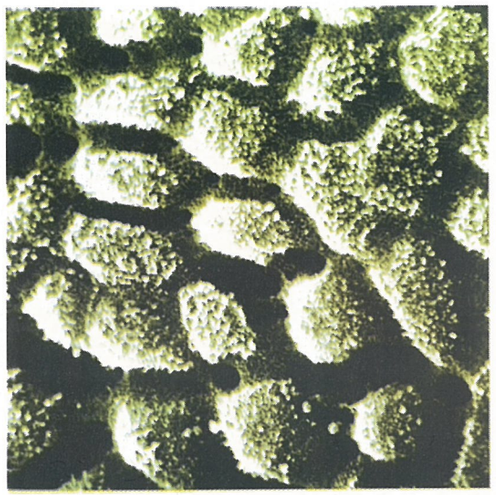
SLIKA 8. Prvi trimestar: uvećanje 5000
FIGURE 8. First trimester: magnification 5000



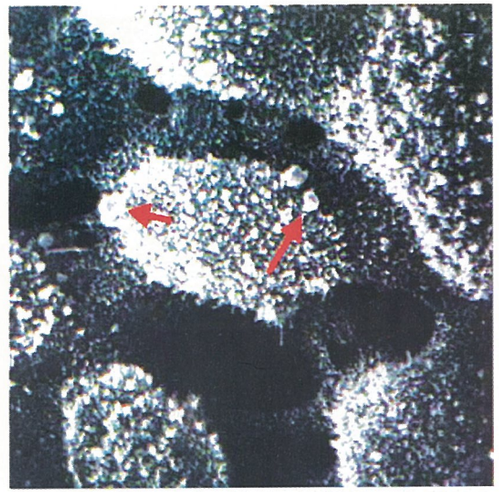
SLIKA 9. Drugi trimestar: uvećanje 2000
FIGURE 9. Second trimester: magnification 2000



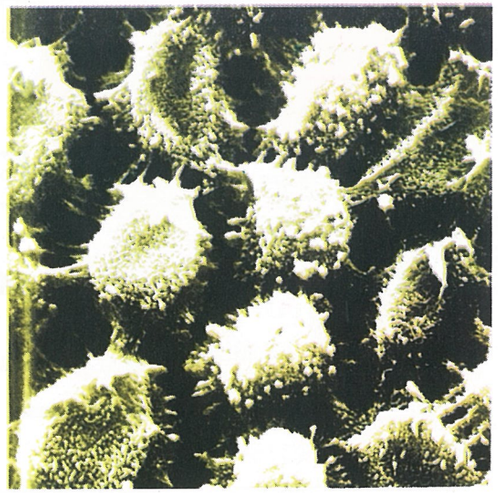
SLIKA 10. Drugi trimestar: uvećanje 5000
FIGURE 10. Second trimester: magnification 5000



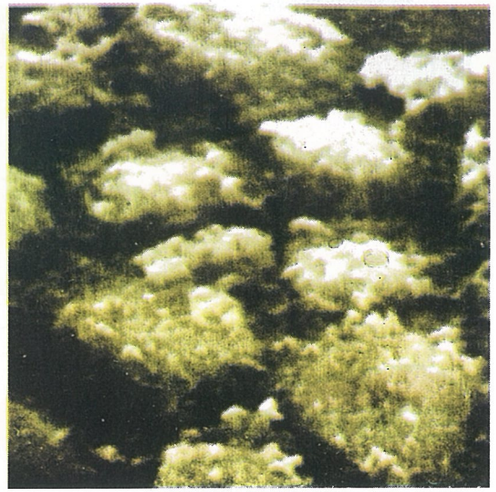
SLIKA 11. Treći trimestar: uvećanje 2000
FIGURE 11. Third trimester: magnification 2000



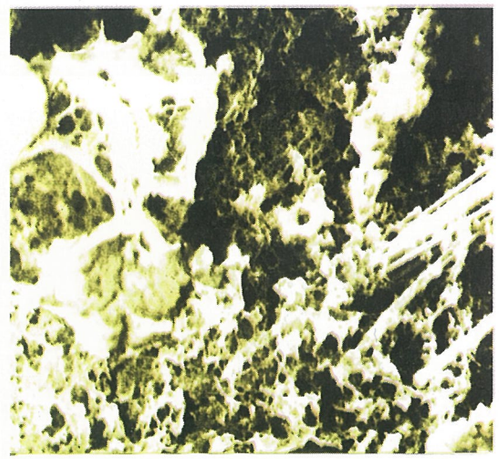
SLIKA 12. Treći trimestar: uvećanje 5000
FIGURE 12. Third trimester: magnification 5000



SLIKA 13. Posle porođaja: uvećanje 2000
FIGURE 13. After delivery: magnification 2000

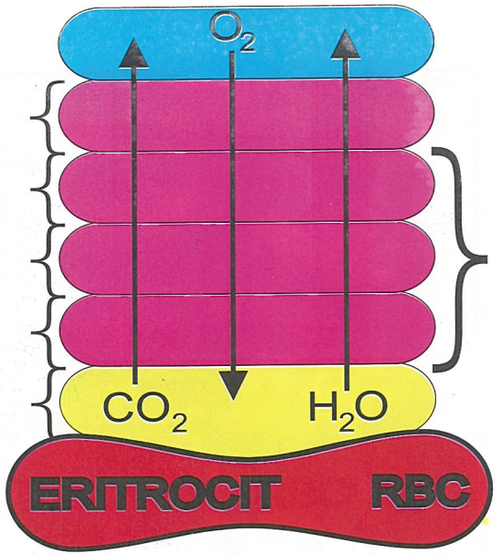


SLIKA 14. Posle porođaja: uvećanje 5000
FIGURE 14. After delivery: magnification 5000

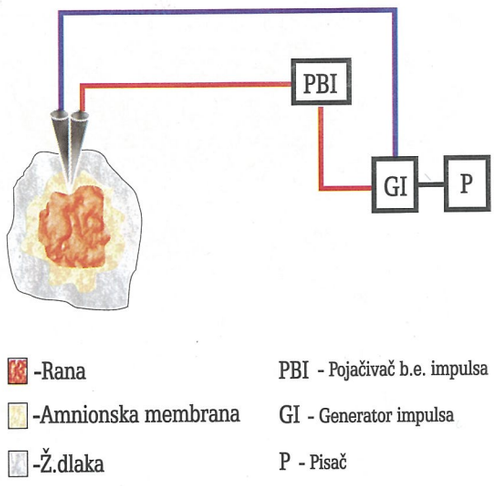


SLIKA 15. Fibroelastična vlakna: uvećanje 5000
FIGURE 15. Fibroelastic fibers: magnification 5000

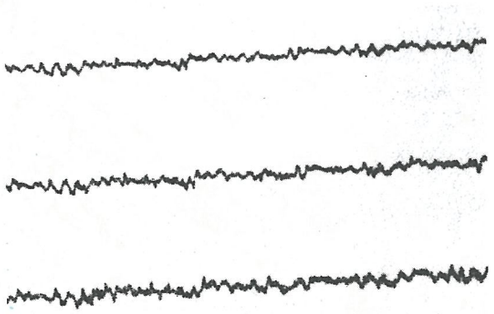
SLIKA 16. Fibroelastična vlakna: uvećanje 10000
FIGURE 16. Fibroelastic fibers: magnification 10000



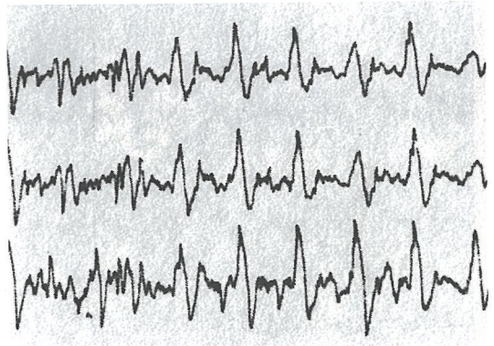
SLIKA 17. Put O_2 do eritrocita.
FIGURE 17. The way of O_2 to RBC.



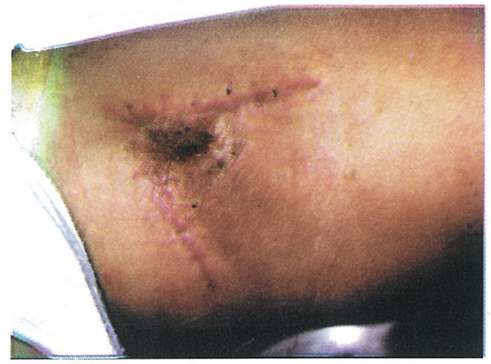
SLIKA 18. Shema aparature za registrovanje bioelektrične aktivnosti.
FIGURE 18. She



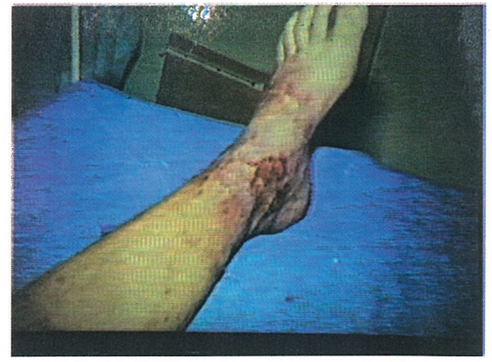
SLIKA 19. Bez bioelektrične aktivnosti.
FIGURE 19. No bioelectric activity.



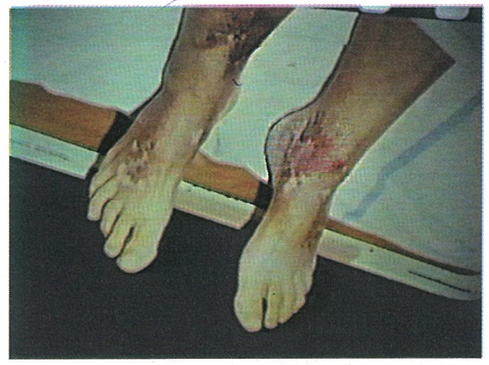
SLIKA 20. Prisutna bioelektrična aktivnost.
FIGURE 20. Bioelectric activity was present.



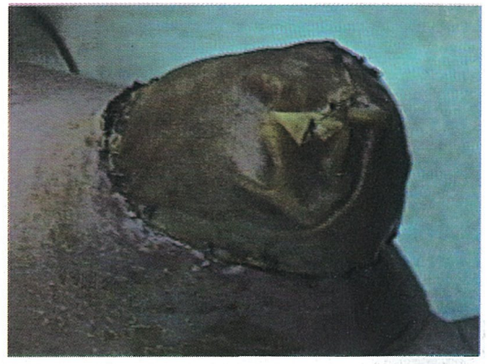
SLIKA 21.
FIGURE 21.



SLIKA 22.
FIGURE 22.

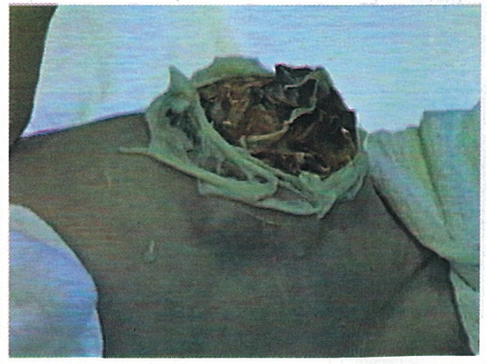


SLIKA 23.
FIGURE 23.

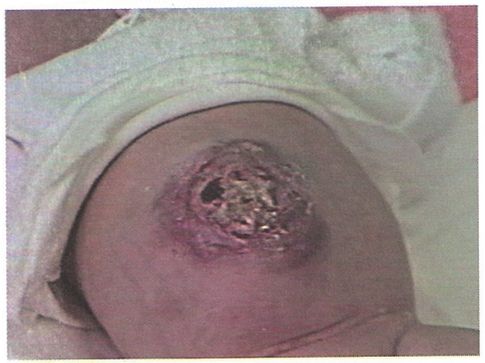


SLIKA 24.
FIGURE 24.

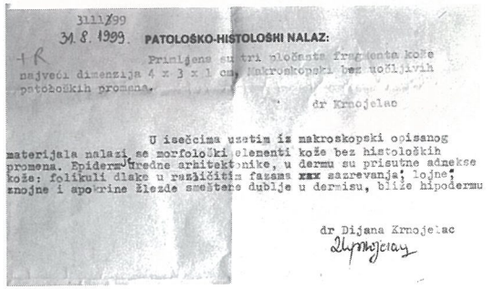
SLIKA 25.
FIGURE 25.



SLIKA 26.
FIGURE 26.



SLIKA 27.
FIGURE 27.



3111/99
31.8.1999. **PATOLOŠKO-HISTOLOŠKI NALAZ:**

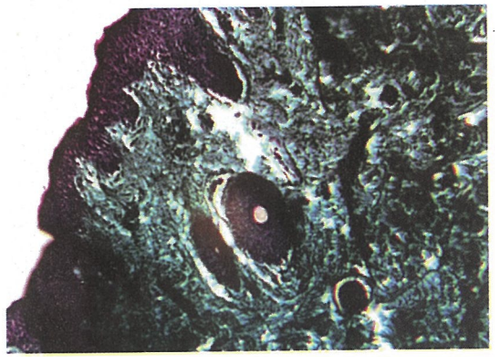
Primljeno su tri pločasta fragmenta kože najveće dimenzije 4 x 3 x 1 cm, makroskopski bez uočljivih patoloških promena.

dr Krnojelac

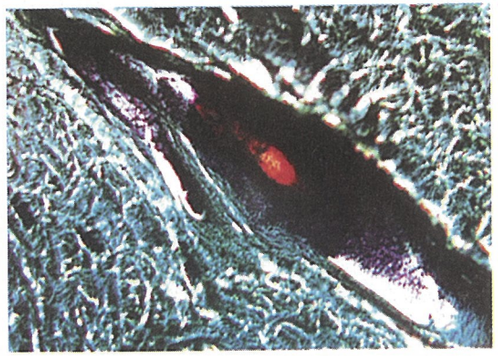
U isečcima uzetim iz makroskopski opisanog materijala nalazi se morfološki elementi kože bez histoloških promena. Epiderm uredne arhitektonike, u dermu su prisutne adneksne kože: folikuli dlake u različitim fazama xxx zazrevanja; lojne; znojne i apokrine žlezde smeštene dublje u dermisu, bliže hipodermu

dr Dijana Krnojelac

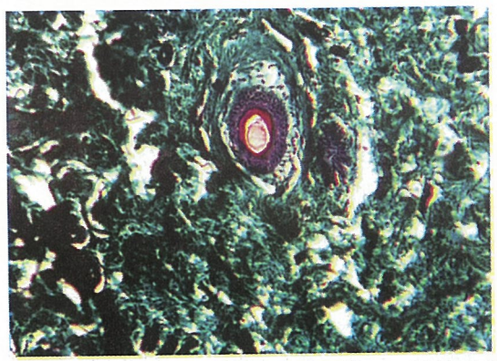
SLIKA 28.
FIGURE 28.



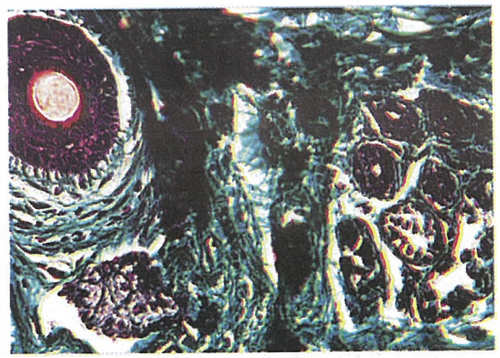
SLIKA 29. **(Mallory 6,3x10)**
FIGURE 29.



SLIKA 30. **(Mallory 6,3x10)**
FIGURE 30.

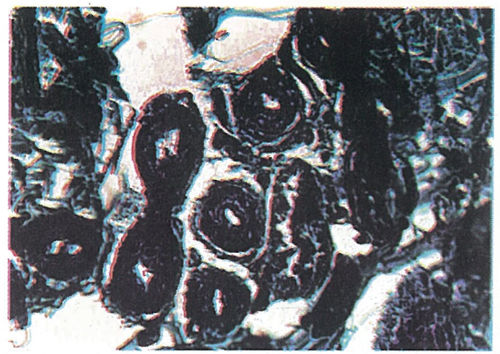


(Mallory 6,3x10)
SLIKA 31.
FIGURE 31.

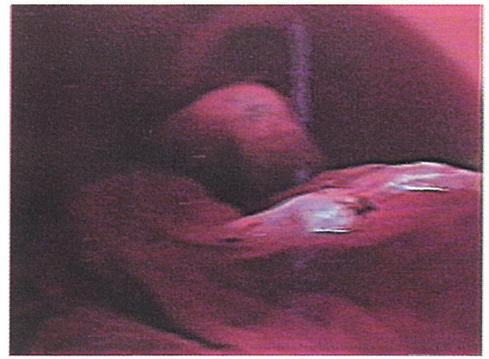


(Mallory 6,3x10)
SLIKA 32.
FIGURE 32.

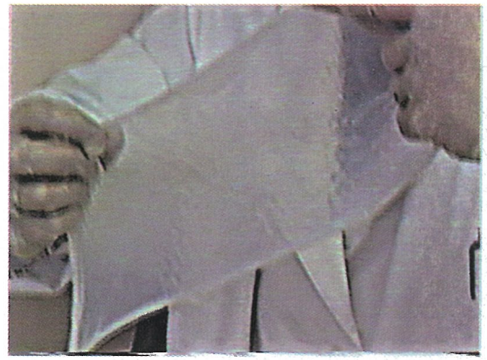
SLIKA 33. **(Mallory 6,3x10)**
FIGURE 33.



SLIKA 34. **(Mallory 6,3x10)**
FIGURE 34.

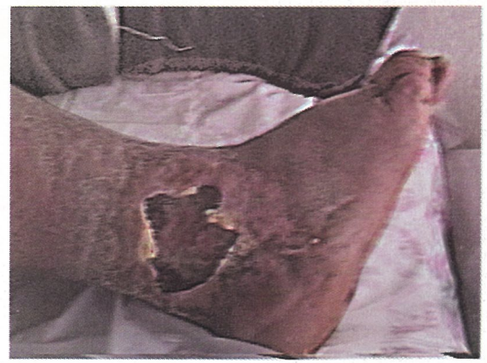


SLIKA 35.
FIGURE 35.

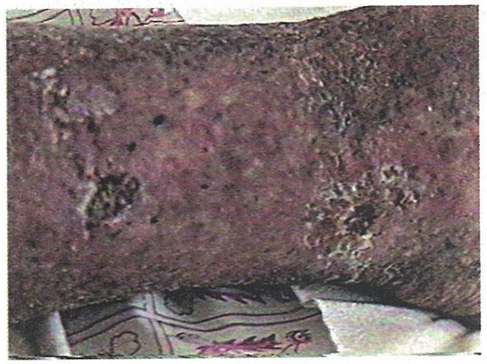


SLIKA 36.
FIGURE 36.

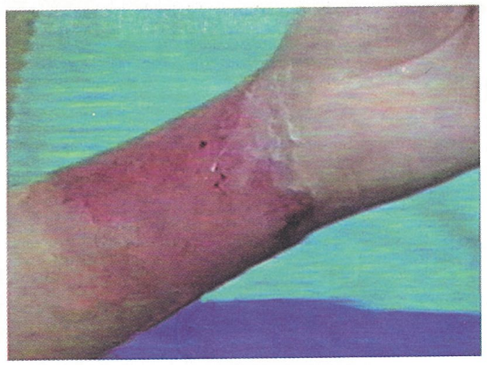
SLIKA 37.
FIGURE 37.



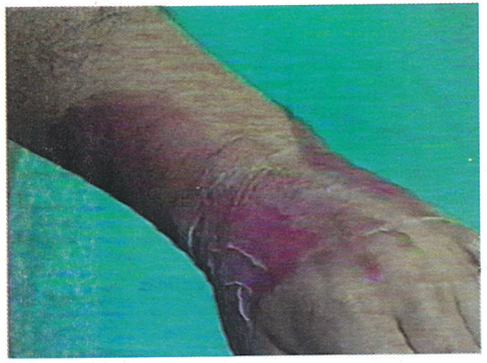
SLIKA 38.
FIGURE 38.



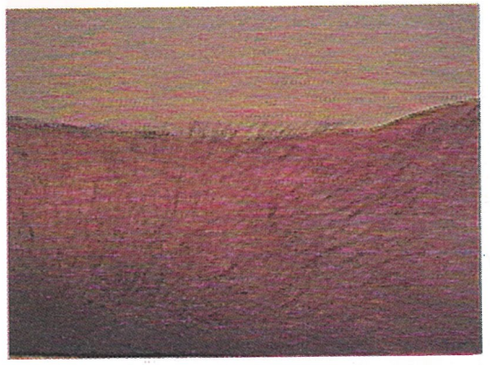
SLIKA 39.
FIGURE 39.



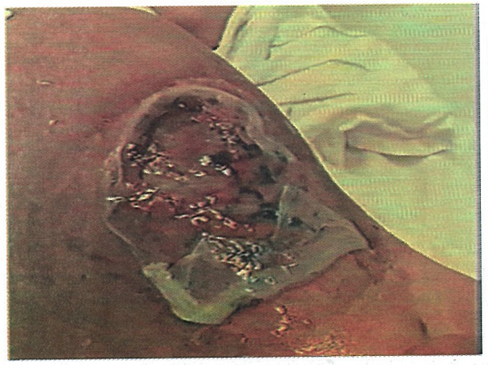
SLIKA 40.
FIGURE 40.



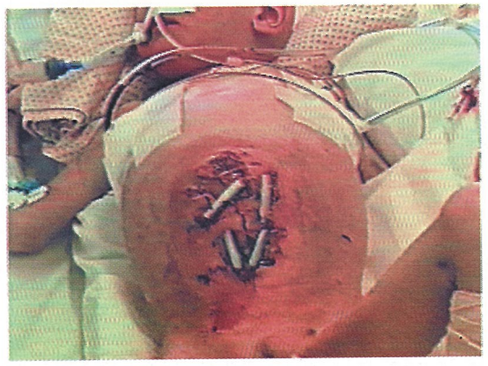
SLIKA 41.
FIGURE 41.



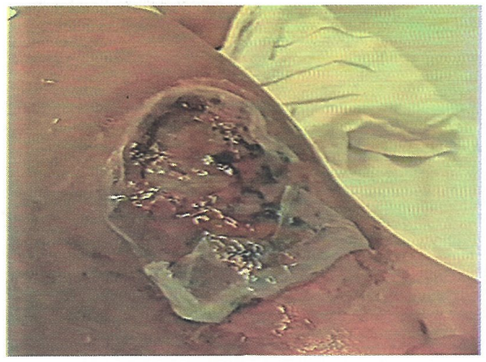
SLIKA 41.
FIGURE 41.



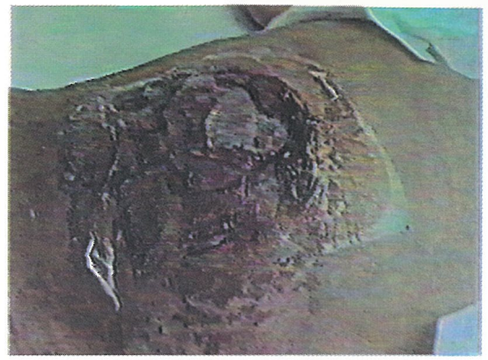
SLIKA 42.
FIGURE 42.



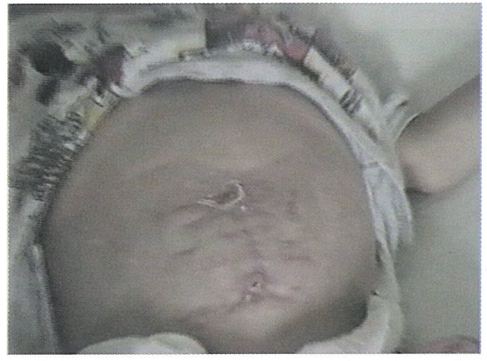
SLIKA 43.
FIGURE 43.



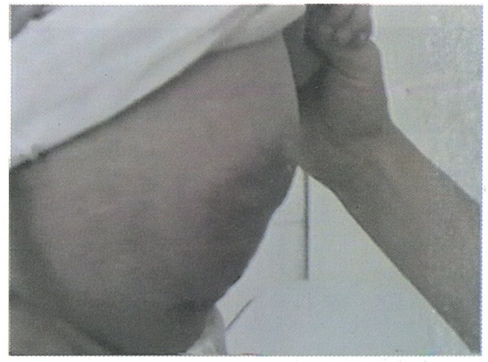
SLIKA 44.
FIGURE 44.



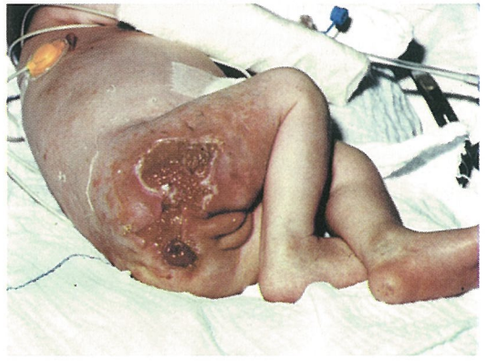
SLIKA 45.
FIGURE 45.



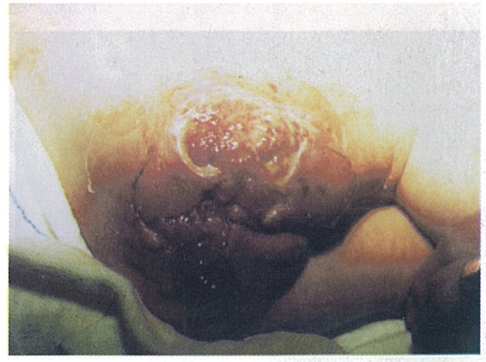
SLIKA 46.
FIGURE 46.



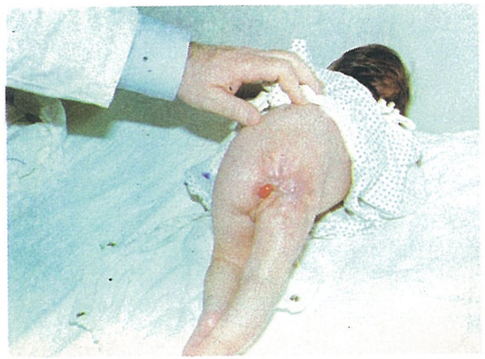
SLIKA 47.
FIGURE 47.



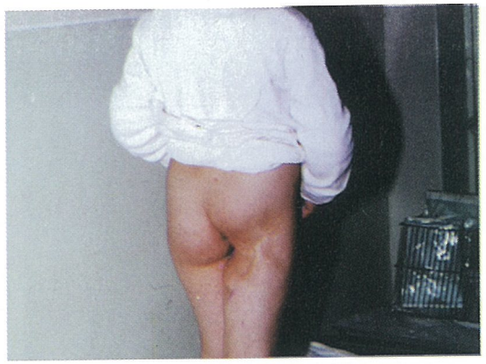
SLIKA 48.
FIGURE 48.



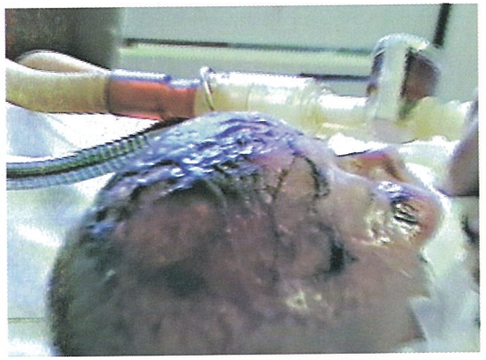
SLIKA 49.
FIGURE 49.



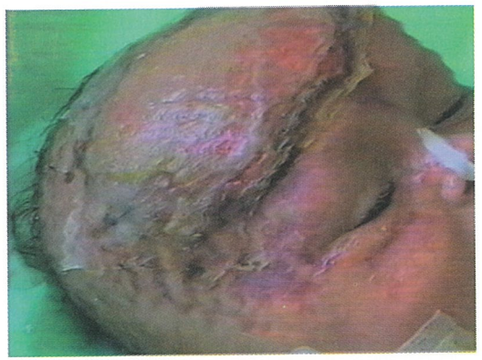
SLIKA 50.
FIGURE 50.



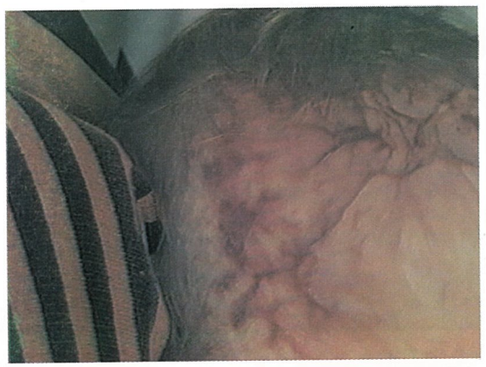
SLIKA 51.
FIGURE 51.



SLIKA 52.
FIGURE 52.



SLIKA 53.
FIGURE 53.



SLIKA 54.
FIGURE 54.