VACCINES: OUR INSURANCE FOR A HEALTHIER LIVING

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Abstract. In the 20th century, vaccination has been possibly the greatest revolution in health. Together with hygiene and antibiotics, vaccination led to the elimination of most childhood infectious diseases and contributed to prolong the disability-free life expectancy that in western societies increased from 50 to 78-85 years [1, 2]. In the 21st century, vaccination will eliminate the remaining childhood infectious diseases such as meningococcal meningitis, respiratory syncytial virus, group A streptococcus, and will address the health challenges of this century such as the aging society, antibiotic resistance, emerging infectious diseases and poverty. However, for this to happen we need to increase the public trust in vaccination so that vaccines can be perceived as the best insurance against most diseases across all ages.
Keywords: vaccines; vaccination; infectious diseases; meningitis

THE NEED TO INCREASE PUBLIC CONFIDENCE IN VACCINATION

Up to very recently vaccines have been developed following the Pasteur example of inactivating and injecting the microorganisms causing the diseases ([3]. These primitive technologies, essentially developed during the first half of the 20th century, led to crude vaccine preparations that have been very successful in the conquest of diseases. However, they were often associated with some safety concerns. For instance, although it was instrumental for the eradication of the disease, the smallpox vaccine, was essentially developed with a technology of 1796, and was associated with cases of generalized vaccinia, encephalitis and myocarditis. The first rabies vaccine, grown in mouse brain cells, was associated with the occasional induction of encephalitis due to vaccine-related autoimmune responses against the brain protein myelin [4]. Even the Sabin oral poliomelitis vaccine, developed during the 1950s, was associated in one case per million with paralytic disease in vaccinees and contacts. Some of other first generation vaccines were also known to exert a significant reactogenicity.

Therefore, it was quite understandable that some public fears were associated with vaccination during the first part of the 20th century. Although, none of these vaccines is used any longer at least in western countries (Table 1), there is still a deficit of public trust, which is hampering the optimal control of some vaccine-preventable diseases. This is due to the perception that vaccines are great tools to fight fatal diseases but may be occasionally dangerous. This is enhanced when the risk of infection is decreasing as a result of generalized vaccination against a particular target disease and it is a paradoxical characteristic of a wealthy society. There is a false perception that some diseases are not or no longer dangerous. For example, many people consider measles as an entirely benign infection and forget the high toll of morbidity and mortality it can cause at our latitudes. Measles epidemics do occur today in European countries due to rejection of vaccination and resulting in insufficient vaccination coverage. Similarly, there was a major epidemic of diphtheria in Russia due to disruption of the health system in former USSR and a reduced level of vaccination. This original sin vaccines are facing fostered the perception that vaccines are great but dangerous, and throughout the entire century people recurrently attributed to vaccination all those diseases of unknown cause. For instance, in the absence of a known cause of the raise of autism in the last decades, many people concluded that had to be caused by vaccination. First, they associated autism with measles, mumps and rubella vaccination. Then, when it was scientifically proven that this association was not there, others associated autism with the use of thimerosal, a mercury compound used until recently to preserve the sterility of vaccines [5]. Now, after the association of thimerosal with autism has been scientifically disproved, there are still some fundamentalists that refuse to accept the scientific evidence and insist that autism is caused by vaccination. Another example is the war veterans. When they come back from the drama of the war with various health problems, such as in the case of the Gulf War [6], people like to attribute their disabilities to vaccines rather than to the brutality of the war. Similar clinical pictures were observed in the soldiers fighting in the Secession War, at a time when vaccines did not exist yet (except one). Another phenomenon that has happened during the last century is the increase of allergy in developed countries. Being of unknown cause, many have associated it with vaccination.

NEW TOOLS TO INCREASE PUBLIC CONFIDENCE

The perception that vaccination may be dangerous has been a major concern for vaccine developers and regu-

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Table 1. Vaccines associated with safety concerns that are no longer used	
Smallpox	*generalized vaccinia, encephalitis, myocardatis*
Sabin oral polio	*paralytic disease in vaccinees and contacts*
Measles high dose	*increased mortality from all causes in females*
Difteria Pertussis and Tetanus - DPT (whole cell Pertussis P)	*febrile seizures and encephalopathy (disproved)*
Rotavirus (Rotashield)	*intersusception (bowel obstruction)*
Bacillus Calmette-Guérin - BCG (tuberculosis)	*disseminated BCG infection*
Thimerosal	*autism, neurodevelopmental delays (disproved)*

Table 2. New tools will continue to improve vaccine safety
Screening for sequences homologous to proteins encoded by the human genome to remove sequences mimicking self antigens
Immunohistochemistry to check cross reactions with human tissues
Multiple cytokine induction to profile the immune response Th1/Th2
Profile of cytokines induced by novel adjuvants and vaccines to predict potential of autoimmunity, induction of expected immune response
Availability of well controlled cell lines to avoid use of undefined non controlled cell substrates for vaccine production such as brain extracts (rabies), whole animals (smallpox), primary monkey kidney cells (polio Sabin). These may induce autoimmunity (brain extracts) or contain undefined viral/prion contaminants
Control of cell lines for prion proteins
Simulation of immune response data from different immunization regimens
Mathematical models of disease, biomarker, immune response kinetics, efficacy, and safety
Mouse-human cross-over studies for understanding role of TLRs
Animal and in vitro models to test disease enhancement (RSV, influenza, measles)
Large phase III and phase IV studies to exclude statistically rare events

latory agencies that during the last few decades have been working hard to improve vaccine safety. First, all those vaccines associated with major safety concerns, such as smallpox, oral polio, whole cell pertussis, high dose measles, eccetera have been discontinued or are going to be discontinued soon (Table 1). Second, the new technologies allowed to minimize the risks associated with the new generation of vaccines. Highly purified components of known molecular entity, recombinant antigens, polysaccharides conjugated to purified proteins, new antigens discovered by genomics allowed the development of a new generation of molecularly tailored vaccines that are well characterized and intrinsically safer than the crude preparations of the 20th century. Live-attenuated vaccines that in the past were derived by random passages and mutagenesis today are replaced by strains with molecularly designed attenuating mutations or by vectors designed to immunize but not replicate. Finally, in the era of the technological revolution, we have plenty of new tools to predict safety risks of new vaccines.

For instance, screening the vaccine candidates for sequence homology with the human genome allows identification and removal of those antigens that may have a risk of inducing autoimmunity that have been often a problem in the past. New tools that will continue to increase vaccine safety are listed in the enclosed Table 2.

However, elimination of the vaccines with safety concerns and minimization of the safety risks in present and future vaccines is not going to be enough to gain the public trust in vaccines. We need to educate people that, even in our rich countries, infectious diseases are still around us and that they are a real thread if our alert is discontinued and if a preventive approach is not undertaken. Therefore, people need to think about vaccines when they are healthy, because vaccination is the best insurance against diseases that will be available in the 21st century. In other words, we need to remove from the mind of people the perception that vaccines are dangerous and are to be avoided, since this mindset is a relict of the 20th century and is not true any longer. In this respect, health policy makers should also actively pursue this perception starting from the consid-

eration that vaccination has contributed more than any other medical intervention in the reduction of human diseases.

21ST CENTURY VACCINES

Vaccines were developed in the 20th century to address the needs of a society where morbidity and mortality caused by infectious diseases in the early years of life was the major health challenge. Thanks to the vaccine success, the 21st century society lives longer and we should consider how vaccination can be redesigned to meet the needs of the health care systems that are struggling with the new reality.

Today vaccines address mostly infant diseases and we have >10 vaccines recommended in western countries for infant vaccination, one (papillomavirus) recommended in adolescent women and one (influenza) recommended in the elderly. In developing countries there are only 5 recommended vaccines, all for infants. However, thanks to the technology revolutions, genomics and the great progress in immunology, today it is possible to design vaccines able to prevent many diseases of modern society. For instance, we could think at a vaccination plan where before birth, pregnant women receive a boost vaccine during the third trimester to generate and transfer to the newborns antibodies against those diseases of the first few days or months of life, such as GBS, tetanus, hepatitis B, meningococcus, pneumococcus, RSV, influenza, using the strategy to protect newborns selected by nature during human evolution. Infants would then be vaccinated starting from 4-5 months of age to build their own active immunity. The next vaccination event would be in adolescents, who would receive those vaccines that prevent those chronic diseases and cancer associated with infectious diseases such as papillomavirus, which is associated with ovarian cancer, hepatitis C, which is associated with liver cancer, chlamydia which is associated with infertility, and those vaccines that would be useful during pregnancy such as CMV, GBS. Some vaccines like CMV and EBV have also the potential to slow the aging of the immune system, one of the major problems beyond the age of 50.

Finally, approximately at the age of 50, when the immune system starts to wane vaccination could be used to fight, delay or eliminate those diseases that are typical of modern aging society. These are resurging infectious diseases such as influenza, pneumococcus, RSV, those diseases associated with the risk of hospitalization (mostly nosocomial diseases) and cancer.

Finally, there are numerous other health risks in modern society that could be minimized by using vaccination as an insurance. Prevention of those infections caused by antibiotic resistant microorganisms that are a major threat during hospitalization such as Staphylococcus aureus, Pseudomonas aeruginosa and Clostridium difficile is a realistic goal. Prevention of pandemic influenza by appropriate pre-pandemic vaccination using vaccines with established safety record is a second one. Vaccines for travelers to areas with diseases not longer present in the country of origin is a third one. In conclusion, there is strong rationale to propose vaccines as the best insurance against the risks of diseases associated with the modern society.

VACCINATION FOR LOW INCOME COUNTRIES

Vaccines can also make a great contribution to reduce and possibly eliminate poverty from our planet. In developing countries, many vaccine-preventable diseases exact a huge toll from the income of each family and throw them into a downward spiral of poverty [7-10]. Currently, five vaccines are recommended for routine use in developing countries, against >10 in western countries. In addition, there is no mechanism to develop those vaccines needed only in developing countries and for which there is no market. Innovative mechanisms to make vaccines available to the people in developing countries must be a priority in the 21st century for western societies and for the governments of development countries. Few projects such as the Advanced Market Commitment, the Meningitis Vaccine Project and the Novartis Vaccines Institute for Global Health [8] are a few promising examples of initiatives that can help funding, developing and deploying vaccines to the poorest people. The new technology is going to offer in the coming years very promising perspectives in the development of "unconventional" vaccines, i.e. vaccines against non infectious diseases (such as cancer, Alzheimer, diabetes, drug addiction, hypertension, autoimmune diseases, etc), to extend then the potential for vaccines to improve the quality of our lives. In any case, a concerted action, involving academic environments working in vaccine research and medical teaching, vaccine manufacturers, public health policy makers, governments, eccetera, will be needed if we want to eliminate poverty from our planet.

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From Lucy to language: our place in nature

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My long-time experience in Africa has not only rewarded me with exceptional discoveries like LUCY, but also given me time to reflect on what I have discovered about our place in nature, and especially what the past tells us about ourselves.

When I was preparing today's comments I focused on one question: What has LUCY taught us since she first made her debut some 32 years ago?

As a young scholar my primary goal was to find fossils, the alpha evidence for human evolution. I was more fortunate that most, but I have grown to realize that while the bones are the primary quarry, it is the message they bring us from some long forgotten time that is most important of all.

Today I am increasingly interested in what LUCY tells us about where we fit into the rhythms of the earth. Is there something that connects us to nature, that transcends time and distance?

Just think, no other species has such a fascination with its origins as we do. No other species has the means to explore and discover fossilized clues to its beginnings. No other species has so much to learn from its past. But, sadly, no other species has so much to lose by ignoring its origins. I trace the roots of this philosophy back to my youth when I read Thomas Henry Huxley's book Man's Place in Nature (1863). I was intrigued by his proposition that humans are just another species, a result of the same process of evolution that brought about all other forms of life. I wanted to find the fossil clues, the Rosetta Stones for helping us decipher the secrets of human origins.

As a paleoanthropologist I knew that the story began in Africa. Darwin was right about that, our closest living relatives, chimps and gorillas live there today, he surmised we must have had a common ancestor.

I know some of you have been on Safari in Africa talk of being bitten by the Africa bug. Africa gets into our blood we say, but as the birthplace of the human family, Africa is already in our blood, in our bones and even in our genes.

Our DNA is 99% identical to that of the African chimpanzee. Imagine if we received a message from some far corner of the universe that astronauts found a hairy, quadrupedal creature so closely related to ourselves. We would be spending millions trying to understand what we could learn from this creature about ourselves. Today the chimp's habitat is quickly disappearing and who knows how many of the mountain gorillas still survive in the Rwanda.

Most of my research has focused on Africa's Great Rift Valley, a stunningly beautiful place. A jagged dueling scar on the planet's face that's the result of the slow grinding and shifting of the earth's crust, a tectonic tug of war.

The Great Rift Valley is a place of pilgrimage for paleoanthropologists; Hadar, Ethiopia is one of its shrines. This is where I found Lucy on November 24th, 1974. She's become an ancestral ambassador of sorts. Introducing me to more people than anyone alive. I always reminded of how much better known she is than her discoverer.

LUCY has been an incredibly successful ambassador. Bringing together people of very diverse backgrounds. We work with Muslims. The Koran doesn't have much of a place for evolution, but the nomadic, Afar understand that the world knows Lucy and they are proud that the first human was an Afar.

Lucy is important for many reasons and continues to be the subject of study by scholars world-wide. She has become an icon for paleoanthropology. The touchstone and benchmark by which all other fossil discoveries are judged. Older than, younger than, more complete than, etc. It is endlessly fascinating to me that after so many years and the discovery of much older and even more complete finds, Lucy is still the focal point of discussion among anthropologists and the lay-public alike.

At a dinner party when someone brings up the idea of human ancestors and people admit they know nothing about human evolution, someone mentions Lucy and people perk up, as if they have brought up the name of a relative and in a funny way she really is a relative, for all of us.

Lucy is the link to our common origin with the African

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apes. But Lucy has an even more important message for us; she is a link to the natural world. The more links we find, the closer we see ourselves to the natural world. We are part of the continuum of life. The more we learn about our closeness to Nature the more we see ourselves as part of Nature and not distinct from it. We cannot turn our backs on the very natural world that created us and all other life on this planet.

Discoveries over the last three decades have shown us that the tree of life is just as Darwin predicted; a tree with many branches, all of which went extinct, except the one that led to us. In this regard I find it fascinating that still so many people think of human evolution as a straight line from ape to angel; and we know who the angel is supposed to be.

Think about for a minute, I am sure you have seen those little depictions of creatures evolving from all fours to upright humans. And who is the pinnacle of human evolution,the White European Male!

Why?

Because white European males drawn those pictures.

I love to say that Lucy is the woman who shook up man's family tree!

Tonight I am not going to debate who's version of the human family tree is the right one, for there are as many interpretations as there are anthropologists. But anthropologists agree on one primary and inescapable conclusion, Africa is the cradle of humankind.

All the major steps in human evolution: bipedal walking, the first stone tools, brain expansion, the first appearance of Homo erectus, the first Homo sapiens, all premiered in Africa.

So, no matter which branch of the human family tree you grasp, the roots, inevitably lead back to Africa, the continent of our origins. We, as a species are united by that past and by accepting this conclusion we realize, in spite of superficial difference, such as skin color, we are one, all with a common origin.

Let me return to extinction. Extinction is the rule in the evolution of life, not the exception. 99% of all species, since the beginning of life on this planet, have gone extinct!

Here is an important message for all of us. Just because Lucy's ancestors stood up four million years ago didn't mean that modern humans were destined to arise, we were not inevitable.

We and our ancestors were susceptible to the same vagaries of climate and environmental change, we could easily have gone extinct, also. As my later colleague Stephan Jay Gould said, if the tape is rewound and played again, there is no guarantee that we would be here tonight talking about OUR origins.

Modern humans are unique, don't' get me wrong, we are separated by a huge gulf from our closest living relatives, the African apes. Sure they use very rudimentary tools and in a laboratory can learn an amazing number of words, but no other species composes operas, writes poetry, paints great art, contemplates the future, or ponders the past, only us.

My work has led me to appreciate our place in the natural world. We belong here as much as any other species, but we have inherited an awesome responsibility as the most intelligent creature on the earth. Whether we like it or not, we are the guardians of the planet! A frightening, but unavoidable conclusion.

Lucy » skeleton (AL 288-1) Australopithecus afarensis,

Humans are consumed by an egocentric view that we are the pinnacle of evolution. But, looking at ourselves in the broader perspective of evolutionary time provides us with a very different and humbling point of view.

Just imagine for one moment, LUCY's species, Australopithecus afarensis, walked the earth for at least a million years, ten times longer that Homo sapiens has existed. Here we sit in the 21st Century and futurists are asking if we as a species will survive the first few decades of the third Millennium. BUT, when we look though the lens of time, to equal the time that LUCY's species spent on earth, modern humans will have to survive not just the next century, but live for another 9,000 centuries beyond that! What a humbling notion!

I am convinced that understanding the ancient, evolutionary legacy that still resides in our modern psyches is vitally important, because such knowledge undoubtedly will play a role in our survival, indeed the survival of all life on this planet.

We may live in modern cities, but in may respects we still operate with a stone-age mind. Our rapidly accelerating technological evolution has far outpaced our genetic evolution that is virtually identical to that of our hunter - gathering ancestors - there is a vital imbalance here.

Can a psyche that evolved in the Paleolithic successfully cope with the modern world? Will we overcome the primitive urges and replace them with enlightened compassion for our fellow man?

While we may be the most intelligent, dangerous and cooperative animals to walk the earth, we are not the final resting point of life's three billion-year adventure. Our species is still an evolutionary work in progress.

The future is upon us now; we have the power to influence the outcome of all life on this planet. It is my hope that is egocentric species will become the introspective species and make choices that will not only benefit us, but all of our fellow travelers in the great mystery of life.

I am hopeful that an increased awareness of the deep biological roots we share with one another, in fact, with the entirety of nature, will point us in the direction of our best dreams rather than our worst nightmares...

Electric Soldani
Fashionable earthquake theories in late eighteenth century Tuscany

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Abstract. Ambrogio Soldani wrote on earthquakes only once, after Siena was struck by one in May 1798. Unlike Soldani's earlier works, the Relazione del terremoto accaduto in Siena il dì 26 Maggio 1798 is not original either for what concerns the seismogenic theory underlying it or the treatment of data. However, it gives the first systematic overview ever made of historical seismicity in the Sienese district. Thus, even in the field of descriptive seismology, Soldani managed to become a groundbreaker, after all.

Fire vs. Electricity

In the second half of the eighteenth century, earthquakes had become a very controversial subject. An ongoing debate about what exactly it was that caused earthquakes to happen had split the international scientific community into the two warring factions of the *Firists* and the *Electricists*.

The former – whose ranks numbered luminaries such as Immanuel Kant and Sir William Hamilton – subscribed to the ancient seismogenic theory set forth by Aristotles, according to which earthquakes are a product of the dynamic processes at work within the insides of the Earth. *Pneuma* (Greek for 'breath'), a kind of underground wind heated by the unquenchable fires burning at the very core of the Earth, is the ultimate earthquake-generating force. Until *Pneuma* remains free to blow around within the vast cavities existing underground, nothing untoward happens on the outside surfac of the Earth. If, however, *Pneuma* does get trapped within some fissure or tunnel in the layer of rock enveloping the planet, then it is forced to rise up- and outwards through these narrow channels, causing the earth surface to quake and shake as it blows out and disperses in the atmosphere. It is interesting to note that faint but unmistakable traces of this venerable theory do still survive nowadays in the popular belief that earthquakes are more likely to occur in hot weather or that localities whose underground is hollow are less prone to earthquake damage (unfortunately an opinion which experience does not bear out).

If *Firists* took an ultra-conservative stand, on the other side *Electricists* went as progressive as they could be. In the mid 1700's electricity was a byword for progress. The scientific studies of electricity and magnetism started in the seventeenth century had produced a considerable amount of empirical known-how and machinery for demonstrating electrical phenomenaand electricity had become popular, finding expression in fashionable drawing room entertainments, therapies, quack treatments, and a few new ideas for pornographers. On a more serious plan, speculations on the still mysterious "electric fluid" had given rise to a new seismogenic theory, according to which the cause of earthquakes was to be looked for over the ground rather than under it, in the state of unbalance between terrestrial and atmospheric electricity. As long as both earth and air were neither too dry nor too damp their electric fluids would be well balanced and nothing untoward would happen. However an unbalance between fluids (such as could ensue from a prolonged drought or an excessive rainfall) would generate a friction between the atmosphere and the earth's surfaces and therefore set off an earthquake. Impending earthquake could be forecasted by a close observation of atmospherical phenomena (vapours, fogs, oddly-shaped clouds, lightnings, fireballs and so on) and sudden climatic or temperature changes. For this reason, electricist literature took special care to recording the occurrence of these phenomena.

Italian Electricists at work

Obviously enough, the electric earthquake theory had to be proved. During the second half of eighteenth century, Italian electricists had many opportunities to set down to this task, as a great many earthquakes occurred in Italy in these years. Among them there are one of the top seismic disasters on record in Italy (the 1783 Calabrian earthquakes), the strong earthquakes which caused heavy damage in extended areas or important towns (1751, Gualdo Tadino; 1781, Faentino-Forlivese and Cagliese; 1786, Riminese), low-intensity seismic sequences which went on for several months generating great panic if not much damage (1779, Bologna:

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1785, Umbro-Marchesan Apennines; 1785-1786, Piediluco) and comparatively minor earthquakes that caused moderate damage in circumscribed areas (1781 and 1787 in the Sienese district). Each of these events gave to supporters of the electric earthquake theory the occasion to make detailed observations and publish them, as pamphlets, books or magazine essays (Fig. 1). By and large, all these literary efforts are planned on the same lines, starting with a meticolous relation of the context in which the earthquake took place and proceeding to record the exact date, time and circumstances in which earthquake shocks manifested themselves; the effects they wreaked on buildings, people and the environment; the extent of the area within which they were observed and, last but not least, the weather conditions, temperature and atmospheric phenomena (if any) that preceded or accompanied them, the latter - as it will be remembered - being intended to give a confirmation of the theory espoused by the author.

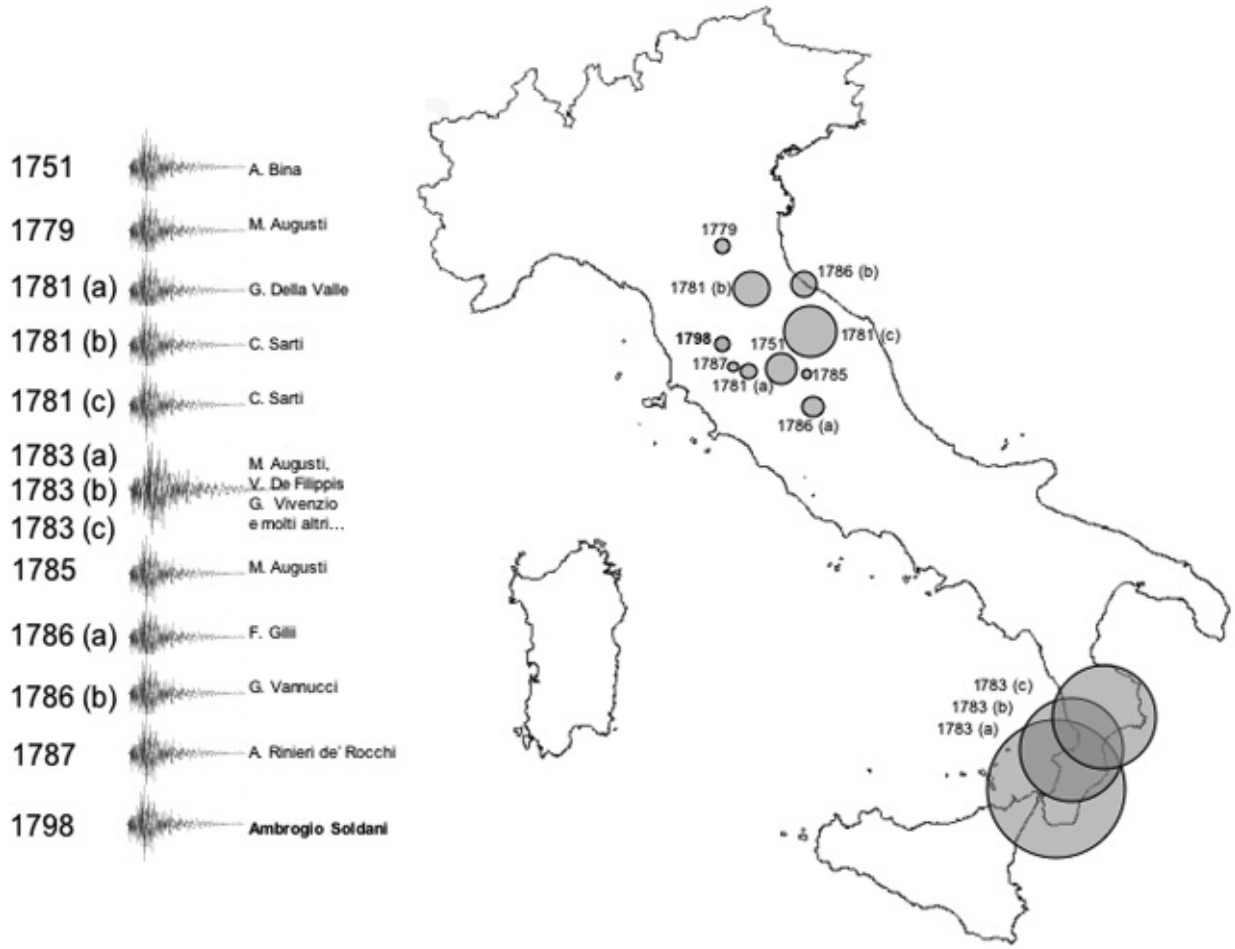
The remarkable similarity existing between so many distinct works should not be wondered at. After all, their authors were part of the same intellectual network, which spread all over Italy and abroad. Often they were personally acquainted or at least had common friends; they read the same magazines, corresponded and sent each other copies of one's own works. Ambrogio Soldani was a full-title member of this erudite milieu, together with other ecclesiastics such as the Olivetan monks Michele Augusti and Piermaria Rosini, the Franciscan friar Guglielmo Della Valle and the *arciprete* Giuseppe Vannucci, university professors such as Cristofano Sarti and mere *dilettanti* like the Sienese nobleman Antonio Rinieri de'Rocchi, who in 1788 suggested to the Fisiocritic Academy that seismic risk in the Sienese Crete could be reduced by planting in the ground iron "earthquake rods" through which earth's electric fluid would be harmlessly discharged in the air.

Thus, when a biggish earthquake struck Siena, on 26 May, 1798, and Ambrogio Soldani was finally in the position to make his contribution to the electrical debate, he had only to follow in the footsteps of many friends and associates, to produce a work written according to a well-established pattern.

SOLDANI'S CONTRIBUTION TO SEISMOLOGY

The *Relazione del terremoto accaduto in Siena il dì 26 Maggio 1798* was printed by the Sienese typographer Giuseppe Pazzini Carli a few months after the earthquake it described. Divided into six "letters", dated between 9

Figure 1.
Electricists at work. Fifty years of earthquakes in Italy and the people who wrote about them (1751-1798).



June and 17 July 1798, the *Relazione* includes a detailed chronicle of the recent earthquake (based on Soldani's own impressions and on the evidence of several reliable eyewitnesses), a précis of the distribution of damage in the urban area, metheorological and geological observations, and a lists of the *«earthquakes that at various times have previously struck Siena»* together with short descriptions of their effects, as derived by an assortment of printed and manuscript historical sources dating back as far as the thirteenth century.

It is with the compilation of this list of historical Sienese earthquakes that Ambrogio Soldani managed to make a unique contribution to Italian seismology. In process of time the electric earthquake theory would be discarded and become no more than a quirky episode in the history of science. Soldani's chronological list of historical earthquakes, on the contrary, did live on.

Descriptive seismology (the collection of evidence of the macroseismic effects of earthquake past and present) is an avocation that attracted many followers in Italy, the earliest of whom seems to have been humanist Giannozzo Manetti that inserted a list of historical earthquakes in his *De Terraemotu* treatise, written in 1457. Thus, along the centuries, a large corpus of descriptive earthquake compilations was accumulated in Italy. When seismologist Mario Baratta started putting together his monumental history of *I terremoti d'Italia* (1901), he relied to a large extent on this considerable national tradition of historical earthquake studies and Ambrogio Soldani's chronological list became its main source of information for Siena and the Sienese territory for the pre-1800 period. Later syill, in the 1970's, Baratta's opus did become, in its turn, the main source of raw data for the compilation of the first generation of Italian parametric earthquake catalogues, an indispensable tool for setting in train the complex procedures necessary to assess the seismic hazard of the country, locate its seismic zones and effect a correct seismic classification of the national territory. Thus, even in a field such as seismology, in which he was not a forerunner – as he was in micropaleontology and in meteorite studies- the outstanding personality of Ambrogio Soldani managed to express itself with a lasting contribution to general knowledge.

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FREE RADICALS, OXIDATIVE STRESS AND ANTIOXIDANTS

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The topic of oxidative stress is deep-routed in some biological paradoxes.

Oxygen is necessary for the life of aerobic organism and acts as terminal oxidant in the mitochondrial respiratory chain, that is the main source of energy. However the univalent reduction of oxygen leads to the formation of one of the reactive oxygen species, the superoxide anion ($O_2^{-\bullet}$). In the mitochondrial electron transport chain about 95% of the consumed oxygen undergoes the cytochrome oxidase catalysed tetravalent reduction to H_2O, but over than 3-5% of the consumed oxygen is released in the form of reactive species, such as $O_2^{-\bullet}$ and hydrogen peroxide (1). Thus reactive oxygen species are produced even in the normal respiratory chain.

Iron is necessary for life too, being an essential component of vital enzymes, such as cytochromes, cytochrome oxidase, catalase and of equally vital complexes such as hemoglobin, myoglobin, ferritin, etc. However iron, when released from these complexes in a free form, as it will be seen below, can react with active oxygen species to yield additional oxy-radicals (through the Fenton reaction). Iron is therefore potentially toxic to biological structures.

Similar considerations are true for cytochrome P_{450} (cyt P_{450}) a key component of the mixed function oxidase system, which is utilized, in its microsomal electron transport chain, by the drug metabolising system. This system, that is basically involved in the detoxification of xenobiotics, can produce harmful radicals or electrophilic intermediates from xenobiotics themselves.

The mechanisms of free-radical induced cell injury include, in summary, (i) reactions with nucleic acids, nucletides, polysaccharides, protein and non protein thiols (thiol oxidation); (ii) covalent binding to membrane components (proteins, lipids, enzymes, receptors, transport systems, etc.); and (iii) initiation of lipid peroxidation, schematically illustrated in Fig.1 (see the legend for summary explanations).

Lipid peroxidation of cellular membranes has been suggested as a common mechanism in a large number

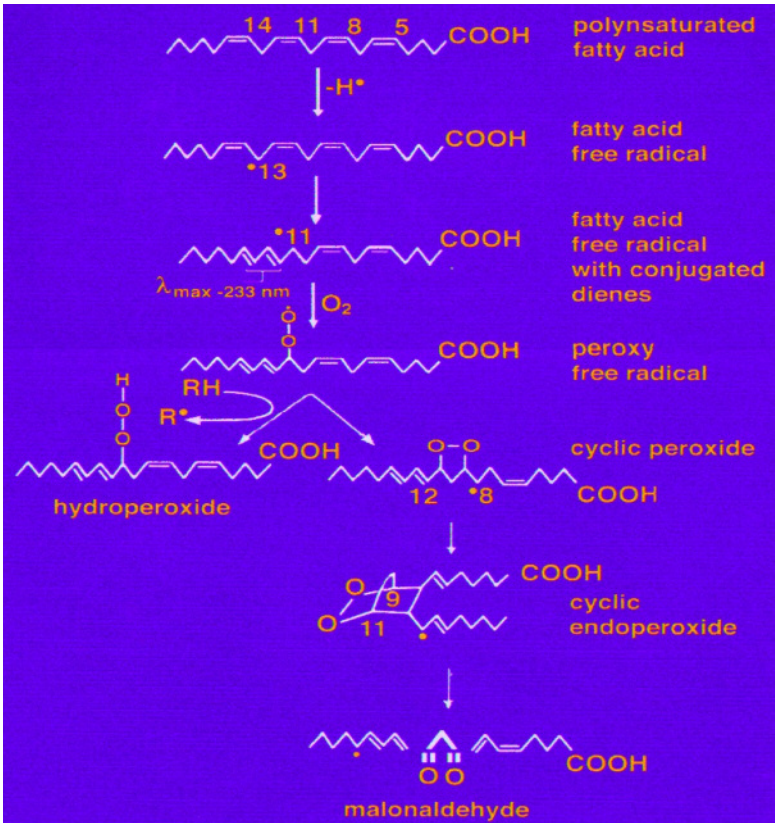

Fig.1 - Scheme of lipid peroxidtion
The free radical attack at a carbon of the methylenic group adiacent to a double bond of the polyunsaturated fatty acid is shown with the consequent formation of a fatty acid free radical. After shift of the double bond because of resonance, conjugated dienes are formed which show both characteristic U.V. absorption at 233 nm and change in the steric conformation of the olefinic chain from the cis to the trans form. The fatty acids free radical then interacts with molecular oxygen and the peroxy free radical is formed which undergoes various pathways shown in the scheme. Finally the fatty acid molecule is broken into various fragments one of which is malonaldehyde as shown in the scheme.

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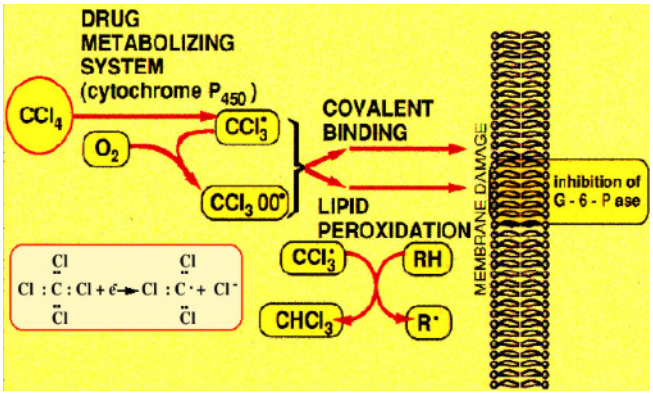
of biopathological conditions. Yet, up to some decades ago lipid peroxidation was only known in the chemistry of oil and fat rancidity, and its interest was confined mainly to the field of food technology. The spreading out of interest in lipid peroxidation in the field of biopathology in the half of the 60's was mainly due to (i) the knowledge that lipid peroxidation can be linked to the microsomal electron transport chain of drug metabolism (2); (ii) the recognition that the metabolism of the model molecule, prototype of experimental pathology of that time, carbon tetrachloride, yields aloalkane free radicals (3); and (iii) our observation that CCl_4 in fact greatly stimulates the peroxidation of liver microsomal lipids (4).

Today it is well established that lipid peroxidation is only one of the reactions set into motion as a consequence of the formation of free radicals in cells and tissues. It was one of the first aspects of abnormal oxidative reactions to be recognized, probably because it represents the most prominent phenomenon of uncontrolled oxidative stress. With the discovery of SOD (5) and with the consequent acquisition that oxy radicals can be rather easily produced in the living tissues, a much more complex spectrum of pathophysiological and pathological oxidations has been progressively recognized, so that the term of "oxidative stress" has been introduced (6), to signify any condition in which the prooxidant/antioxidant balance is shifted in favour of oxidations.

When over fifty years ago, I started to work in oxidative stress (but at that time only the term lipid peroxidation or the "thiobarbituric acid reaction" was mentioned) it was only known (7) that liver homogenates from vit. E deficient animals produced, after aerobic incubation, a higher amount of malonic dialdehyde (MDA) with respect to controls. Today formation of free radicals, particularly from oxygen, and initiation of oxidation and peroxidation processes are implicated in a very large number of conditions of cellular damage belonging not only to experimental pathology but even to human spontaneous pathology (8).

Our studies of oxidative stress have been carried out through the use of various models of experimental pathology; for the sake of brevity, I will only give some flashes on that of carbon tetrachloride hepatotoxicity, on that of GSH depleting agents' toxicity and on that of the release of iron in a free form and its relationships with erythrocyte ageing. With regard to the CCl_4-model, Fig.2 shows the homolytic cleavage of the carbon-halogen bond occurring during the metabolism of the molecule in the drug metabolism of the hepatocyte, with formation of CCl_4 radicals (3) (you can see the one electron reduction of the molecule catalysed by cytochrome P_{450}, the latter being maintained in the reduced form by NADPH, the general cofactor of drug metabolism).

Fig.2 - Metabolism of CCl_4 showing the homolytic cleavage of the carbon-halogen bond and the consequent formation of the trichloromethyl radical (CCl3•).



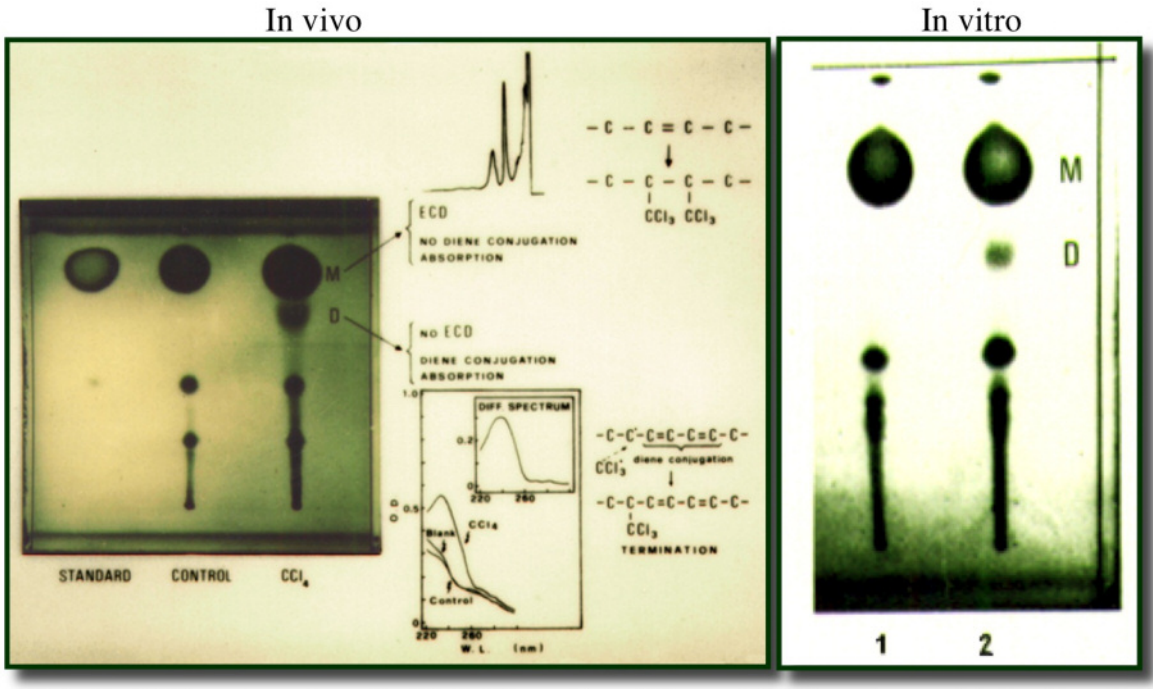
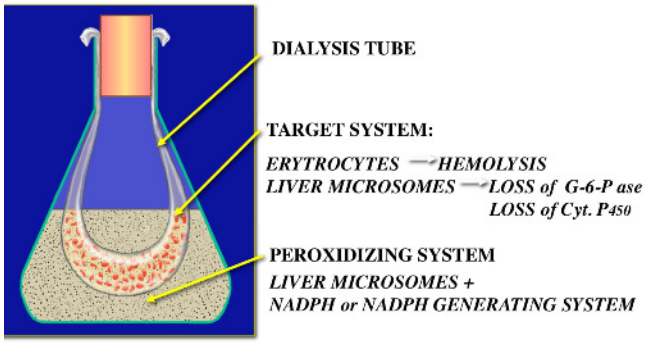


*Fig.3 - **In vivo:** thin layer chromathography (TLC) of fatty acids methyl esters (M) of liver microsomal lipids of control and CCl4-intoxicated (250 µl/100 g body wt) rats (sacrifice at 1 hr). The GLC analysis with electron capture detection (ECD) is reported as well as the absolute absence of U.V. absorption of conjugated dienes. (D) The spot containing the entire U.V. absorption of conjugated dienes and the absolute absence of the response of GLC with ECD is shown.*

***In vitro:** TLC of fatty acid methyl esters (M) of lipids of liver microsomes incubated under anaerobic conditions in the presence of CCl_4 (~150 µg/ml of incubation mixture). Both band M and band D are clearly visible.It must be pointed out that in this anaerobic in vitro system no decrease in microsomal G-6-Pase was observed. (See refs. 12, 13, 14)*

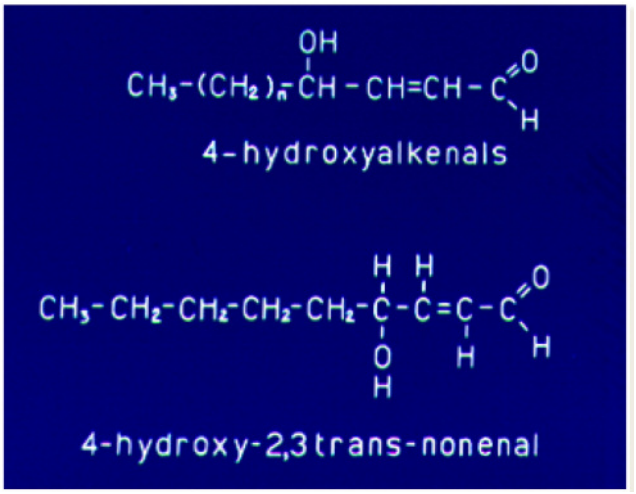
The trichloromethyl radical (CCl3·) is thus generated, which in the presence of oxygen can form the trichloromethyl peroxy radical (Fig. 2). These radicals, whose formation has been then demonstrated in vitro and in vivo (9-11), can act both by covalently binding to lipids and proteins of endoplasmatic reticulum and by initiating lipid peroxidation, which was in fact our pioneristic demonstration (4,12) with liver preparations containing the microsomal fraction.
By dissociating the two pathways in vitro, we showed that at least some early effects of CCl_4, such as the inactivation of microsomal glucose-6-phosphatase (G-6-Pase) (assumed as a test membrane enzyme) are mediated by lipid peroxidation rather than by covalent binding. This because in anaerobic systems it was possible to reproduce (Fig. 3) the molecular alterations induced by CCl_4 in the fatty acids of microsomal lipids, such as conjugated dienes and chloromethyldienes (13,14), without any enzyme inactivation (15).
Therefore some product downstream conjugated dienes in the peroxidative cascade after the intervention of oxygen, seemed to be responsible for the enzyme inactivation (16). These studies, therefore, led to the hypothesis that in addition to the local membrane damage, toxic products originate from lipid peroxidation of cellular membranes, diffuse and act at distant loci, as second toxicological messengers of cell injury (16).
In this line of studies, we showed (17) that products originating from the peroxidation of liver microsomal lipids are capable of inducing cytopathological effects (hemolysis, inhibition of microsomal enzymes, such as G-6-Pase and cyt. P_{450}) in revealing or target systems (red blood cells, or liver microsomes, respectively), which were separated from the peroxidizing system by a dialysis membrane (Fig. 4).

Fig.4 - Scheme of the cytopathological effects brought about by dialysable products originayting from the peroxidation of liver microsomal lipids.



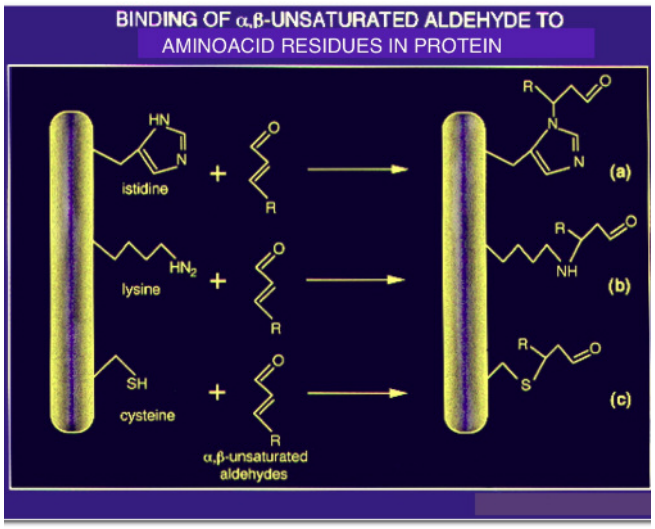
Thus we had to do with metastable products (much more stable than the CCl_4 radicals), capable of crossing a dialysis membrane and of inducing pathological effects at a distance.
In further studies (18) some of these toxic products were separated from the dialysate and characterized. Of particular importance was the identification (19) of an aldehyde of the class of 4-hydroxyalkenals, namely 4-hydroxy-2,3-trans-nonenal (4-HNE) (Fig. 5).

Fig.5 - Four-hydroxyalkenals and 4-hydroxynonenal (4-HNE)



These aldehydes are provided with an extremely high biological reactivity, mainly due to their capacity to bind to nucleophilic groups by their double bonds in α-β position; and the reaction, that is a Micheal addition, occurs whit –SH groups (thioether linkage with C_3), with $-NH_2$ groups and with - NH groups (himidazolic ring of histidine) (Fig. 6).

Fig.6 - Reactivity of 4-hydroxyalkenals with -SH and amino groups of mcromolecules

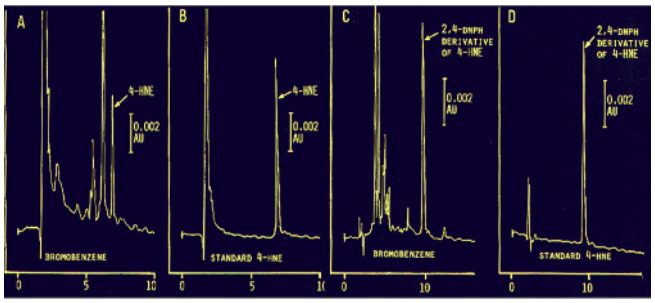


4-HNE and related aldehydes show inhibitory effects on an extremely large number of biological functions (20) and can be considered mediators of the cellular damage produced by agents which, like CCl_4, promote lipid peroxidation. In further studies (21,22) we demonstrated (Fig. 7) that 4-HNE and related aldehydes are in fact formed in vivo in the liver of animals intoxicated with various prooxidants. Also, this aldehyde

(4-HNE) has been extensively studied (20) and regarded as the model molecule of oxidative stress.

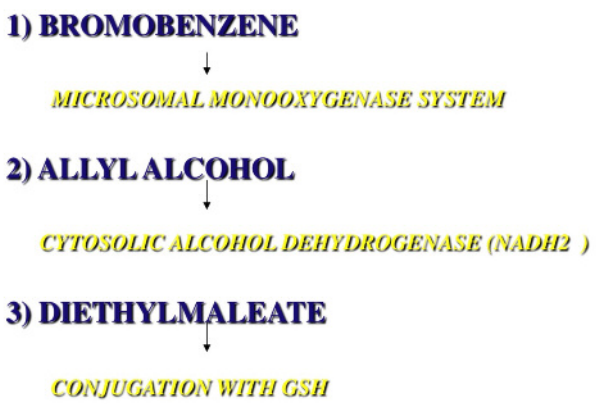
Fig.7 - Detection by high pressure liquid chromatography (HPLC) of 4-hydroxynonenal (4-HNE), as free aldehyde (A,B) or 2,4-dinitrophenylhydrazone (2,4-DNP) derivative (C,D), in the liver (≈5mg of protein) of bromobenzene-poisoned mice. See ref.21,22 for operating conditions.

(A) 4-HNE in the liver of bromobenzene-treated mice.
(B) Standard 4-NHE (5pmol).
(C) 2,4-DNP derivative of 4-HNE in the liver of bromobenzene-treated mice.
(D) 2,4-DNPH derivative of standard 4-HNE.
Control mice showed no 4-HNE



Coming to the second model of oxidative stress mentioned above, that concerned with GSH depleting agents (Fig. 8), we have studied (23,24) the intoxications with bromobenzene and analogues, allyl alcohol and diethylmaleate, which (as shown in Fig. 8), follow different metabolic pathways in the hepatocyte, all producing GSH depletion.

Fig.8 - Glutathione (GSH) depleting agents used as experimental models of oxydative stress and the relative pathways of liver metabolism.



In a typical experiment of bromobenzene intoxication of the mouse (Fig. 9) a very marked hepatic GSH depletion occurs within the first hours (or minutes in the case of allyl alcohol) (23,24). Subsequently (15-18 hr with bromobenzene, 2-4 hr in the case of allyl alcohol) severe lipid peroxidation develops and is accompanied by a likewise severe liver necrosis.

Fig.9 - Typical experiments of bromobenzene or allyl alcohol intoxications in the mouse with the time-course of hepatic GSH depletion, hepatic lipid peroxidation (MDA) and liver necrosis (serum SGPT).

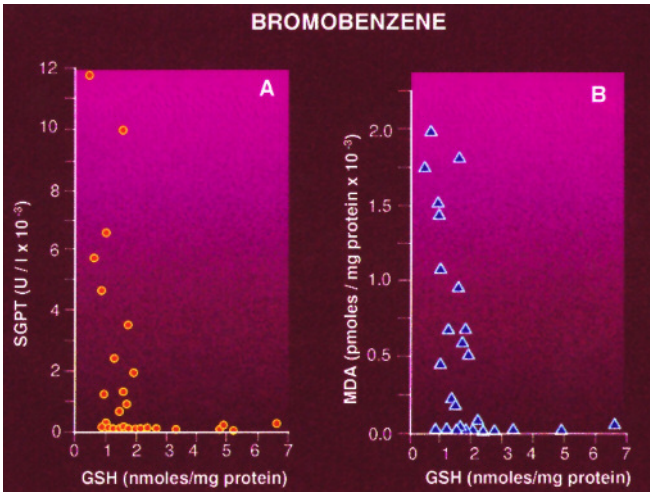
BROMOBENZENE
(13 mmol/Kg b.w., per os)

Time after intoxication	0 time	3 hr	12 hr	15 hr	18 hr
GSH (nmol/mg protein)	24.3 ± 1.7	3.7 ± 0.2	2.2 ± 0.2	2.4 ± 0.3	1.9 ± 0.3
Hepatic MDA content (pmol/mg protein)	---	0	3 ± 2	189 ± 85	1097 ± 406
SGPT (units/L)	46 ± 5	48 ± 30	63 ± 15	2578 ± 1389	4669 ± 1545

ALLYL ALCOHOL
(1.5 mmol/Kg b.w., per i.p.)

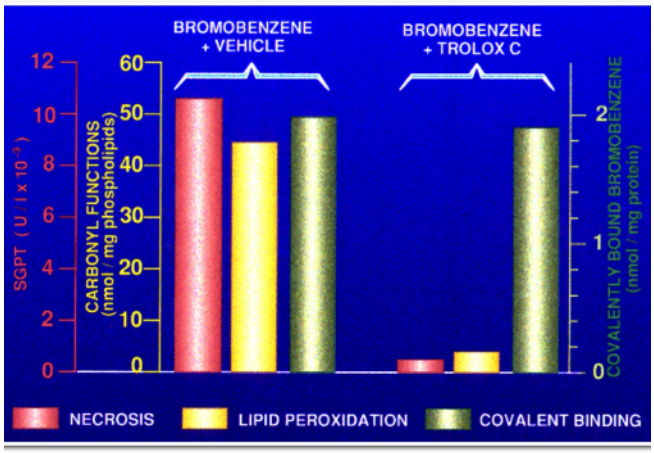
Time after intoxication	0 time	30 min	2 hr	4 hr
GSH (nmol/mg protein)	22.3 ± 0.8	2.4 ± 0.2	3.3 ± 0.4	6.3 ± 1.0
Hepatic MDA content (pmol/mg protein)	---	12 ± 2	55 ± 16	225 ± 63
SGPT (units/L)	36 ± 7	116 ± 39	591 ± 206	1639 ± 310

Fig.10 - Plot of the individual values for lipid peroxidation (MDA) or liver necrosis (serum GPT) against the corresponding hepatic GSH levels in bromobenzene-intoxicated mice.



The plot (Fig. 10) of the individual values for lipid peroxidation and for serum transaminases (liver necrosis) against the corresponding hepatic GSH levels shows that the two phenomena develop only when the hepatic GSH depletion has reached critical or threshold values (23). The treatment of the animals (Fig. 11), even after the intoxication, with antioxidants (Trolox C in this case, but even with other antioxidants) completely prevents both lipid peroxidation and liver necrosis, while not changing at all the extent of the covalent binding of bromobenzene metabolites to liver protein (23).

Fig.11 - Effects of the treatment with the antioxidant Trolox C on liver necrosis (serum GPT), lipid peroxidation (carbonyl functions in liver phospholipids) and covalently bound bromobenzene to liver protein in bromobenzene-intoxicated mice.



These studies therefore indicated that, contrary to what assumed for a long time, covalent binding is not a factor in this type of hepatotoxicity, rather that lipid peroxidation seems to be implicated.

The third model of oxidative stress mentioned above is that related to iron release from its macromolecular complexes. Iron redox cycling, in fact, is at the base of the Fenton reaction which produces the potent oxidant, hydroxyl radical.

Normally iron is transported and stored in specific proteins (transferrin, ferritin, haem proteins) that prevent its reaction with reduced oxygen species. Thus, to be redox cycling active, iron has to be released from these complexes. Studies from our laboratory have shown (25) that iron is released in a free (desferrioxamine (DFO) chelatable) form when mouse erythrocytes are incubated with a number of oxidizing agents, such as phenylhydrazine, divicine, isouramil, acrolein, phenylhydroxylamine and dapsone hydroxylamine.

The results (25) obtained with phenylhydrazine are reported in Fig.12: as can be seen, a consistent release of iron occurs; iron is released from hemoglobin or heme (26) and the release is accompanied by methemoglobin formation. If the erythrocytes are depleted of GSH (which can be easily obtained by a short preincubation with diethylmaleate, DEM), the release of iron is also accompanied by lipid peroxidation and hemolysis (25).

A similar release of iron also occurs (Fig.13) during the erythrocyte ageing, here experimentally reproduced by a prolonged (24-48-60hr) aerobic incubation in buffer (a model of rapid in vitro ageing of erythrocytes), and it is accompanied by methemoglobin formation (27); whereas no substantial iron release nor so massive methemoglobin formation occurs when the incubation is carried out under anaerobic conditions (Fig.13), which again suggests the involvement of oxidative stress in iron release.

Fig.12 (right) - Iron release (DFO-chelatable iron), methemoglobin (Met-HB), gluthatione (GSH) decrease, lipid peroxidation (MDA) and hemolysis in erithrocytes incubated with phenylhydrazine (Phz) or preincubated with diethylmaleate (DEM) and then incubated with phenylhydrazine.

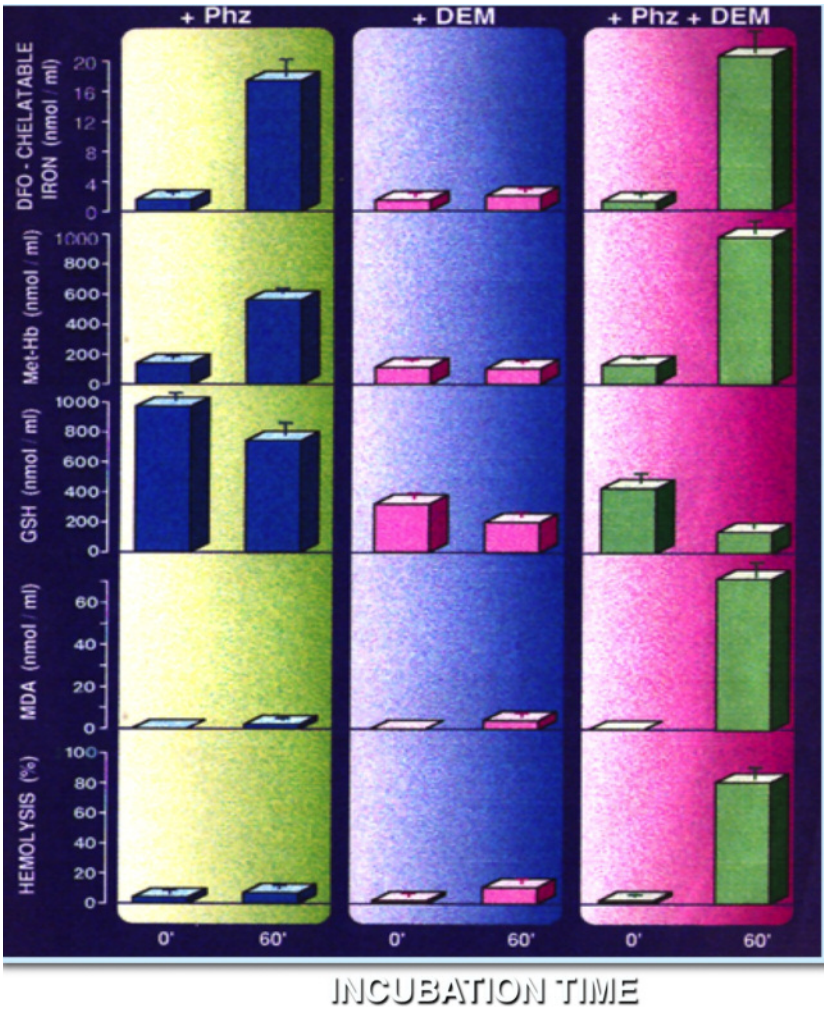


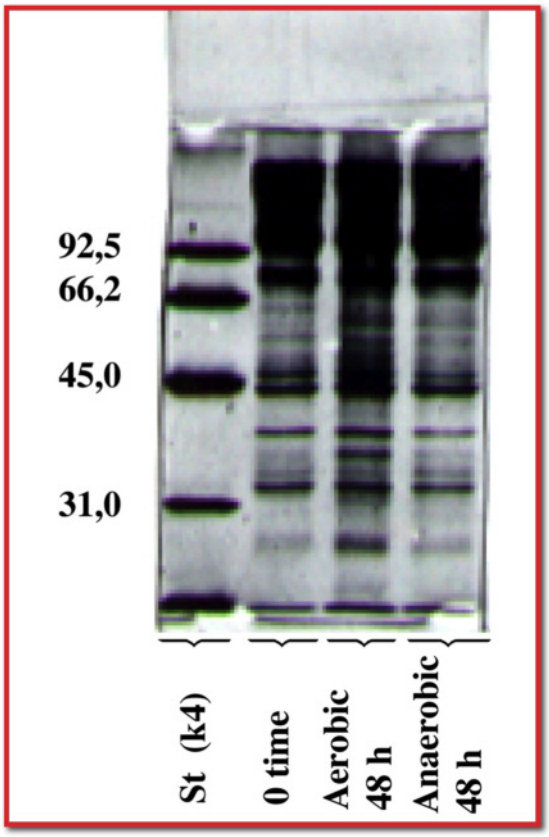
Fig.13 - Iron release and methemoglobin (Met-Hb) formatioin during erythrocyte ageing, experimentally reproduced by a prolonged aerobic incubation in buffer (model of rapid in vitro ageing of erythrocytes). The results of the anaerobic incubation are reported for comparison.

Release of iron (DFO-chelatable iron) and methemoglobin (Met-Hb) formation in calf erytrocytes incubated in phosphate buffer under aerobic or anaerobic condition

	Incubation time (h)	"free iron" (nmol/ml)	Met-Hb (nmol/ml)
Aerobic Incubation	0	1.7 ± 0.3	169 ±6
	24	5.2 ± 0.9	460 ±30
	48	11.6 ±1.7	1690 ±60
	60	36.0 ± 2.0	3866 ±121
Anaerobic incubation	24	2.2 ± 0.3	130 ±30
	48	3.1 ± 0.9	247± 37
	60	5.0 ± 0.9	324± 45

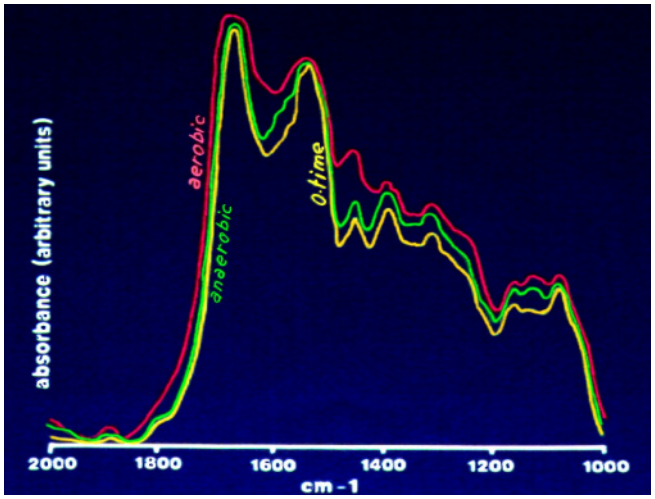
The release of iron is also accompanied by oxidative alterations of membrane proteins (27) and these alterations have been detected by both PAGE electrophoresis (Fig.14) and infrared spectroscopy (IR).

Fig.14 - PAGE electrophoresis of membrane proteins of erythocytes incubated aerobically or anaerobically as in Fig.13



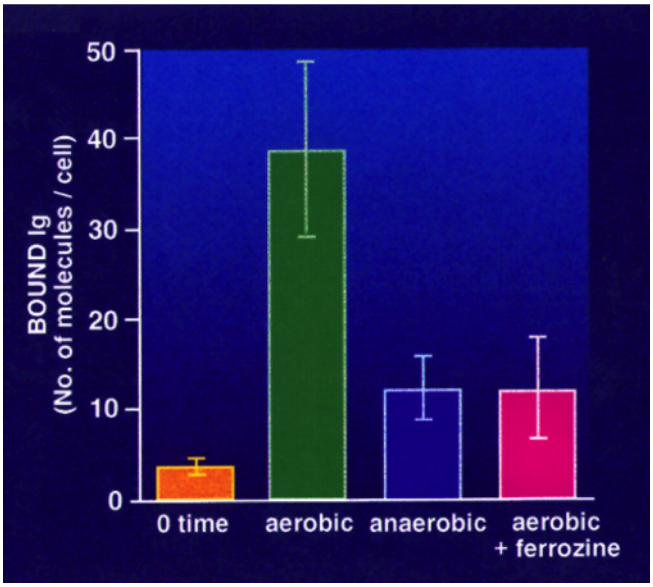
The latter (Fig.15) shows (27) a consistent increase in carbonyl groups (absorption increase in the 1685-1650 cm^{-1} range) and other alterations of the spectrum all related to protein oxidation.

Fig.15 - Infrared (IR) spectroscopy of membrane proteins of erythrocytes incubated under aerobic or anaerobic conditions, as in Fig.13



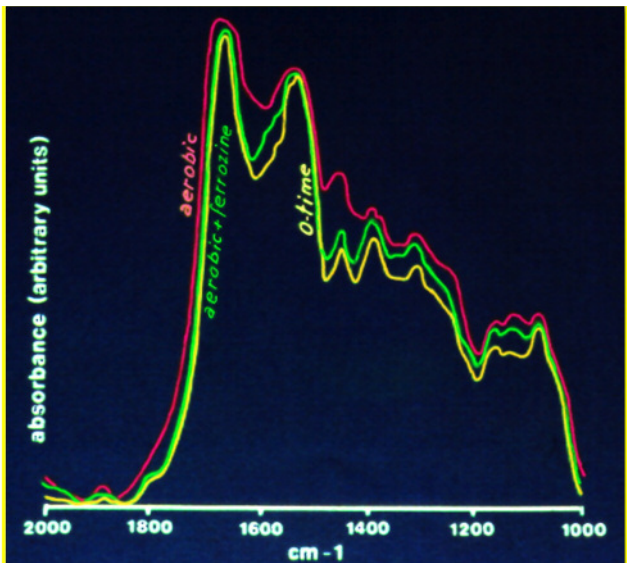
It is generally accepted that senescent cell antigen is generated as a result of oxidative modification of some membrane proteins, particularly band 3, and acts as a specific signal for termination of old cells, by initiating the binding of autologous IgG and subsequent removal by phagocytes.

Fig.16 - Binding of autologous IgG to membrane proteins of erythrocytes incubated aerobically or anaerobically, as in Fig. 13, or incubated aerobically in the presence of the iron chelator ferrozine



As can be seen in Fig.16, in the membranes of aerobically incubated cell (as reported in Fig. 13), the senescent antigen is formed as shown by the binding of autologous IgG, which substantially does not occur after the anaerobic incubation (27). The addition of the iron chelator ferrozine (which freely enters the cells) during the aerobic incubation prevents both the formation of senescent antigen (27), that is the binding of autologous IgG (Fig.16), and the membrane protein oxidative alterations previously (Fig.15) seen in the infrared spectroscopy (the green spectrum of the aerobic plus ferrozine sample is very close to the yellow one of the control, 0 time sample (Fig.17)) (27).

Fig.17 - Infrared (IR) spectroscopy of membrane proteins of erythrocytes incubated aerobically in the presence of the iron chelator ferrozine



These results strongly suggest that ferrozine is capable of chelating, at intracellular level, the iron released during the aerobic incubation and to prevent in such way the alterations of membrane proteins, probably produced by the redox cycling of the released iron and related to the formation of senescent antigen.
Moreover, even more suggestive results have been obtained (28) by using, as protective agent, a synthetic acyl hydrazone, pyridoxal fluor benzoyl hydrazone, which is an iron chelator (as shown in Fig. 18) and enters the cells.

Fig.18 - Pyridoxal fluor benzoyl hydrazone (PFBH) and its iron chelation.

This chemical prevented the formation of senescent cell antigen in human erythrocytes incubated aerobically for 48-60 hr. The autologous IgG binding was detected, in these experiments (28), by using an anti-IgG antibody labelled with fluorescein and by examing the cells for fluorescence with confocal microscopy. Fig. 19 shows the non-incubated (control, 0 time) cells (panel A) (only minor fluorescence can be seen); the aerobically incubated cells (panel B) showing extensive fluorescence that is extensive binding; and the cells incubated aerobically in the presence of the acyl hydrazone (panel C and D), which show much less fluorescence (much less binding).
Finally, a progressive iron release (Fig. 20) occurs in human erythrocytes stored in their own plasma at 4°C for 15 and 35 days (29). Therefore an iron release seems to be really related to the ageing of red blood cells and it is likely to occur even under physiological conditions.
In summary the overall scheme would be the following: an oxidative stress in the erythrocytes will promote iron release, which in turn will promote oxidation of membrane proteins and the consequent formation of senescent cell antigen, that is the ageing of erythrocytes.

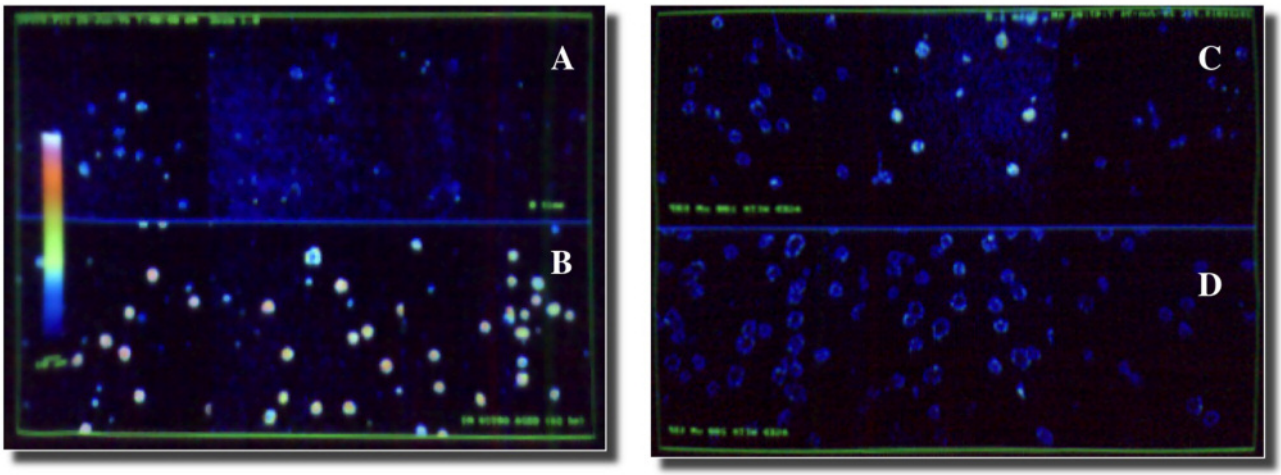


Fig.19 - Protection by PFBH of erythrocyte ageing. The erythocytes were incubated aerobically for 48-60 h. The autologous IgG binding was detected, in these experiments, by using an anti-IgG antibody labelled with fluorescein and by examining the cells for fluorescence at confocal microscopy.

A) non incubated (0 time, control) cells;
B) aerobically incubated cells;
C) aerobically incubated cells in the presence of PFBH (100 µM);
D) aerobically incubated cells in the presence of PFBH (200 µM).
A', B', C' and D', same experiment at higher magnification.

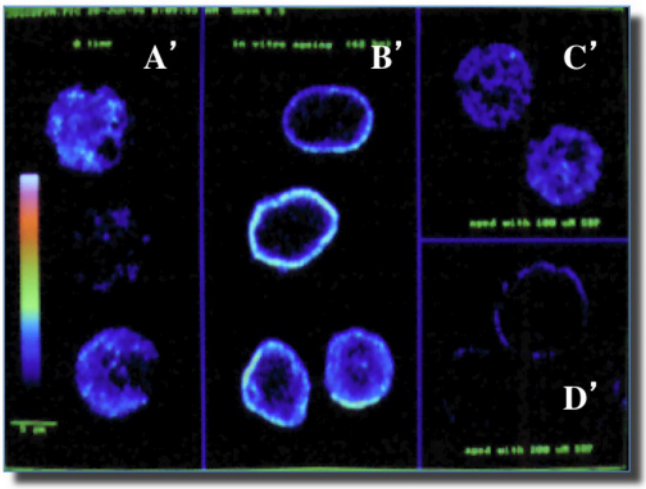


Fig.20 - Release of DFO-chelatable iron and methemoglobin (Met-HB) formation in human erythrocytes stored in their own plasma at 4°C

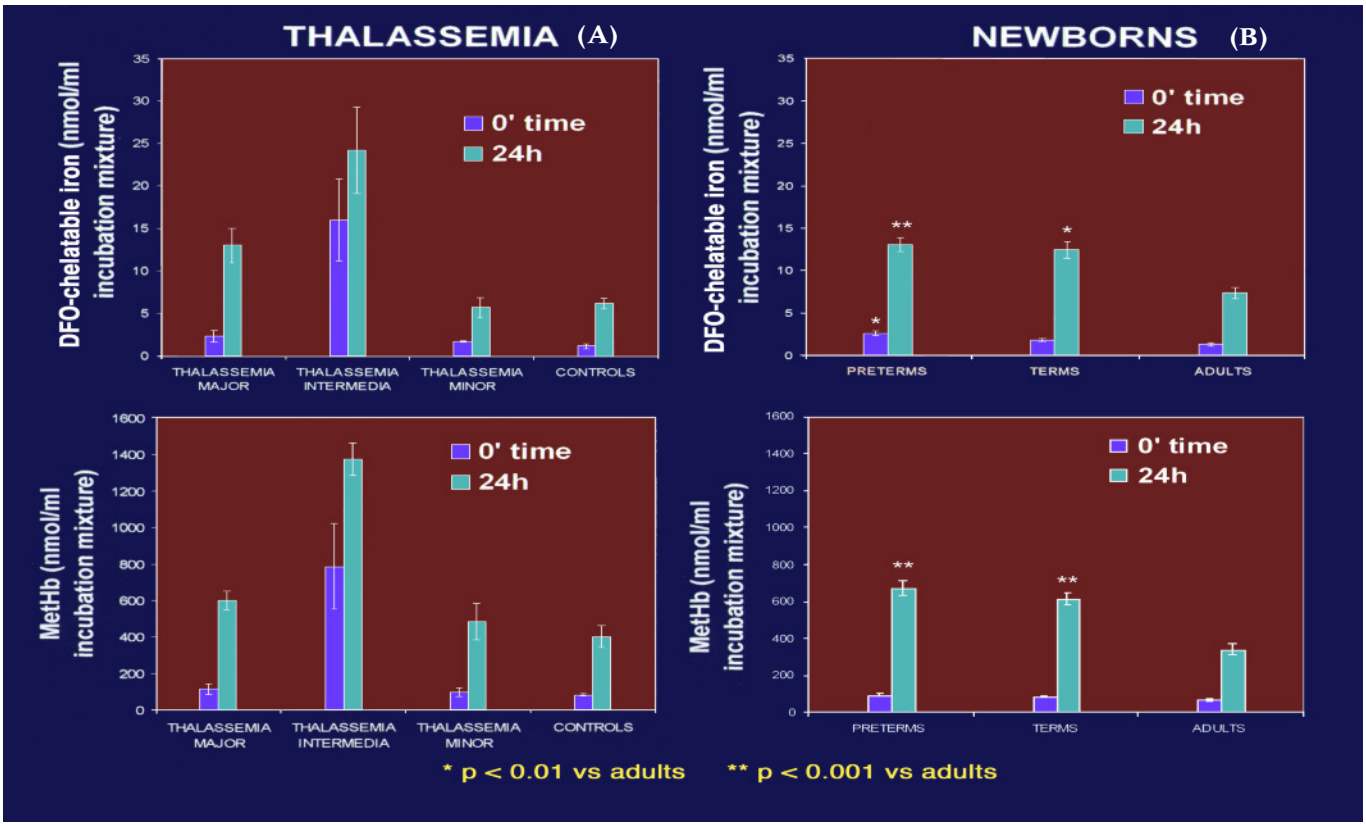
	Ageing Time (days)	Free Iron (nmol/ml)	MetHb (nmol/ml)
Storing in plasma at 4°C	0	1,7±0,03	115±7
	15	6,6±1,6	141±24
	35	15,4±3,2	179±40

An increased release of iron after aerobic incubation is also observed (30) in erythrocytes from subjects with β-thalassemia (major and intermedia) (Fig.21) and in erythrocytes from neonates (31). In both cases an increased susceptibility to oxidative stress, an accelerated removal from the blood stream and a marked increase of hemoglobin F (HbF) occurs. In thalassemic erythrocytes, besides the release (24 h incubation), the content of free iron (at 0 time) is also increased (Fig.21A). Both values are correlated with HbF content (30). In the erythrocytes from newborns (31) the release is increased in both term and preterm newborns (Fig.21B) and it is correlated (31) with the levels of plasma non protein-bound iron (NPBI), a form of non bound iron of uncertain origin, which appears in plasma of newborns, thalassemic and hemochromatotic patients. Both iron release and non protein bound iron are inversely correlated to the pH values of cord blood (31), which suggests that both values are more elevated when hypoxic conditions occur.

Recently the so called "senescent cell antigen" binding autologous IgG has been shown (32) to consist of dimers or larger aggregates of band 3. We have shown (33) that such an oxidatively modified band 3 is present in as much as 74% of preterm, 21% of term newborns and 10% of adults, which explains the accelerated removal of erythrocytes in the perinatal period. After in vitro ageing of erythrocytes (that is aerobic incubation), the band increases in frequency and intensity in all the erythrocytes (33) and the increase is almost completely paralleled by iron release.

The great deal of studies on oxidative stress, although bringing forth very interesting results, had not however allowed the evaluation of oxidative stress in human pathology, at least on a large scale. This was due to the fact that a reliable and non invasive method to monitor lipid peroxidation in vivo with the only use of blood and urine was lacking. In fact all the methods used (detection of conjugated dienes, lipoperoxides and aldehydes) are poorly reproducible and reliable when carried out in plasma due to the extreme reactivity and instability of the species which are going to be detected; or they imply the use of tissues and therefore hardly feasible in men. Some years ago, however, the group of Morrow and Roberts in U.S.A. demonstrated (34,35) the production of a series of prostaglandin F_2-like compounds, named F2-isoprostanes (Fig. 22), that

Fig.21 - . Iron release and Meth-Hb formation in aerobically incubated erythrocytes from subjects with β-thalassemia (major, intermedia and minor) (A) and from newborns (preterm and term) (B). In thalassemic erythrocytes, besides the release, the content of free iron (at 0 time) in also increased. Both values are correlated with the HbF levels.



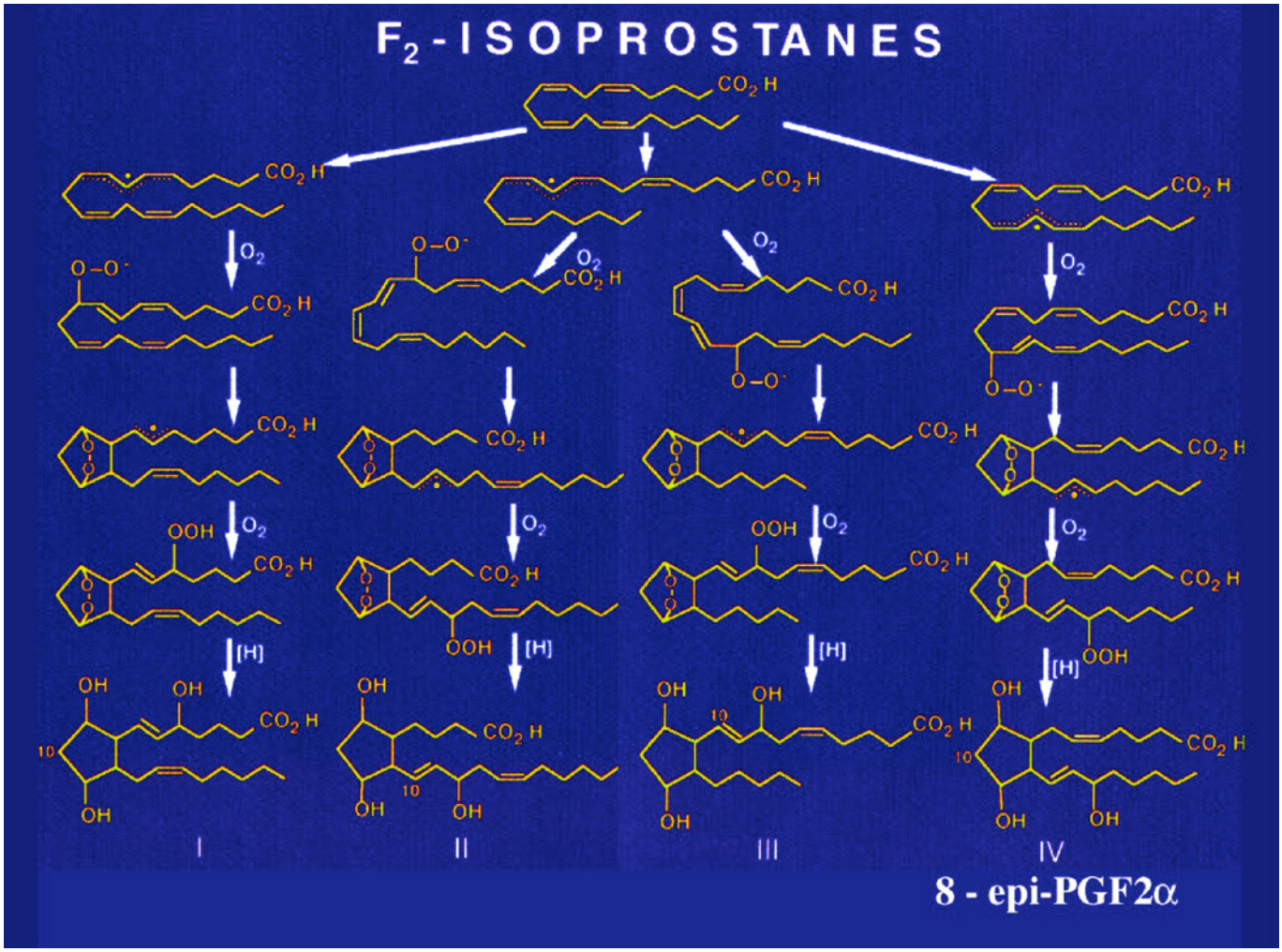


Fig.22 - *F_2-isoprostanes (F_2-iso) derived from the peroxidation of arachidonic acid (from Morrow et al. Analyt. Biochem., 184, 1-10, 1990).*

are formed in vivo and in vitro by free radical-catalyzed peroxidation of phospholipid bound arachidonic acid, a pathway which is independent of the cyclooxygenase pathway. Since isoprostanes, initially formed in situ on phospholipids (36), are released into the circulation and since these prostanoids are less reactive than other lipid peroxidation products such as lipid hydroperoxides and aldehydes, they can be found more easily in plasma and urine. Therefore, F_2-isoprostanes can nowadays be considered as the most reliable markers of oxidative stress (lipid peroxidation) and can be used to evaluate the oxidative status in a number of human pathologies. Elevated levels of plasma and/or urinary isoprostanes have been reported (37) in several diseases such as diabetes, alcoholic liver disease, ARDS, Alzheimer disease, rethinopathy of prematurity and many others.

We currently use, for the determination of F_2-isoprostanes, the gass-mass technique which, as also recommended by Roberts and Morrow (37), is the most reliable method to detect these products. The procedure of Noouroz-Zadeh (38) for the preparative technique before gass-mass is also used. Yet, before any approach to human pathology, we investigated whether elevated levels of plasma F_2-isoprostanes could be measured in our experimental models of oxidative stress, mentioned above. The results have shown (Fig. 23) dramatically elevated levels of plasma isoprostanes in the acute CCl4 intoxication (39) and even (although to a lesser extent) in the acute ethanol intoxication, thus confirming both our previous studies and the more recent results of Morrow et al. (40) (and also our early studies on oxidative stress in ethanol toxicity (41)).

Fig.23

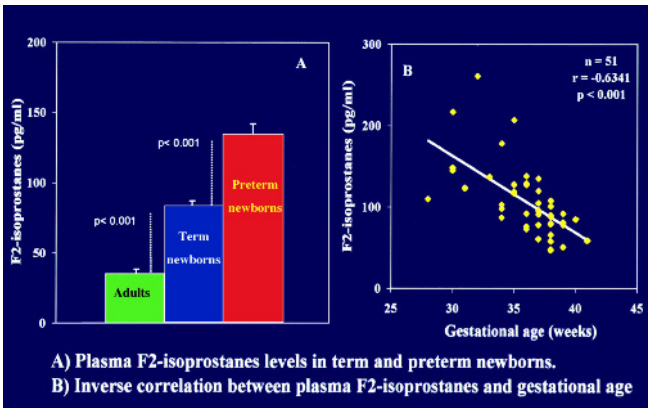
PLASMA F_2-ISOPROSTANE LEVELS IN CONTROL AND CCl_4 OR ETHANOL INTOXICATED RATS

	F_2-ISOPROSTANES (pg/ml)
controls	126 ± 24 (11)
CCl_4	4506 ± 451 (11)*
Ethanol	882 ± 185 (6)*

Starved rats were intoxicated intragastrically with CCl_4 (0.2 ml/100 g body weight) or with ethanol (7 g/kg body weight) and killed at 4 or 6 hr after the intoxication, respectively. Control rats received the same volume of saline.
*$p < 0.001$ versus controls values

Since it has been repeatedly suggested that newborns are exposed to conditions of oxidative stress resulting from the change from a low oxygen pressure in utero to a relatively high oxygen pressure at birth, we have evaluated the oxidative status in human newborns (42). Plasma isoprostanes were significantly higher in newborns as compared to healthy adults, and the highest values were found in preterm newborns in whom F_2-isoprostanes were higher as compared to term babies (Fig.24A). Moreover, a highly significant inverse correlation was found (42) between the plasma levels of isoprostanes and the gestational age (Fig.24B).

Fig.24 (A) - (B)



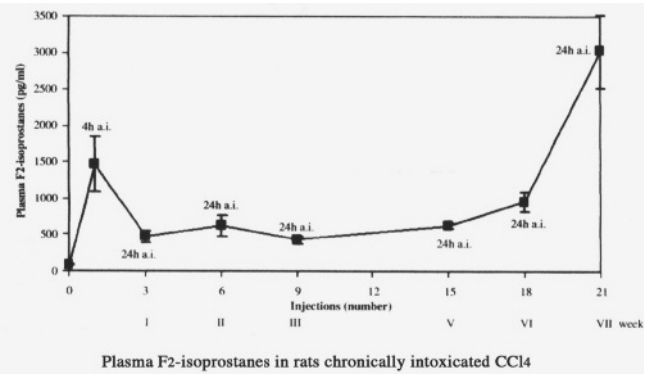
This suggests that some form of oxidative stress is active during the prenatal life and that it is going to attenuate during the last periods of gestation. This also confirms the occurrence of the so called "free radical disease of the neonate".

Finally we have recently suggested (43) that F_2-isoprostanes are potent agonists in experimentally induced liver fibrosis. The connection between oxidative stress and collagen hyperproduction was firstly proposed by Chojker et al. (44) who observed that the addition of ascorbic acid and iron to human cultured fibroblasts strongly stimulates lipid peroxidation and, at the same time, the production of collagen and procollagen α-1 (I) mRNA; the effects are reproduced by the addition to the same fibroblasts of malonaldeyde (MDA), one of the end products of lipid peroxidation. Also, 4-HNE has been reported (45) to stimulate collagen synthesis in hepatic stellate cells (HSC) (previously known as Ito cells or lipocytes, the most important source of collagen and other matrix proteins in the liver) and TGF β synthesis in cultured lineages of macrophages (46). Since F_2-isoprostanes proved to be mediators of important biological effects, we investigated whether collagen synthesis was stimulated in HSC by F_2-isoprostanes, which possess receptors able to induce specific signal transduction pathways, while aldehydes (such as 4-HNE) can interact with cellular macromolecules by addition or alkylation processes only.

Since, as above mentioned, we have shown that plasma F_2-isoprostanes are extremely elevated in the acute CCl_4 intoxication, we examined the levels of plasma F_2-isoprostanes in a model of chronic CCl_4 intoxication (47) leading to liver cirrhosis, we observed (43) that such levels are maintained elevated during the whole period of experimental treatment (Fig. 25) and correlated to the hepatic content of collagen and in parallel studies we investigated (43) the effects of isoprostanes on cultured HSC.

Fig.25 - Model of chronic intoxication with CCL_4 leading to fibrosis

Rats were injected intraperitoneally three times weekly with 125µl/100g body wt of a CCl_4/mineral oil solution (1:1, v/v) (= 62.5µl CCl_4/100g body wt). Groups of 4 animals were sacrificed 24hr after the last injection at 1, 2, 3, 5, 6 and 7 weeks, respectively. Blood was drawn from the abdominal aorta and analysed for plasma F_2-iso.



The latter were isolated (48) from normal livers and cultured in suitable media. At the seventh day of culture all the cells showed (Fig. 26) the typical transformation to the myofibroblast-like phenotype

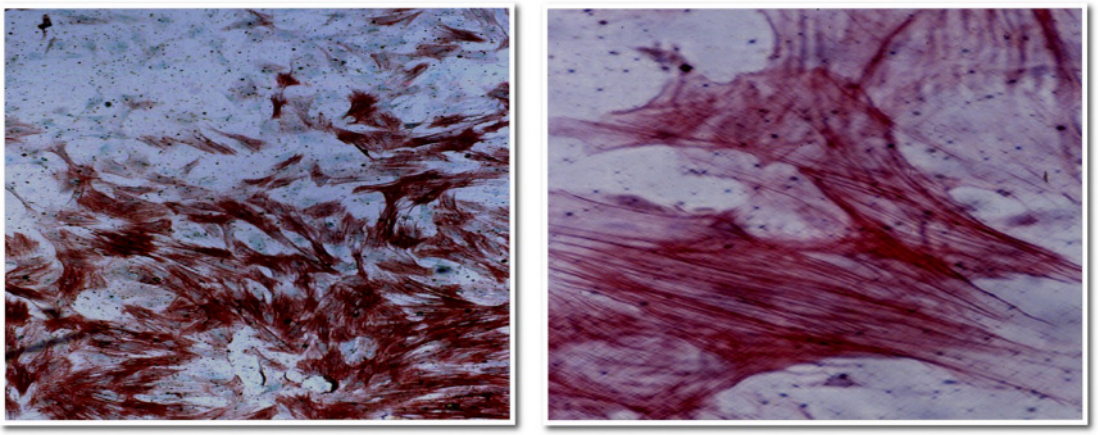


***Fig.26** - Hepatic stellate cells (HSC) isolated from normal rat livers and their activation (expression of α-smooth muscle actin (α-SMA)) at 7 days of culture.*

(expression of α-smooth muscle actin (α-SMA)). The cells were deprived of serum and then treated for 48 hr with F_2-isoprostanes in the range of concentrations seen in the in vivo experiments (10^{-8} to 10^{-10} M).

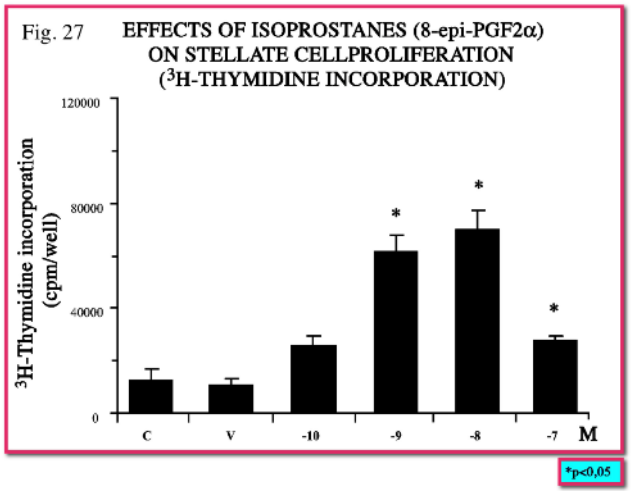
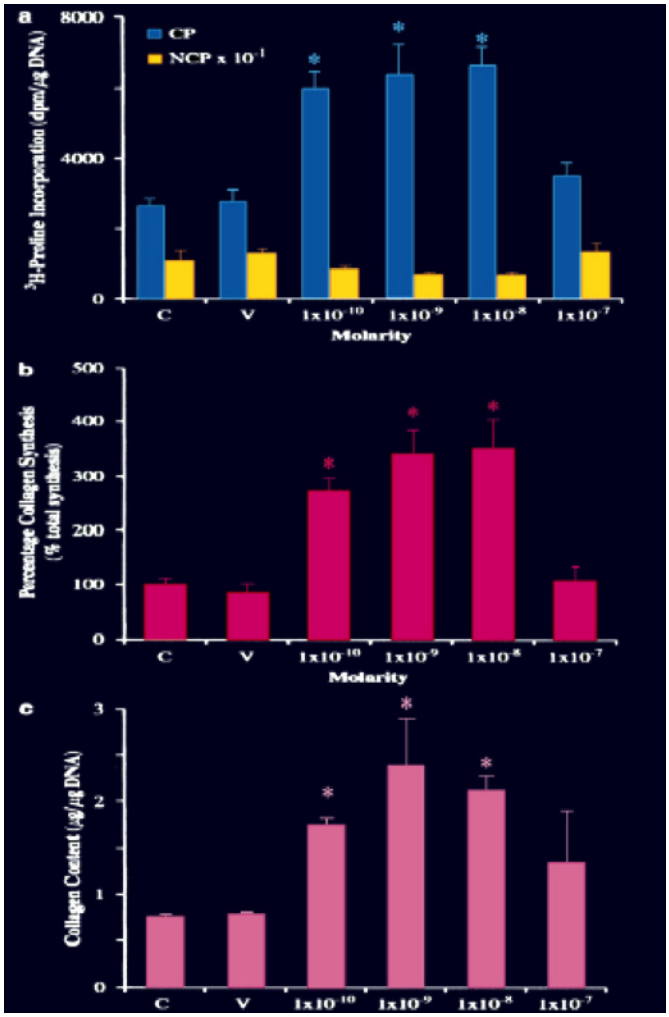
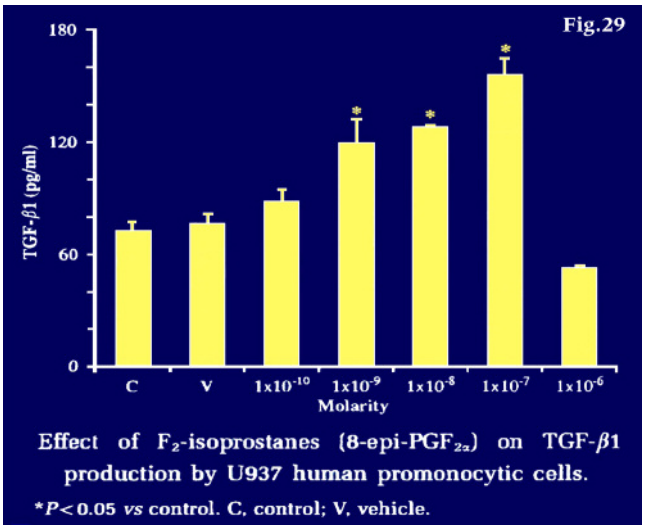


Fig.28 (a),(b),(c) - Effects of F_2-iso (8-epi-PGF$_{2\alpha}$) addition to HSC on collagen synthesis as measured by 3H-proline incorporation.
a) proline incorporation
b) percentage of collagen production overtotal protein production (collagenic plus non collagenic protein)
c) total collagen contents of the cultures.



The isoprostane (actually 8-epi-PGF2α the most represented isomer of the series) addition to HSC induced (43) a marked increase in DNA synthesis (Fig.27), as measured by tritiated thymidine incorporation, and of cell proliferation (as measured by the cell count, not shown in the Figure), as well as a striking increase (Fig.28A,B,C) in collagen synthesis, as measured by tritiated proline incorporation (43). The relative collagen production, that is the percentage of collagen production over the total protein production (collagenic plus non collagenic proteins) was increased by 3.0-3.5 fold (Fig. 28B). Total collagen content of the culture was similarly increased (Fig. 28C). The most active concentrations were between 10^{-8} and 10^{-9} M (10 nM and 1 nM), exactly as those found in the in vivo intoxication (3000-500 pg/ml = 9.0 – 1.5 pmol/ml = 9.0-1.5 nM).

Since it is generally believed (49-51) that activation of HSC follows the release of soluble factors (cytokines, mainly TGF-β1) by cells of macrophage linkages, the effects of F_2-isoprostanes on TGF-β1 release by the human promonocyte cell line U937, assumed as a model for Kupffer cells or liver macrophages, was also studied. F_2-isoprostanes increased (43) the production of TGF-β1 by U937 cells (Fig.29) and this would suggest an alternative pathway of stimulation of HSC, through TGF-β1, with consequent increase in collagen synthesis.



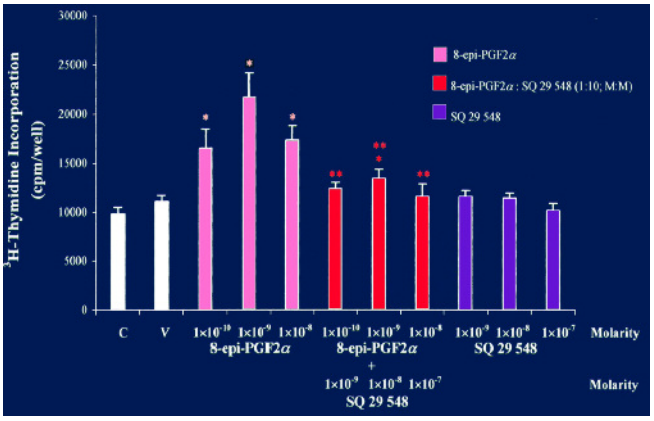
Effect of F_2-isoprostanes (8-epi-PGF$_{2\alpha}$) on TGF-β1 production by U937 human promonocytic cells.
*$P < 0.05$ *vs* control. C, control; V, vehicle.

In summary we propose here that the plasma F_2-isoprostanes generated by lipid peroxidation in hepatocytes mediate the collagen hyperproduction in this model of hepatic fibrosis.

For such an hypothesis to stand up it had to be demonstrated that receptors for F_2-isoprostanes are present in HSC. Besides being markers of oxidative stress, F2-isoprostanes appeared to be mediators of important biological effects. The first one to be revealed (52) was the vasoconstriction of renal glomerular arterioles, an effect which is believed to be very important in the explanation of the hepato-renal syndrome. The effect is mediated through the activation of receptors analogous or identical to those for tromboxane A_2 (TxA_2r) (53).

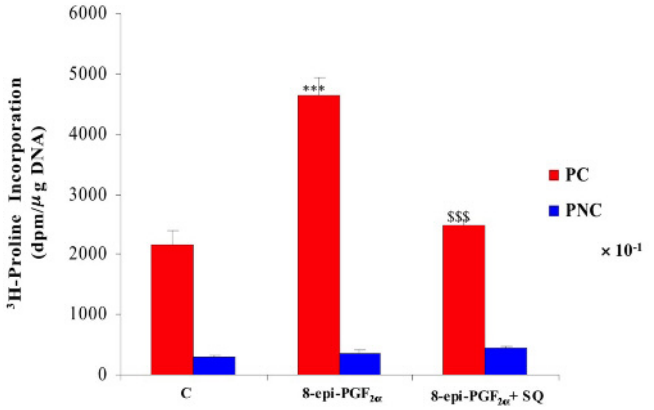
Many other biological effects of F_2-isoprostanes have been described and most of them are very likely due to activation of receptors related to TxA_2r (54-59). Therefore, we investigated whether the specific antagonist of TxA_2r, the molecule named SQ29548, was able to antagonize the effects of isoprostanes in HSC. As can be seen (Fig.30) the isoprostane-induced stimulation of DNA synthesis is almost completely abolished by SQ29548 (43,60).

Fig.30 - Effect of SQ29 548 on the stimulation of DNA synthesis (^{3}H-thymidine incorporation) in HSC treated with F_2-isoprostane (8-epi-PGF2α).



Also the other effect of isoprostanes (8-epi-PGF2α) on HSC, i.e. the stimulation of collagen synthesis is completely antagonized (Fig.31) by SQ29548 (60).

Fig.31 - Effect of SQ 29 548 on the stimolation of collagen synthesis (^{3}H-thymidine incorporation) in HSC treated with F2-iso (8-epi-PGF2α).



Moreover, an effect similar to that of 8-epi-PGF2α (although to a lower extent, but anyhow statistically significant) is brought about by the molecule named I-BOP (Fig.32), the specific agonist of TxA_2r, and even this effect, still on DNA synthesis, is abrogated by SQ29548 (60). The effect of isoprostanes on HSC seemed therefore to be mediated by TxA_2r and then this receptor should occur in HSC.

Finally by using an antibody raised against C-terminal aminoacids of human TxA_2r (TPr) we carried an immunoblot analysis of membrane proteins of HSC and we obtained (60) a single band of 55 kd (Fig.33), quite analogous to that obtained (still at 55 kd) with membrane proteins of human platelets used here as positive control (Fig.33) since it is known (61) that such membranes are rich of TxA_2r.

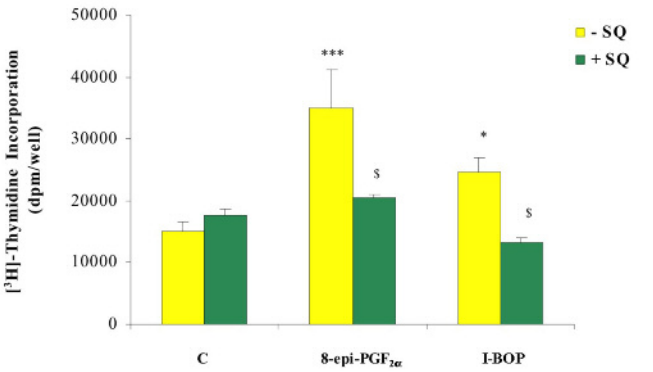
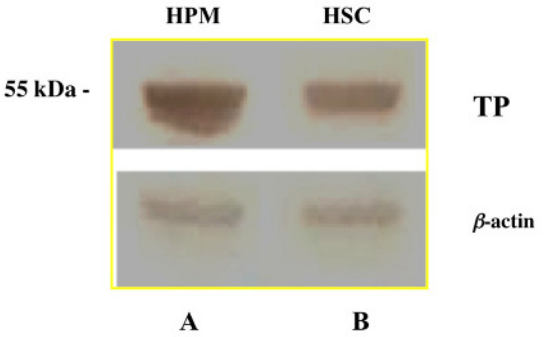


Fig.32 (above) - Effect of F2-iso (8-epi-PGF2α) and of I-BOP on DNA synthesis (3H-tymidine incorporation) in HSC and the antagonizing activity of SQ 29 548.

Fig.33 (below) - Immunoblot identification of TxA_2r in HSC membrane protein lysates. Incubation with an antibody raised against the terminal aminoacids of human TxA_2r (TPr) (1:1000 dilution) revealed immunoreactivy for a 55 kDa protein in HSC membrane lysates (lane B). Lysates from human platelet membranes (HPM) (lane A) were used as positive control. Blot were reprobed with β-actin to assess equal loading.



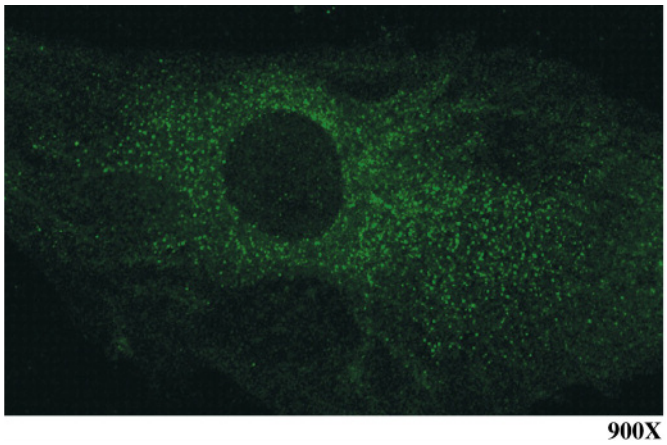
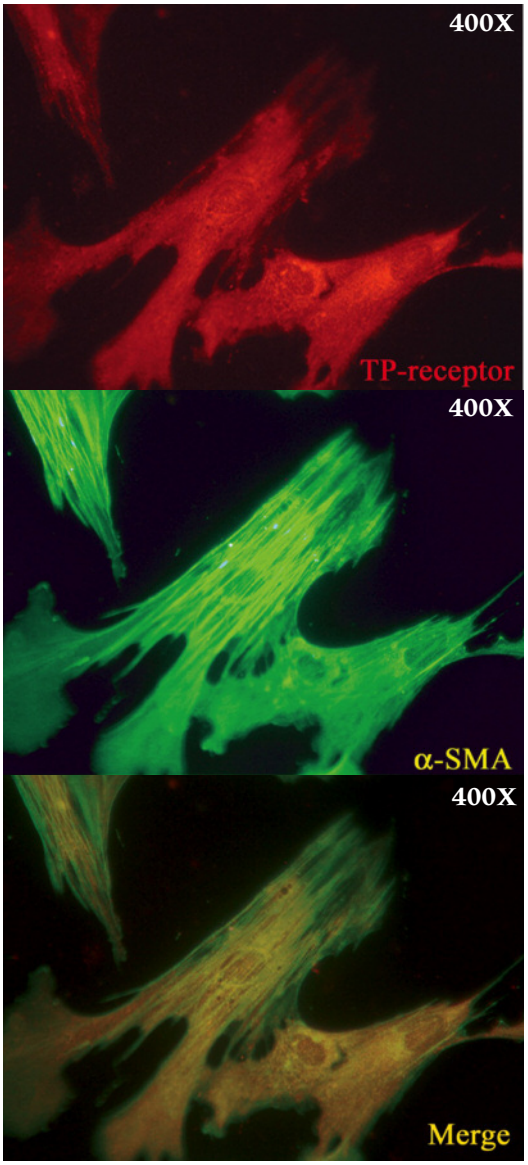


Fig.34 - Subcellular localization of TxA_2r (TPr) in HSC. Cells were permeabilized, incubated with anti-TxA_2r antibody and then with FITC (fluorescein isothiocyanate) conjugated anti-rabbit IgG. Confocal image shows that TxA_2r (TPr) is predominantly expressed in the area around the nucleus.

Immunocytochemical studies (60) carried out by using the same antibody (as primary antibody) together with a secundary anti-rabbit IgG antibody conjugated with the fluorescent dye FITC (fluoresceine isothyocanate) showed at confocal microscopy the presence of the same receptor (TxA_2r) in HSC (Fig.34) and its major expression in the perinuclear region. Lastly, in colocalization studies (Fig.35) carried out by using the same primary antibody together with a secundary anti-rabbit IgG antibody conjugated with the fluorescent dye TRITC (tetramethylrodamine esothyocianate) (red) we obtained (60) in red the TxA_2r (or TP receptor); and by using another primary antibody (anti α-SMA) together with a secundary anti-mouse IgG antibody conjugated with FITC we obtained (60) α-SMA in green. In merge images (Fig. 35) it was possible to appreciate the exact colocalization. It must therefore be concluded that not only HSC express the TxA_2r but also that such receptor is expressed at the same extent as α-SMA, which is the protein marking the activation of these cells.

Fig.35 *- Co-localization of TxA_2r (TPr) and α-SMA in HSC. Activated HSC expressed both TxA_2r (TPr),revealed by TRITC (tetrametylrhodamine isothiocyanate) (red labeled) and α-SMA, revealed by FITC (fluorescein isothiocyanate) (green labeled). Merged images (yellow) show the high degree of co-localization.*



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CHILDHOOD BLEPHAROPTOSIS: A REVIEW OF 113 CASES

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Purpose. The aim of this study is to describe the etiology, clinical patterns, surgical management and outcome of a series of 113 pediatric ptosis patients. *Methods.* To determine the visual and cosmetic outcome following surgical correction of congenital ptosis we reviewed the charts of 113 patients observed in our Department between January 2001 and December 2005. All patients undergo pre-op complete ophthalmological check, visual acuity, ocular motility imbalance, levator fuction and height of the palpebral fissure. Regarding the surgical procedure levator resection was performed in patients with a good function of the levator; a frontalis sospension was performed in patients with a poor levator function.
Results. 93 cases (82%) were unilateral, 20 (18%) bilateral; 21 patients (18%) had family history of ptosis. The etiology was myogenic in 74%, neurogenic in 11%, the jaw- wink ptosis was observed in 7%, aponeurotic in 4,5% and blepharophimosis in 3,5%. Regarding the eighths palpebral fissure, 34 cases (30%) presented severe ptosis, 63 cases (56%) moderate, 16 cases (14%) mild. Regarding the levator function 25 cases (22%) presented poor function or levator, 46 cases (41%) moderate, 42 cases (37%) good. Levator resection was performed in 77 cases (68%), frontalis suspension in 36 cases (32%). The postoperative outcome was poor in 15 cases (13%), suboptimal 12 cases (11%) and good in 86 cases (76%). The incidence of amblyopia was 25%. *Conclusion.* If a careful preoperative evaluation is performed, the surgical outcome in childhood can offer a very good prognosis.
Key Words. Blepharoptosis, Amblyopia, Ptosis, BPES, Blepharophimosis

INTRODUCTION

The aim of this study is to describe the etiology, clinical patterns, surgical manage-ment and outcome of a series of 113 ptosis paediatric patients. In our observations about 50% of all blepharoptosis are in the paediatric age group. The predominant pattern of the congenital ptosis, particularly in unilateral or in asymmetric bilateral cases, is a variable degree of amblyopia related to both visual deprivation and significant astigmatism: management requires repeated follow up for early detection and introduction of occlusion therapy; in spite of this, especially in unilateral cases and when the ptosis is associated with an ocular motility imbalance, amblyopia can be severe (1).

METHODS

The study was a retrospective of 113 patients, 71 males (63%), 42 (37%) females, median age 7 years (2-16 years), who underwent ptosis surgery in the Department Dentistry and Ophtalmology Section of Ophtalmology, University of Siena, under the care of the one of the authors (G.L.) between January 2001 and December 2005, median follow-up 36 months (24-52 months). All patients undergo pre-op complete ophthalmological check, visual acuity, ocular motility imbalance, levator fuction and height of the palpebral fissure.
In unilateral cases the amount of ptosis was calculated as the difference in mm be-tween the heights of the palpebral fissure: severe ptosis was defined as 4mm or lower than the desired eyelid level, moderate between 2 and 4mm, mild as 2mm or lower. In bilateral cases the ptosis was classified as mild if the palpebral fissures were 6 mm or more, as moderate between 4mm and 6 mm, as severe if less than or equal to 4mm. The levator function was measured as the maximum lid excursion from maximal downgaze to upgaze: this was classified as poor if less than 4mm, as moderate between 4–8mm, and good if more than 8 mm. The surgical procedure performed was related to the height of the palpebral fissure and to levator function (2): levator resection was performed in the patients with mild to moderate ptosis with levator function of more than 4mm. In all these cases the surgical approach was an anterior approach through the lid crease (3). For patients with moderate to severe ptosis with poor levator function our choice was a frontalis suspension (4) utilising a double armed 3-O Supramid suture (S.Jackson Inc®). The epicanthic folds and telecanthus in blepharophimotic patients were corrected with a Y - V plasty before ptosis correction was undertaken (5).
For moderate to severe jaw-winking ptosis, frontalis suspension, after levator exci-sion, generally provided satisfactory correction of both jaw- winking and ptosis (6). All patients underwent surgery under general anaesthesia (10 patients under 3 years old for visual obstruction) and all patients were assessed preoperatively for corneal anaesthesia and Bell's phenomenon. The postoperative outcome was classified as poor if was re-

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quired another operation, suboptimal if the difference in lid height was more than 1mm, and good if the lids were within 1mm height.

Results

In our sample: 93 cases (82%) were unilateral, 20 (18%) bilateral; 21 patients (18%) had family history of ptosis. The etiology was myogenic in 84 cases (74%); neurogenic in 12 cases (11%), the jaw-wink ptosis was observed in 8 cases (7%), aponeurotic in 5 cases (4,5%) and blepharophimosis in 4 cases (3,5%) (Table I).

Table I. Childhood blepharoptosis: 113 cases

	Cases	*%*
Males	*71*	*63*
Females	*42*	*37*
Unilateral	*93*	*82*
Bilateral	*20*	*18*
Myogenic	*84*	*74*
Neurogenic	*12*	*11*
Aponeurotic	*5*	*4,5*
Associated with Jaw-wink	*8*	*7*
Associated with Blepharophimosis	*4*	*3,5*

Regarding the heights of palpebral fissure, 34 cases (30%) presented severe ptosis, 63 cases (56%) moderate, 16 cases (14%) mild (Table II).

Table II. The amount of ptosis was calculated as the difference in mm between the eighths of palpebral fissure

Amount of ptosis	*Difference between the eighths of palpebral fissure*	*Cases*	*%*
Severe	*≥4mm*	*34*	*30*
Moderate	*>2,<4mm*	*63*	*56*
Mild	*≤2mm*	*16*	*14*

Regarding the levator function 25 cases (22%) presented poor function or levator, 46 cases (41%) moderate, 42 cases (37%) good (Table III).

Table III. The levator function was measured as the maximum lid excursion from maximal downgaze to upgaze

Levator function	*mm*	*Cases*	*%*
Poor	*≤4*	*25*	*22*
Moderate	*4-8*	*46*	*41*
Good	*≥8*	*42*	*37*

Levator resection was performed in 77 cases (68%), frontalis suspension in 36 cases (32%).
The postoperative outcome was poor in 15 cases (13%), suboptimal 12 cases (11%) and good in 86 cases (76%). The incidence of ambylopia was 25%.

Discussion

The main difference in the surgical procedure correcting blepharoptosis in adult patients and in the childhood is related to the predictability of lid height that in adult patients could be enhanced using local anaesthesia (7); this option is not available in the childhood, therefore, more care must be taken in evaluating two predictor patterns like the amount of the lid fissure and the levator function: in other words the surgeon must not fit all cases to his favourite technique but the surgery must be strictly related to the clinical patterns.
Cosmesis was the predominant indication for surgery in our pediatric sample, particurarly on parents's demand (8). The main indication for precocious surgery in our opinion is related to preventing amblyopia (9). Regarding the surgical procedures, we did not experience employing fascia lata (10); a double armend (skin needle) 3-O Supramid worked very well in our suspension procedure. The anterior approach was always our favourite choice in the levator resection procedure. This approach allowed us to create or enhance a nice lid crease (3) Regarding the complications we had only a transient (recovered in a couple of weeks) corneal exposure in 3 brothers with congenital external Ophthalmoplegia . The prevalence in our cases with a poor outcome (11) was not related to the surgical technique or to the choice of suspensors materials, but was related to the severity of clinical features like the patients with congenital external Ophthalmoplegia (3 cases), or with Blepharophimosis, Ptosis, Epicantus inversus syndrome (BPES 4 cases) . In conclusion, if a carefull preoperative evaluation is performed, the surgical outcome in childhood Blepharoptosis can offer a very good prognosis.

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SUPERIOR OBLIQUE PALSY: A REVIEW OF 135 CASES

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Purpose. Paresis of the superior oblique muscle is a common isolated palsy of an extraocular muscle. The aim of this study is to investigate clinical findings and surgical approaches to this ocular motility imbalance.
Methods. We reviewed the records of 135 patients with superior oblique palsy who were examined in our Department of Ophthalmology over the period 2000- 2005 to evaluate the etiology, clinical patterns, surgical management and outcome.
Results. Congenital palsy were mostly unilateral (79%), traumatic palsy bilateral (70%). An excyclotropia of greater than 15 degrees was highly suggestive of bilaterality. Our patients reported diplopia more frequently in acquired (60%) than in congenital (21%) superior oblique palsy. The surgical procedures were performed according to Knapp's classification of superior oblique palsy. In 47 cases (35%) tucking of superior oblique alone corrected the full deviation. In 14 cases tucking of superior oblique was combined with weakening of inferior oblique when the deviation exceeded 30 prism dioptres. In 60 cases (45%; Knapp class 8th) tucking the superior oblique was performed concurrently with recession of superior rectus.
Conclusion. In our experience surgical treatment of superior oblique palsy, according to Knapp's classification, is not as difficult or unpredictable as that of horizontal strabismus; in fact if one uses a well thought out plan of diagnosis and treatment the results are most gratifying to the patient and to the surgeon.
Key Words. Superior oblique palsy, Strabismus, Excyclotropia

INTRODUCTION

Paresis of the superior oblique muscle is a common isolated palsy of an extraocular muscle (1); because of its long intracranial course, the IV nerve is highly vulnerable to insult. The aim of this study is to investigate, the etiology, clinical findings surgical approach and outcome to this ocular motility imbalance since then haven't there been any studies evaluating the surgical correction of superior oblique palsy according to Knapp's classification.
We reviewed the records of 135 patients with superior oblique palsy who were examined in our Department over the period 2000-2005 to evaluate the etiology, clinical patterns, surgical management and outcomes.

METHODS

The study was a retrospective interventional case series report. The age of our patients ranged between 5 and 65 years. All patients undergo pre op head tilt, torsion with the Maddox double rod test, and a prism cover test in the 9 positions of gaze in relation to fitting all cases into Knapp's classification, divided cases of superior oblique palsy according to where the deviation was greatest and assigned a surgical plan to each class. The age of the patients with diplopia ranged between 13 and 65 years. Patients with a history of a previous surgery were excluded from this study. The etiology of superior oblique palsy is listed in Table I.

Table I. Etiology of superior oblique palsy: 135 cases

	Number	Unilateral	Bilateral	Male	Female
Traumatic	40 (30%)	12 (30%)	28 (70%)	35 (87%)	5 (13%)
Congenital	88 (65%)	70 (79%)	18 (21%)	50 (56%)	38 (44%)
Idiopathic	7 (5%)	5 (71%)	2 (29%)	5 (71%)	2 (29%)
Total	135	87	48	90	45

In congenital cases were also included examinated longstanding and decompensating deviations and congenital cases examinerd while the patients were still children. In our series childhood photograph of head posture was helpful in many cases. The congenital preponderance was related to those patients in whom the onset of strabismus or torticollis dates back to infancy as per history or photographs. The male preponderance extended not only to the traumatic group but was present in all other etiologic categories as well. Congenital palsy were mostly unilateral (79%), traumatic palsy were mostly bilateral (70%); some patients were initially diagnosed as having unilateral palsy, the bilaterality became apparent after surgery. The main criteria in order to confirm a bilateral superior oblique palsy were reported in Table II.

Table II. Clinical patterns in 48 cases of superior oblique paresis

V pattern	35 (73%)
HTT positive to either side	40 (83%)
Left hypertropia to the right Right hypertropia to the left	48 (100%)
Excyclotropia > 15 degrees	46 (96%)

All patients with bilateral palsy had a right hypertropia in left gaze and a left hypertropia in right gaze. In our experience because of typical asymmetrical involvement of superior oblique muscle the diagnosis may be missed if careful measurements in the lower fields of

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gaze at near are not performed. Because of V pattern the typical head posture in our patients was a chin depressed assumed so that the patients can fuse in upgaze.

Our patients reported diplopia more frequently in acquired (60%) than in congenital (21%) superior oblique palsy. The prevalence of head tilt in our series (70%) is similar to that encountered by other authors (3). Our surgical procedures, followed Knapp's classification of superior oblique palsy and its surgical management and are (4, 5) reported in table III: tucking of superior oblique tendon on the temporal side of the superior rectus was the procedure of choice (89% in our series). Recession of the ipsilateral superior rectus (combined with tucking the superior oblique) was performed in 67 cases (50%; Knapp class 8th). The 8th class consisted of a large vertical deviation in all fields, frequently more on the non paretic side. (Personal communication 1987).

Results

A positive head tilt test to either side was elicited in 83% of patients with bilateral palsy; the presence of a V pattern (73% in our cohort) strongly suggests bilaterality (2) (Table II). Excyclotropia of greater than 15 degrees is highly suggestive of bilaterality. Bilateral paresis of superior oblique in our series was usually related to a close head trauma; the patients complained diplopia due to esotropia in downward gaze; he rarely spontaneously awared of torsional components.

In 47 cases (35%) tucking of superior oblique alone corrected the full deviation. In 14 cases the tucking of superior oblique was associated with a weakening of the inferior oblique when the deviation exceeded 30 Prism dioptres. In 60 (45%) cases tucking of superior oblique was associated with the recession of the superior rectus according to Knapp class 8th . An overcorrection of tucking was not observed at anytime in the follow up period. In two cases the initial postoperative measurements showed a mild overcorrection that spontaneously recovered in few weeks. Weakening the inferior oblique alone was performed in 7 cases (Knapp class 1). In two cases recession of the contralateral inferior rectus was performed; the final result was an overcorrection and this procedure was not performed in subsequent cases. When a V-pattern exceeded 40 prism dioptres (5 cases in our sample, 3,5%), surgery on all four oblique muscles was necessary to eliminate the pattern (Table III).

Table III. Surgery performed in superior oblique paresis: 135 cases

Unilateral superior oblique Tucking	47	35 %
Unilateral superior oblique Tucking + Unilateral Disinsertion inferior oblique	14	10 %
Unilateral superior oblique Tucking + Unilateral Recession superior rectus	60	45 %
Unilateral Disinserzion inferior oblique	7	5 %
Bilateral superior oblique Tucking + Bilateral Disinserzion oblique superior (V pattern more than 40 p.d.)	5	3,5 %
Unilateral Recession yoke inferior rectus	2	1,5 %

The percentage reduction in deviation between the pre op and the post op was about 90% (this goal was set for a success). Any patient required re-operation.

Discussion

In this series tucking superior oblique tendon (89%), alone or in combination with other surgical procedures, was the procedure of choice according to Knapp's classification of superior oblique palsy. Recession of the ipsilateral superior rectus muscle (50% in our cohort), was always performed in conjunction with a superior oblique tuck according to Knapp class 8th . Advantages of the superior oblique tucking operation are that it straightens the head, reduces hyper-deviation and it has no effect on the width of the lid fissure. In our series overcorrection is rare and unusual (6).Under-correction is due to failure in pre-op testing to elicit full deviation that had been hidden by fusion, or can be related to the fear of overcorrection or to not knowing how much to tuck. Relatively few precautions must be taken to avoid the so-called Brown's syndrome, limiting elevation in adduction. Sometimes a tuck of the superior oblique always produces a transient Brown's syndrome. In our series with a tuck alone, not combined with a disinsertion of the inferior oblique, if care is taken to tuck the tendon alone, the limitation in elevation in adduction is mild and transient: in other words care must be taken that the various layers of subconjunctival tissue are kept out of the tuck. In cases where a gentle search fails to find superior oblique temporally to the superior rectus, the tendon can be searched medially and then moved and tucked temporally. If this is done one can do the proper amount of tuck (until there is resistance in tightening up the tucker). If one can not find the tendon after such a search, it is wiser to stop and do an alternative procedure rather than producing scar tissue in this area.

In conclusion in our experience, the surgical treatment of superior oblique palsy according to Knapp's classification is not as difficult and unpredictable as that of horizontal strabismus; in fact, if one uses a well thought-out diagnosis and treatment plan the results are most gratifying to the patient and to the surgeon.

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Ranibizumab (Lucentis®) injection by anterior chamber in aphakic eyes with myopic choroidal neovascularization

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Purpose. macular choroidal neovascularization (CNV) is one of the most vision-threatening complications of myopia, which can lead to severe vision loss. Our purpose was to evaluate the safety and efficacy of trans-corneal injection of ranibizumab in the treatment of myopic CNV in aphakic patients.
Materials And Methods. ten eyes of 10 aphakic patients with CNV secondary to pathologic myopia treated with three trans-corneal injection of ranibizumab were evaluated. A complete ophthalmologic examination including best-corrected visual acuity (BCVA) and fundus biomicroscopy, specular microscopy, fundus optical coherence tomography (OCT), fluorescein angiography (FA) were performed at baseline and monthly for all patients. Mean time of follow-up was 6 months.
Results. The mean axial length was 27,6 mm (range, 25.7-31.3 mm). The mean initial visual acuity (VA) was 0.19 (decimal equivalent). A statistically significant improvement to a mean VA of 0.33 decimal equivalent (log-MAR:0.48) was demonstrated at the final follow-up. VA improved by a mean of 2.86 lines. Mean central macular thickness (CMT) measured with OCT was 340 µm (range, 179-663 µm) at the baseline, and was reduced significantly at the final follow-up to 212µm (range, 125-455 µm). No injection complications or drug-related side effects were noted during the follow-up period.
Conclusions. in this small series of aphakic eyes with limited follow-up, ranibizumab by anterior chamber administration seems to be a safe and effective treatment for CNV secondary to pathologic myopia (PM), without any complications. Further studies to evaluate the safety and efficacy are justified.
Keywords. Ranibizumab, safety, efficacy, myopic CNVs, aphakia, anterior chamber.

Introduction

Pathological myopia (PM) is one of the leading causes of visual disability in the world from 20–50 years of age[1,2]. Choroidal neovascularization (CNV) is one of the most important vision-threatening complications of PM and occurs in 5–10% of myopic patients, with a positive correlation between risk and degree of myopia. Among myopic patients with pre-existing CNV, more than 30% will develop CNV in the fellow eye within 8 years[3,4] .

The process of angiogenesis is multi-factorial and highly complex, but vascular endothelial growth factor (VEGF) is considered critical both in physiological and in pathological angiogenesis[5,6].

Ranibizumab (Lucentis®, Novartis, Basel, Switzerland) is an anti-VEGF antibody recommended as an option for the treatment of wet age-related macular degeneration (AMD). Usually it's inserted with intravitreal (IVT) injection via pars plana and the recommended dose is 0,5 mg (0,05 ml)[7]. Several studies have reported promising short-term results with off-label use of the intravitreal anti-VEGF drug ranibizumab for the treatment of CNV in PM[8,9,10].

The IVT injection procedure as such is not without risks for serious complications such as endophthalmitis[11,12], retinal detachment, perivenous retinal haemorrhages[13,14,15], vasculitis and increased intraocular pressure (IOP). An anterior chamber ocular inflammation is possible: it's dose-dependent, relatively rapid (peaked at day 2 post injection) and transient. In the vitreous, it was later (peaked at week 1 post injection) and more persistent[16]. Retinal detachment is more frequent in hypermyopic patients with peripheral retinal degeneration[17,18].

The purpose of this study was to evaluate safety and efficacy of trans-corneal injection of ranibizumab by anterior chamber in 10 hypermyopic and aphakic patients with CNV and with peripheral retinal degeneration or retinal tears (treated by argon laser) or previous retinal detachment in the fellow eye.

Materials And Methods

We conducted a prospective, consecutive, non-randomized, interventional study of 10 eyes of 10 patients with CNV secondary to pathologic myopia treated with trans-corneal injection of ranibizumab in St.Maria Scotte Siena Hospital. Best-corrected visual acuity (BCVA), slit-lamp examination, specular microscopy, optical coherence tomography (OCT), and fluorescein angiography (FA) were performed at baseline and monthly for all patients.

Inclusion criteria were retinal signs of PM, 6 months of follow-up, evidence of an active CNV on the basis of the presence of leakage on fluorescein angiography (FA)and/or intra-retinal or sub-retinal fluid on optical coherence tomography (OCT); all eyes were aphakic

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and had myopic retinal abnormalities such as peripheral retinal degeneration or retinal tears (treated by argon laser). Two case showed previous retinal detachment in the fellow eye.

Patient age, sex, affected eye, spherical equivalent refraction and any previous treatment administered were recorded.

The mean age of the patients was 53 years (range, 37 - 78 years); mean time of follow-up was 6 months and the patients were followed every month. Angiographic features in all cases demonstrated active CNV and patients with active CNV secondary to PM were offered treatment with intravitreal ranibizumab in an 'off-label' fashion. The potential benefits and side effects were discussed with patients and relatives: an informed consent was obtained from all patients after a thorough discussion before each injection. The number of intravitreal injections administered for each patient was 3.

All injections were performed by the same surgeon (Esposti Pier Luigi) using the same technique. Ranibizumab 0.5 mg (0.05 ml) was administered by trans-corneal injection under sterile conditions in the operating theatre. Before injection, tetracaine 0.5% was applied topically. Povidone iodine 5% was applied to eyelid margins, eyelashes, and conjunctival surface, and a lid speculum was placed. An additional drop of povidone iodine was applied to site of injection. Using a 30-gauge needle, 0.05 ml ranibizumab was injected through temporal clear cornea injection with the head of needle within the limits of the center of the iris. Postoperatively, a topical antibiotic (ofloxacin) was administered four times daily for 7 days.

Results

The patients were followed every month. Both initial ophthalmic examination and each follow-up included: evaluation of best-corrected distant VA using an EDTRS chart, slit-lamp examination, a fundus exam with dilated pupils, specular microscopy, FA, evaluation of retinal architecture and measurement of foveal thickness using the OCT, evaluation of worsening of subjective metamorphopsia. Any ocular or systemic adverse events were also recorded.

There were four (40%) male and six (60%) female patients. The mean axial length was 27,6 mm (range, 25.7-31.3 mm). The mean initial VA was 0.19 (decimal equivalent), with a range from 0.06 to 0.5. A statistically significant improvement to a mean VA of 0.33 decimal equivalent (log-MAR:0.48) was demonstrated at the final follow-up.

The patient presented asymptomatically without eye pain, redness, tearing or photophobia during our follow-up.

On slit-lamp examination, there was no anterior chamber cell or flare and vitreous examination revealed no cell. There was not ciliary injection, small keratic precipitates, anterior chamber cell or flare. Vitreous cell was not detected.

Corneal endothelial cell counts were done using a non-contact specular microscope: there wasn't a significant decrease in postoperative endothelial cell densities when compared to preoperative values. Mean preoperative endothelial cell densities were 2035 +/-270 cells/mm2; mean endothelial cell densities were 2022 +/-281 cells/mm2 at the final follow-up; the difference was not statistically significant.

All patients had retinal abnormalities consistent with pathologic myopia: all eyes were aphakic and had myopic retinal abnormalities such as peripheral retinal degeneration or retinal tears (treated by argon laser).Two case showed previous retinal detachment in the fellow eye.

The number of intravitreal injections administered for each patient was 3. None additional monthly injections was performed in eyes because we had not patients with persistent CNV leakage after 3 months: all eyes had angiographic closure after 3 monthly trans-corneal injections of ranibizumab.

The OCT results also showed significant reduction in CMT after treatment. Mean central macular thickness (CMT) measured with OCT was 340 µm (range, 179-663 µm) at the baseline, and was reduced significantly at the final follow-up to 212µm (range, 125-455 µm).

It is interesting to notice that none patient had worsening of subjective metamorphopsia.

One potential risk that should be considered in the treatment of myopic CNVs with anti-VEGF is the possible formation of marginal crack lines after treatment-related contraction of the myopic CNVs, which was considered an indication of early damage of retinal pigment epithelium that might lead to expanding macular chorioretinal atrophy [19]. No marginal crack lines were noted during our follow-up.

No ocular or systemic complications were noted after our injections (Table 1).

Table I. Pars plana injection Vs our trans-corneal injection: number of complications.

Adverse event/ Complication	*Intravitreal administration via pars plana Studies: FVF2598g(MARINA) FVF2587g(ANCHOR) FVF3192(PIER)*	*Administration by anterior chamber (our method)*
IOP increased	15.7%(ANCHOR)-16.3%(MARINA)	10%
Retinal detachment	0.4%(MARINA)-1.4%(ANCHOR)	0%
Retinal haemorrhage	16.7%(MARINA)-18.6%(ANCHOR)	0%
Subretinal fibrosis	4.2%(MARINA)-12.9%(ANCHOR)	0%
Uveitis	0.4%(MARINA)-0.7%(ANCHOR)	0%
Vitritis	5.5%(MARINA)-8.6%(ANCHOR)	0%
Endophthalmitis	0%(PIER)-0.4%(MARINA)-0.7%(ANCHOR)	0%
Retinal artery occlusion	0%(MARINA-ANCHOR)	0%
Retinal tear	0%(ANCHOR)-0.4%(MARINA)	0%

Discussion

No generally accepted and satisfactory treatment protocol exists for patients with CNV secondary to PM. In former years, before anti-VEGF drugs became available, PDT was considered as the only treatment option. Angiogenesis is regulated by several proangiogenic and antiangiogenic factors, many of which have now been identified. VEGF has been found to be one of the key elements in angiogenesis. Tong et al [20] showed that the VEGF concentrations in aqueous humour were markedly increased in patients with CNV secondary to PM when compared with the controls. Several studies demonstrated promising short term results for the treatment of CNV in PM with off-label use of the intravitreal anti-VEGF drug bevacizumab [21,22,23,24,25].

Recently, several authors reported the benefit of intravitreal ranibizumab for myopic CNV[8,9,10].

The primary objective for our study was to evaluate the efficacy of ranibizumab by anterior chamber injections in preventing vision loss and to evaluate the safety and tolerability of our trans-corneal injections of ranibizumab (administered monthly). We prove the same efficacy of trans-corneal ranibizumab vs. intravitreal ranibizumab administration: the 6-month outcomes suggest trans-corneal ranibizumab to be a promising treatment method for CNV secondary to PM in aphakic patients, resulting in both visual and anatomic improvements, without any complications (TABLE 1). It is interesting to notice that we did not observe peripheral retinal break and/or retinal detachment after trans-corneal injection in our predisposed hypermyopic patients: retinal detachment is more frequent in hypermyopic patients with peripheral retinal degeneration.

One patient had intra-ocular pressure (IOP) rise, but the elevated IOP was manageable, easily monitored and not serious. Therefore, this adverse event is clearly outweighed by the superior efficacy of the study drug.

In conclusion we show that in this small series of eyes with limited follow-up ranibizumab by anterior chamber administration seems to be a safe and effective treatment for CNV secondary to pathologic myopia.

Further studies to evaluate the safety, efficacy and optimal treatment regimen are justified.

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Adjustable suture strabismus surgery: a review of 850 cases

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Purpose. The aim of the study is to investigate the relationship between the different kinds of squint and the adjustament required in post-op.
Methods. Our personal case histories concerns 850 patients that underwent adjustable suture strabismus surgery during the last ten years, examinated in our Department of Ophtalmology over the period 2000-2009. The same surgeon (G.L.) performed adjustable suture strabismus surgery as routine duties, whether on horizontal or vertical rectus muscles, but never on oblique muscles. Adjustments were always performed in the 24 postoperative hours.
With regard to the relationship between previous surgeries, and requested regulation: 669 cases not underwent previous surgery; 181 cases peviously underwent surgery one or more times.
Results. First we seek for the statistical significant difference between the various results found, computing X2, ρ and the relative risk. We can conclude by saying that who made a vertical muscle squint surgery presents a relative risk of 3 times greater to take hypocorrection rather than hypercorrection and that who made before squint surgery presents a relative risk of 2,72 times greater to take regulation.
Conclusions. We predicted this result because it confirms that the use of adjustable suture is the more frequent as the muscle fibrosis is the more significant. And the fibrosis is an usual outcome of previous squint surgery.
Keywords. Strabismus, Squint surgery, Adjustable suture

Introduction

The wide fields of action of adjustable sutures have been and still today are the purpose of an inquiry by the squint-surgeons: if some surgeons think that the adjustable suture can be useful only in selected cases, other ones usually apply this technique (1,2,3,4) .
In our case we believe that we can apply this technique in every kind of squint as routine. But above all we would like to demonstrate that it is irreplaceable in the resurgeries and in all the situations in which the anatomic muscle structure presents great pathologic anatomical changes due to extensive cicatricial and synechial instances. In practice in all that conditions in which we cannot predict the reaction to the muscle reinforcement or weakening(5,6,7).
So we focalized our attention searching for correlations between previous or not squint surgery, and the necessity of adjustable suture regulation (8,9,10,11), seeking for the statistical significant difference between the various results found, computing X2, ρ and the relative risk.

Methods and materials

The peculiarity of strabismus surgery is strictly connected with the precise quantification of weakening or reinforcement practice that we apply to the concerned muscles to obtain the complete correction of the deviation angle and, when it's possible, the reinstatement of binocular vision.
The outcome of surgery practice is conditioned by two main factors. On one hand the innervational factors, difficult to quantify because related to the emotional condition of the patient, responsible for the so-called "muscle tone", and completely eliminable only under general anaesthesia(12,13,14).
On the other hand we have the mechanical factors that derive from the pathologic anatomical changes of the muscle (both for congenital and acquired diseases) that however modify the tension-length ratio of the muscle and consequently the muscular strength. The mechanical factors gain more importance in the presence of extensive cicatricial and synechial instances, as the outcome of previous muscle surgery. Obviously using topical anesthesia only the mechanical factors will act (15,16,17,18). Concluding, while on one hand the surgery enables us to continuously monitor the innervational condition using topical anesthesia, on the other hand only the use of general anaesthetics enables us to quantify the mechanical factors in the right way.
Consequently, the ideal solution is to evaluate both pa-

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rameters. So we have to fully valuate the mechanical factors in the absence of the so-called "muscle tone"; but we also have to verify and correct the surgery outcome in a second time when we will have the restarting of the innervational factors, that is at the end of the general anaesthesia (when the muscle tone is active again) using the adjustable suture strabismus surgery.
We usually execute a limbical-conjunctival approach, completed by radial section (19,20,21).
An absorbable double-armed suture (Vicryl 6-0) is passed on both sides of the muscle insertion in the recession procedure, or at a distance of few millimetres from the muscle insertion in the resection procedure. Therefore the muscle is disconnected from the globe, and, if it is necessary to strengthen its work, it is also shortened. The two ends of the double-armed suture are passed back through the sclera at the insertional stump, and if it is necessary to weaken the muscular action strength, the muscle insertion is replaced at a distance of few millimetres from the original place, towards the equator. Therefore the two ends of the double-armed suture are tied making first an overhand-knot, and then a bow that can be simply loose at the regulation time to precede or to recede the muscle.
The conjunctiva must be suture to the sclera in such a way to exhibit the knot and to facilitate the regulation of the adjustable suture. Usually we make it after 24 hours from the surgery, sometimes later, never before: in fact the regulation must be always done when we are sure that the muscle tone is completely retake (22,23,24,25).
In fact we uncover the patient's eye and we instill some drops of antibiotic, of anti-inflammatory steroid and finally of topic anesthetic into the fornix of the operated eye. Now we evaluate the patient to find the quantification of the deviation angle (to discover possible hypercorrections or hypocorrections); usually we make it from far and near, always with the patient's optics correction. On the basis of this evaluation the knot must be release and the muscle must be further advance or recede till the complete angle's correction.
Only when we obtain both the optimum conditions and the motor fusion (in an enough roomy space arch) we can definitively shut the suture's knot and the surgical technique can be considered complete.

REPORT OF CASES

Our personal case histories concerns 850 patients that underwent adjustable suture strabismus surgery during the last ten years, in our Department of Ophthalmology, Siena (Table I). All the surgeries were performed by the same surgeon (G.L.), to guarantee the surgery technique homogeneousness.
Adjustments were always performed in the 24 postoperative hours, with the exception of those cases in which we made use of adhesive stuffs, so that the timing was prolonged.
We performed adjustable suture strabismus surgery as routine duties, aside from the strabismus pathogenesis; obviously we practiced an exception to the rule with regard to those patients that just do not offer enough guaranty of cooperation.
We applied adjustable suture whether on horizontal or vertical rectus muscles (26,27), but never on oblique muscles. The patients age were between 7 and 82 years, middle age was about 37 years; 452 were males and 398 were females; 585 horizontal rectus muscles and 265 vertical ones.
With regard to the pathogenesy:

- 568 cases of non paralytic strabismus;
- 133 cases of paralytic strabismus;
- 149 cases of muscle fibrosis (above all referable to dysthyroid orbitopathy).

The evaluation of the surgical outcome has produced the follow results:

- about the horizontal rectus muscles we obtained 287 hypercorrections, 100 hypocorrections, and 198 cases in which no adjustable suture regulation was necessary;
- about the vertical rectus muscles we obtained 48 hypercorrections, 147 hypocorrections , and 70 cases in which no adjustable suture regulation was necessary.

With regard to the relationship between previous surgeries, typology of deviation and requested regulation:

- 669 cases (467 horizontal rectus and 202 vertical ones) not underwent previous surgery, adjustable suture regulation was necessary in 233 (of wich 145 horizontal rectus and 88 vertical ones);
- 181 cases (118 horizontal rectus and 63 vertical ones) previously underwent surgery one or more times, adjustable suture regulation was necessary in 172 (of which 116 horizontal rectus and 56 vertical ones).

Only in one case the deviation angle correction produced the incidence of diplopia; it was so hard that we had to reproduce the pre-surgery angle of deviation. It is interesting to note that the anamnestic reconstruction of the patient history revealed behaviour disorders and psychotropic drugs dependency.

Table I. Adjustable strabismus surgery: 850 cases

		HORIZONTAL RECTUS	VERTICAL RECTUS
NONE PREVIOUS SURGERY	REGULATION NECESSARY	**145**	**88**
	REGULATION NOT NECESSARY	**322**	**114**
	TOT.	**467**	**202**
PREVIOUS SURGERY		HORIZONTAL RECTUS	VERTICAL RECTUS
	REGULATION NECESSARY	**116**	**56**
	REGULATION NOT NECESSARY	**2**	**7**
	TOT.	**118**	**63**

We never had symptoms certainly referible to the oculocardiac reflex, but 7 patients exhibited other vagal responses (lipothymia) that spontaneously resolved without the use of specific drugs, but simply positioning the patient in Trendeleburg.
In 6 cases the complete absence of cooperation (at the regulation time) made useful the use of topic anaesthetics to obtain definitive knot closure.
In 13 cases, at the regulation time, the bow was not completely tight and the muscle was glided back towards the equator.

Results

First study
During the data processing, we focalized our attention searching for correlations between previous or not squint surgery, and the necessity of adjustable suture regulation. First of all we compute the relative risk obtaining the following results.
Who made before squint surgery to correct an horizontal strabismus presents a relative risk of 3,17 times greater to take regulation (Table II) and who made before squint surgery to correct a vertical strabismus presents a relative risk of 2,04 times greater to take regulation, in comparison with who did not make previous squint surgery (Table III).

Table II. First study: horizontal rectus muscle.

	REG.+	REG.-	TOT.
Previous surgery +	116	2	118
Not previous surgery -	145	322	467
Patients who underwent previous surgery to correct an horizontal strabismus show a relative risk of 3,17 times greater to need adjustment versus patients who did not.			

Table III. First study: vertical rectus muscle.

	Adiustment needed+	Not adjustment needed.-	TOT.
Previous surgery +	56	7	63
Not previous surgery -	88	114	202
Patients who underwent previous surgery to correct a vertical strabismus show a relative risk of 2,04 times greater to need adjustment versus patients who did not.			

Finally we can conclude by saying that who made before squint surgery presents a relative risk of 2,72 times greater to take regulation (Table IV).

Table IV. First study: overall estimation

	REG.+	REG.-	TOT.
Previous surgery +	172	9	181
Not previous surgery -	233	436	669
Tot	405	445	850
Patients who underwent previous surgery show a relative risk of 2,72 times greater to need adjustment (the fibrosis is an usual outcome of previous squint surgery).			

We predicted this result because it confirms that the use of adjustable suture is the more frequent as the muscle fibrosis is the more significant. And the fibrosis is an usual outcome of previous squint surgery.

Second study
Subsequently we evaluated if there is correspondence between making surgery on an horizontal rectus muscle, a vertical one and the type of necessary correction. So, who made a horizontal muscle squint surgery presents a relative risk of 1,113 times greater to take hypo- or hypercorrection (that is to need an adjustable suture surgery) (Table V) and that who made a vertical muscle squint surgery presents a relative risk of 3 times greater to take hypocorrection rather than hypercorrection.

Table V. Corrispondence between surgery on an horizontal rectus muscles, a vertical one, and the adjustment needed.

	HYPO/ HYPERCORRECTION	*NO ADJUSTEMENT REQUIRED*	TOT.
Vertical rectus operated	195	70	265
Horizontal rectus operated	387	198	585
Surgery on a horizontal muscle shows a relative risk of 1,113 times greater to need adjustment.			

Third study
With regard to the relationship between previous surgeries, typology of deviation and requested regulation:

1) 669 cases (467 horizontal rectus and 202 vertical ones) not underwent previous surgery, adjustable suture regulation was necessary in 233 (of which 145 horizontal rectus and 88 vertical ones);
2) 181 cases (118 horizontal rectus and 63 vertical ones) previously underwent surgery one or more times, adjustable suture regulation was necessary in 172 (of which 116 horizontal rectus and 56 vertical ones).
3) Then we seek for the statistical significant difference between the various results found, computing χ^2 and ρ.

Statistical significant difference regarding previous surgery or not, horizontal or vertical muscles, computing χ^2 and ρ.

No previous surgery (1)

	Horizontal rectus	Vertical rectus
ADJUSTMENT REQUIRED	145	88
ADJUSTMENT NOT REQUIRED	322	114
TOT.	467	202

χ^2=9,730, ρ =0,018
The difference is a statistical significant difference because p<0,05.

Previous surgery (2)

	Horizontal rectus	Vertical rectus
ADJUSTMENT REQUIRED	116	56
ADJUSTMENT NOT REQUIRED	2	7
TOT.	118	63

χ^2=7,707, ρ=0,0055
The difference is a statistical significant difference because p<0,05.

Difference between adjustable suture squint surgery not applied on vertical and horizontal rectus, apart from previous surgery or not (3)
χ^2=11,874
ρ = 0,0006
The difference is a statistical significant difference.

CONCLUSIONS

The adjustable strabismus surgery can be considered safe as any usual surgical technique. Certainly this surgical procedure is not proposable in the teen age; we can performe this technique in every kind of squint as routine, but we think it is mandatory in the reoperations and in all the cases in which the muscle's structure shows greatest metaplasya.
The adjustable sutures help to gain the desired alignment in the short term follow up, but not in the long term follow up; in fact the final result is related to many factors, particularly to the sensorial imputs.

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A CASE OF HELLP SYNDROME: THE IMPORTANCE OF ANGIOGRAPHY WITH INDOCIANINE GREEN FOR DIAGNOSIS OF RETINIC AND CHOROIDAL VASCULAR OCCLUSION.

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Purpose. To present a case of retinal and choroidal vascular occlusion in conjunction with HELLP syndrome.
Methods. A 35 years-old woman presented with HELLP syndrome underwent cesarean delivery for preeclampsia. After 2 months she had a decrease of vision in OD. She had performed an eye examination with FAG and ICG.
Results. The FAG shows at the posterior pole in the perifoveola temporal area different areas of hyperfluorescence. ICG reveals frameworks of hypoperfusion in perimacular areas, for the probable presence of ischemic foci. After about 5 mounths she presented an improvement in visual acuity and a complete resolution of Ophtalmoscopic findings.
Conclusions. Ophtalmic complications are possible during this syndrome. This is the first description of a patient suffering a retinal and choroidal vascolar occlusion after HELLP syndrome, and we report the important of ICG for diagnosis of choroidal ischemia.
Key words: HELLP syndrome, preeclampsia, retinal and choroidal vascular occlusion, thrombotic microangiopathic vasculopathy

Case report

A 35-year-old woman presented with HELLP syndrome underwent cesarean delivery for preeclampsia, which she has been treated for the previous months. She was admitted to hospital complaining of severe headache, oliguria and non-specific visual disturbance. Her medical and obstetric history was negative. Blood pressure was found to be 190/110 mmHg. Clinical laboratory tests showed elevated white blood cells (16,700 mm3 with 80 % of neutrophils), elevated erythrocyte sedimentation rate (76 mm in the first hour), abnormal liver function with elevation of aspartate aminotransferase (AST / SGOT =166 IU/l) and lactate dehydrogenase (LDH =1,112 IU/l) and severe proteinuria (50 g/l). Analysis of the coagulation system revealed thrombocytopenia (platelets 40,000/mm3), decreased plasma fibrinogen (1,88 g/l) and a short activated partial thromboplastin time (27 seconds). After 2 months from the delivery, she had a decrease of vision, discovered accidentally turning a blind eye, and she has noticed it after an ICU admission for pancreatitis, so advice she was sought from ophthalmology.
The visit found a significative cystoid macular oedema in OD and was prescribed a treatment with Acetazolamide and systemic Steroid. Blood pressure was normal (110/70 mmHg) and the intraocular pressure was normal in both eyes.
After 5 month of previous hospitalization, the patient has performed an eye examination that has found a visual acuity of 20/25, FAG and a Fundus Fluorescein Angiography with Indocianina Green (ICG) .
The FAG shows at the posterior pole in the perifoveola temporal area different areas of hyperfluorescence, which increase in extension in late times with an attitude of cystoid oedema. ICG reveals frameworks of hypoperfusion in perimacular areas, for the probable presence of ischemic foci .
The best corrected visual acuity (BCVA) is 20/25 in the right eye. Systemic steroid and oral Acetazolamide therapy was continuated, but visual acuity did not improve.
After 2 months the best corrected visual acuity(BCVA) is 20/20 in both eyes, and there was a reduction of oedema.
But after 5 months the BCVA in the right eye is 20/32 and 20/20 in the left eye. Optical coherence tomography (OCT) of the right macula detected a foveal elevation (FT 267 µm) and thickening of the temporal foveal section with diffuse hyper-reflectivity of the neuroepithelium.
After a week visual acuity improves, 20/20 in both eyes, and the patient was discharged with home therapy (systemic Steroid, Acetazolamide).

Discussion

During normal pregnancy and immediate puerperium there is an increase in the level of clotting factors and in clotting activity and there are also a number of pathologic sources of thrombosis and of emboli. This increased risk may also shows itself by occurrence of retinal and choroidal vascular occlusion. The earlier literature reports that retinal vein occlusions occur during or immediately after pregnancy.
The HELLP syndrome is a thrombotic microangiopathic vasculopathy that may be present in pregnancy and puerperium. The etiology and pathogenesis of this syndrome has not been elucidated, but it has been forwarded that dysequilibrium in prostanoid metabolism exists. Frequently, the natural evolution of HELLP syndrome is one disseminated intravascular coagulation. Blood pressure values, the plasmatic bloodcloatting factors and platled count in our patient were found in the normal range at the time of retinal vein occlusion, but HELLP syndrome is characterized by unpredictable occurrence of severe maternal complications during and soon after the syndrome (3). The retinal vein occlusion and the choroidal damage are reported causes of visual disturbance observed in HELLP syndrome (4). Choroidal vascular changes, choroidal vasoconstriction, and the ischemia are responsible for the most retinal damage seen in the pregnancy-induced hypertension. FAG shows the presence of macular oedema and Indocyanene Green Angiography suggest that in the hypertensive choroidopathy endogenous vasoconstrictor agents leak freely from the choriocapillaries and act on the walls of the choroidal vessels resulting in choroidal vasoconstriction and ischaemia.
The Indocyanene Green Angiography is an important tool that assist the determination of choroidal Ischemia.

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Bare sclera closure: a surgical approach in congenital esotropia

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Background. The purpose of the authors is to evaluate the efficacy of the bare sclera closure (conjunctival and episcleral tissue recession in association with bimedial recession) in a large cohort with congenital esotropia.
Methods. The charts of 140 patients with congenital esotropia, operated on with this tech-nique, were analysed; each patient was assigned to a single group on the basis of their pre-op angle, respectively between 35 and 40 pd (36 cases); between 40 and 50 pd (66 cases); more than 50 pd (38 cases).
Results. The results were statistically analysed; the p value was statistically significant in the group of patients with a pre-op deviation between 35 and 40 pd with a follow up of 12 months.
Conclusions. This technique is a safe and effective procedure and can improve the surgical result through a uniform approach (2 muscles operated on) versus a selective approach (3 or more muscles operated on).

Introduction

The main aim in congenital esotropia surgery, and on the whole strabismus surgery, is to ob-tain the best ocular alignment through the least traumatic surgical approach. This assertion is particularly truthful in regard to congenital esotropia where the large angle needs a large amount of surgery.
Some Authors perform surgery on two muscles initially, regardless of the preoperative devia-tion (uniform approach); other surgeons approach is to operate on three or four muscles, tai-loring the amount of surgery to the pre operative deviation (selective approach). Another problem concerns the measurement of recession[1] from the muscle insertion or from the limbus; the medial rectus muscle insertion site is close to the limbus in childhood, then it recedes until it is 5.5mm from the limbus in adulthood.
According to Barsoum- Hosmy1 we can divide the growth of the globe into three phases (figure 1): a postnatal phase of rapid growth between birth and 18 months of age during which the axial length of the globe increases by 4mm; a phase of moderate growth between 2 and 5 years of age during which the length increases by 1,1 and 1,2mm; and a phase of slow growth between 5 and 13 years of age, during which the length increases by 1,3 to 1,4mm. Some Au-thors have shown that larger 2 muscle recession of 6- 7, and even 8mm can be performed in patients with larger angle congenital esotropia with results similar to that obtained with simul-taneously performed surgery on 3 or 4 muscles. The disadvantage of augmented bimedial re-cession can be an increased incidence of late consecutive exodeviations and a severe limitation of adduction. We report our experience in congenital esotropia with a bimedial recession aug-mented with a bare sclera closure (BSC) (recession of the conjunctiva and the episclera).

Subjects and methods

We reviewed medical records of 140 consecutive patients with congenital esotropia who un-derwent bimedial rectus recession plus BSC. The patients'age were between 24 months and 4 years. Patients were excluded from this study for one or more of the following reasons: onset of the deviation after 6 months; pre-op deviation less than 35 pd; A and V patterns, accom-modative factors (hyperopia greater than 2 diopters); follow up less than 1 year: significant neurological deficit. Each patient was assigned to their group on the basis of their pre-op angle, respectively between 35 and 40 pd (36 cases); between 40 and 50 pd (66 cases); more than 50 pd (38 cases).
We used alternate prism and cover test with the child fixating on an accommodative target at distance and at near. Because of the young age of our patients the Krimsky test was used in most cases. Cycloplegic refraction was performed using tropicamide 1% or atropine 0.5%. All patients in the first group (35 – 40 pd) underwent 5.5mm bimedial recession in addition to BSC; all patients in the second group (40 – 50 pd) underwent 5.5mm to 6.5mm bimedial in addition to BSC; all patients in the third group (50pd) underwent 6.5mm to 7mm bimedial re-cession in addition to BSC. Measurements were taken from the limbus after disinsertion of the muscle. The conjunctiva and the episclera

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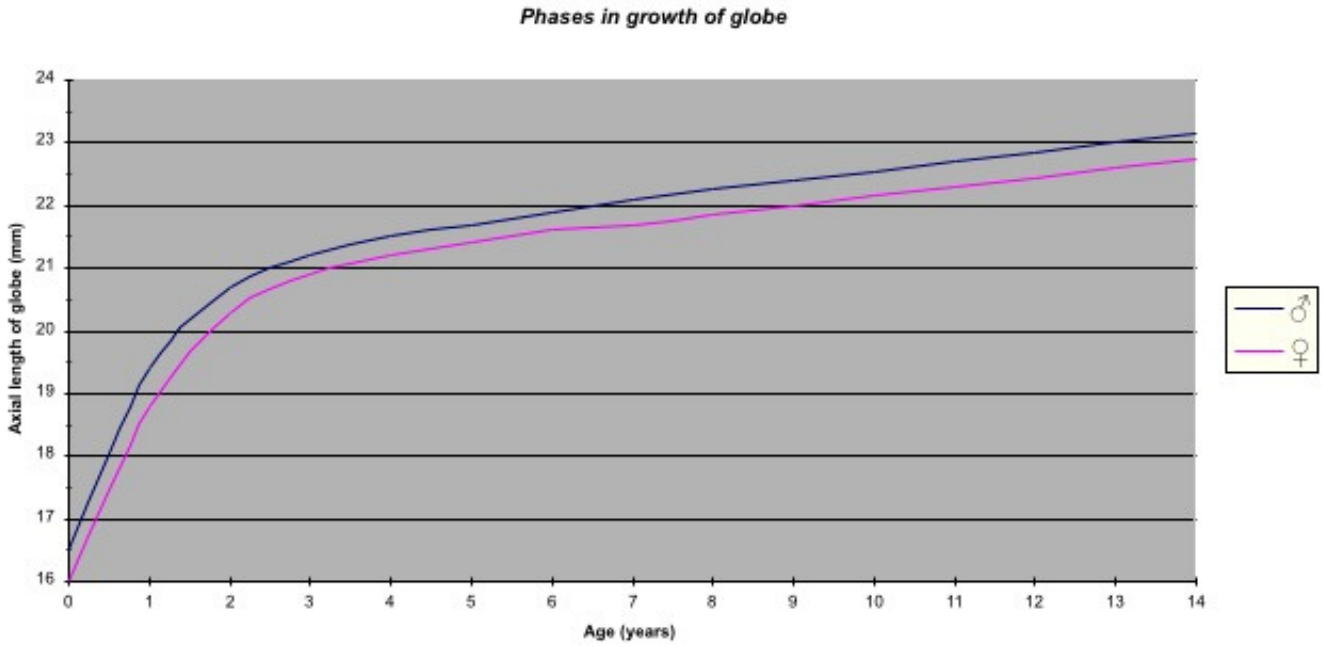
Fig.1: The growth of the globe



were recessed to the original muscle insertion (BSC). The patients were checked at 24 hours, 3, 6 and 12 months in the post-op period.

RESULTS

Our results are reported in table 1. We tested our data with a statistical analysis (Z test) (table 2): we found a p value statistically significant (p= 0.001) in the first group (follow up 12 months); in the second and in the third group the p value was not statistically significant in any of the cases. The trend is toward a late stability of the angle in the post-op period (6 – 12 months). A noteworthy finding was a conjunctival cyst growth early in the post op period in five patients ; a similar finding was not found at anytime in our adjustable strabismus sur-gery (800 cases) where a BSC is routinelly performed to adjust the sutures 24 hours after surgery.

In 4 cases the cyst required surgical excision; in 1 case the cyst vanished spontaneously.

DISCUSSION

Several Authors2 have advocated exceeding the traditional mm recession in large or even moderate angle esotropias. Others have avoided large recession because of concerns of adduc-tion deficiency, convergence weakness and consecutive exotropia with prolonged follow up. Furthermore, high rates of undercorrection in large angle congenital esotropia with traditional bimadial rectus recession have led many surgeons to prefer three to four muscle procedures as initial treatment in these cases. Ing et Al 3 reported a success rate for 5mm bimedial recession of only 30% in 40 patients with esotropia of 50 pd or greater. Scott et al.4 reported a success rate of 37.3% in 57 congenital esotropes with deviations of 50 or more undergoing bimedial rectus recession. With three or four muscles, their success rate was 64.5% in 48 esotropes with comparable preoperative deviation. Lee et al.5 recommend bilateral medial rectus reces-sion and bilateral rectus resection in congenital esotropes with deviation in excess of 50 pd. They report 61% of their patients had straight fixation after only one operation. Forster et al.6 reported a 79.4% success rate with one procedure operating on three to six muscles. However, only 32.5% of these patients had an esodeviation of 50 pd or greater. More recent studies have suggested that results comparable to three or four muscle procedure can be obtained in large angle esotropia with 6mm to 7mm bimedial rectus recession. Hess and Calhoun7 were early proponents of large bimedial rectus recession, reporting a 60% success in 10 patients with esodeviation from 60 to 100 pd undergoing 7mm bimedial rectus recession. Preito Diaz, 8 also a proponent of large recessions, reported an 80% success rate in congenital esotropia with bimedial rectus recession from 6 to 8mm with a 3 year follow up (in these cases the amount of pre-op deviation is not mentioned). Szymd et al.9 reported a 91% success rate at 6 weeks us-ing 6 to 7mm recessions in 45 congenital esotropes with deviations exceeding 50 pd. Nelson et al.10 reported an 83.5% success rate in 97 congenital esotropes with greater than 50 pd of esotropia undergoing graduated 6 to 7mm bimedial rectus recession with a mean follow up of 23.4 months.

We believe our results with 5.5mm to 6.5mm bimedial rectus recession with BSC compare favourably to three or four muscle processes for congenital esotropia. We feel this is a safe and effective surgical treatment in

Table1. Surgical results of 140 consecutive cases of congenital esotropia operated with bimedial recession and bare sclera closure

140 cases	Pre-op pd	Post-op pd 24 h	Post-op pd 3 months	Post-op pd 6 months	Post-op pd 12 months
36 cases Bim. Rec. 5.5mm B.S.C.	35 – 40	<10 (20%) 10-20 (80%)	<10 (59%) 10-20 (41%)	<10 (80%) 10-20 (20%)	Exo (2%) <10 (81%) 10–20 (17%)
66 cases Bim. Rec 5.5-6.5mm B.S.C.	40 – 50	< 10 (27%) 10–20 (68%) > 20 (5%)	<10 (33%) 10-20 (55%) > 20 (12%)	< 10 (68%) 10-20 (26%) >20 (6%)	Exo (1%) < 10 (67%) 10-20 (26%) >20 (6%)
38 cases Bim. Rec 6.5-7mm B.S.C.	>50	10–20 (36%) >20 (64%)	10-20 (49%) >20 (51%)	10-20 (59%) >20 (41%)	10-20 (59%) > 20 (41%)

Pre-op pd: Preoperative prismatic diopter
Post-op pd: Postoperative prismatic diopter
Bim. Rec.: Bimedial Recession
B.S.C.: Bare Sclera Closure

Table 2. Statistical analysis of 140 consecutive cases of congenital esotropia operated with bimedial recession with bare sclera closure

Pre-op 35-40 p.d. (MR rec 5mm. + B.S.C.)

Follow up 24 h.	m=15	p= 0.43
Follow up 12 months	m=10	p=0.001

Pre op 40-50 p.d. (MR rec 5.5 - 6.5mm + B.S.C.)

Follow up 24 h	m=15	p= 0.26
Follow up12 months	m=10	p= 0.15

Pre op >50 p.d. (MR rec 6.5 – 7mm + B.S.C.)

Follow up 24 h	m=15	p=1
	m=20	p= 0.99
	m= 25	p= 0.05
Follow up12 months	m=10	p= 1
	m=15	p=0.99
	m=20	p=066

Pre-op pd: preoperative prismatic diopter
MR rec: Medial Rectus recession
B.S.C.: Bare sclera closure
pd: prismatic diopter

congenital esotropia, particularly if the pre-op doesn't exceed 40 pd; in these cases the success rate increases between the 6th and 12th months in the post-op follow-up. This procedure does not appear to increase the rate of re-operation. Advantages of this method include a quicker, simpler and less traumatic procedure, which leaves the lateral rectus muscles unoperated on for the patients who need further sur-gery. Though this appears to be a promising procedure, we do feel that longer follow-up is needed to determine the true incidence of under and overcorrection.

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Quali-quantitative analysis of eight Rosmarinus officinalis essential oils of different origin. First report.

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Aim. It is well known that the pharmacological activity of essential oils depends on their major components, which may vary enormously. The aim of the present study was to determine the chemical composition of samples of essential oil of rosemary of different origins, in order to identify the main therapeutic constituents, according to European Pharmacopoeian (EP).
Material and Methods. Analytical GC/MS was carried out on a total of eight samples of essential oil of rosemary: seven samples were commercial products from producers located in different geographical areas; the last sample was prepared in our laboratory from fresh flowering terminal sprigs of rosemary collected in Siena's Province.
Results. The most representative constituents of the essential oils tested, were 1,8-cineole and camphor. Other components also occurred in significant quantities in some samples, for example and α- and β-pinene, limonene and caryophyllene, indicating clear phytochemical differences among samples.
Discussion. The high quantity of eucalyptol and camphor detected in the samples made them particularly suited for treating minor respiratory disorders. Eucalyptol is expectorant and liquefies bronchial secretions; camphor increases the interval between inspiration and expiration and increases the activity of the parasympathetic nervous system, facilitating respiration. On the other hand, the essential oils analyzed by us were not suitable for perfume production, because they contained little or no positive aromatic components.
Key words. Rosmarinus officinalis, essential oil, 1,8-cineole, camphor.

Introduction

Rosmarinus officinalis L. is a xeromorphic species that grows spontaneously on sand, cliffs and stony places near the sea in Europe, Africa and Asia (1). In Italy it is spontaneous along all coasts except the northern Adriatic (1). Italian production of essential oil of rosemary is supplemented with imports from Spain, Morocco, ex-Yugoslavia region and Tunisia (2).
The plant is of economic importance because of essential oils extracted by steam distillation from fresh leaves. The European Pharmacopeia (3) lists the following constituents of "Rosemary Oil": α- and β-pinene, camphor, 1,8-cineole, camphene, limonene and borneol.
Distillation of essential oil is mainly carried out in Spain, France, Tunisia, Morocco, ex-Yugoslavia, Dalmatia, Sardinia and Sicily. Spain produces the largest quantity of this essence, the quality of which may vary considerably (4).
Conventional medicine recognizes various therapeutic properties of rosemary oil, principally those of antispasmodic (5), antiseptic and antimicrobic (5, 6, 7), especially in respiratory diseases (8) and also those of stomachic, stimulant, revulsive and hyperemizing agent (4, 9).
It is well known that the pharmacological activity of essential oils depends on their major components, which may vary enormously. The aim of the present study was to determine the chemical composition of eight samples of essential oil of rosemary of different origins, in order to identify the main therapeutic constituents, according to European Pharmacopoeian (EP).

Materials and methods

Essential oils

A total of eight samples of essential oil of rosemary were analysed: seven samples were commercial products from producers located in different geographical areas. The oils were labelled as being steam distilled from fresh flowering sprigs of R. officinalis as described in EP. The other sample was prepared in the same way by us from fresh flowering terminal sprigs of rosemary collected in the municipality of S. Quirico d'Orcia (Siena, Italy).

Gas chromatography-mass spectrometry

Analytical GC/MS was carried out on a Varian 3800 (Varian, Walnut Creek, CA) gas chromatograph interfaced with a Varian Saturn 2000 mass spectrometer. A Rtx-5MS (Restek Bellefonte, PA, USA) column (30 m x 0.25 mm, 0.25 µm film thickness) was employed, with helium as carrier gas (flow rate 1.0 mL/min). Samples were injected using the split sampling technique, ratio 1:10; 1.0 µl of sample (diluted 1:10 in chloroform) was injected. Oven temperature was held at 60°C for 8 min, then programmed at 3°C/min to 180°C, held there for 5 min. The MS operating parameters were: electron ionization 70 eV; scan m/z range 40-650. Identification of the constituents was carried out by comparing the retention times with those of reference compounds or by peak-matching library search using the NBS/NIST library and comparison of the MS data with those published in references works (10, 11).

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RESULTS

The volatile compounds identified in the eight rosemary samples are shown in Table 1, together with their respective percentages, in order of elution from the column.

Compound	percentage concentration (%)							
Sample no.	1	2	3	4	5	6	7	8
α-pinene	13.88	8.91	18.81	13.78	12.18	20.47	7.14	4.55
camphene	3.71	4.69	6.92	3.53	4.29	3.91	2.60	2.93
β-pinene	9.05	6.94	5.72	9.11	7.05	3.25	5.32	6.62
myrcene	1.78	1.15	1.58	1.79	0.61	0	tr	0.53
limonene	3.55	2.32	3.50	3.38	1.18	0	tr	tr
1,8-cineole	48.25	46.65	37.11	46.82	50.08	38.59	60.28	45.22
linalool	0.27	1.55	1.26	0.37	0	0	tr	0.89
camphor	6.89	14.87	8.80	12.39	17.12	27.72	10.80	15.90
borneol	tr	4.29	1.58	0.78	0	0	0.05	1.86
bornyl acetate	1.96	1.82	10.06	2.40	0.54	2.84	6.22	11.09
caryophyllene	5.11	5.91	4.31	5.68	6.92	3.21	7.65	10.41

DISCUSSION

Under "Rosmarini aetheroleum", the EP (3) lists eleven principal components characteristic of rosemary-type essence obtained from plants grown in Morocco, Tunisia and Spain. To characterize the samples of the present study, we therefore indicate the components listed in the EP as well as a further component, identified as caryophyllene, which we found in all samples in quantities sufficient to be considered representative. The EP specifies that essence from Spain is low in 1,8-cineole (1-2.2%), but high in α-pinene (18-26%) and camphor (13-21%), whereas essence from north Africa are much richer in 1,8-cineole (38-55%), with quantities of borneol, camphor and α-pinene around 10%. This is the most widely available essential oil.

In the analyzed samples , the most representative components were 1,8-cineole and camphor. According to Lugli and co-workers (5), a good essential oil of rosemary should contain 20-50% eucalyptol. Our samples had contents of 1,8-cineole from 37.11% (sample 3) to 60.28% (sample 7), and were therefore similar to African essences. They were also particularly rich in camphor, with values ranging from 6.89% (sample 1) to 27.72% (sample 6). According to the EP, Spanish essences have a camphor content in the range 13-21% in contrast with the lower content (5-15%) of African essences. However, the negative note of camphor is partly "neutralized" (in the organoleptic sense) by the good quantity of compounds imparting positive notes, for example the pungent but pleasant borneol, the floral fragrance of linalool and the sweet taste of bornyl acetate (samples 6 and 8). Sample 3, labelled as "bornyl acetate chemotype" did indeed contain the highest percentage of this component, except for sample 8 distilled by us.

The results of analysis showed that the essential oils were of the 1,8-cineole chemotype. The high quantity of eucalyptol and camphor detected in the samples made them particularly suited for treating minor respiratory disorders. Eucalyptol is expectorant and liquefies bronchial secretions; camphor increases the interval between inspiration and expiration and increases the activity of the parasympathetic nervous system, facilitating respiration. Eucalyptol and camphor are both revulsive agents, useful for circulatory disorders and rheumatic pain.

A camphor chemotype is mentioned for the first time in a recent aromatherapy text (4). Its parameters are: camphor up to 20%, 1,8-cineole 15%, borneol 5%. Essences of this type have mainly been distilled in southeast France and Spain. This composition has marked tonic effects on muscles and circulation.

Only samples 3, 7 and 8 had the parameters of the bornyl acetate chemotype, which must contain at least 5% of this component. Bornyl acetate has colagog, coleretic and hepatoregenerative properties, especially when associated with phenolic essences (such as thyme oil), and facilitates intestinal peristalsis. The bornyl acetate chemotype is indicated as a coleretic, colagog, regulator of intestinal flora and general stimulant. However, the essential oils analyzed by us were not suitable for perfume production because they contained little or no positive aromatic components.

More extensive studies are needed to define the chemical characteristics of the various types of essential oil of Rosmarinus officinalis. The present study shows the need for greater clarity in differentiating 1,8-cineole, bornyl acetate and camphor chemotypes, in order to establish the pharmacological differences between the various components so as to provide products tailored to the disorders to be treated.

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Vescico-ureteral reflux: endoscopic treatment, management and long-term follow-up.

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Introduction. Vescicoureteral reflux (VUR) is a dynamic event in which there is a retrograde passage of urine from the bladder to the ureters. It is the most common urological disease in the childhood and it manifests with recurrent urinary tract infections (UTI). The therapeutic strategy provides an antibiotic prophylaxis, invasive surgery (Cohen reimplantation) and minimally invasive surgery (endoscopic subureteral injection). Recently, Endoscopic Subureteral Injection with Deflux became the treatment of choice. The purpose of this work was to conduce a retrospective study of our patients with VUR to evaluate the role of endoscopic technique (Deflux and Macroplastique) in the treatment.
Materials and Methods. Forty two patients with VUR were treated by us in 65 refluxing units. Twenty were males (47.6%) and 22 (52.4%) females. Sex, age of infiltration, presence of associated diseases, unilaterality and bilaterality, side of presentation of VUR and substance used for injection were considered. The follow-up study included urine cultures and periodic renal ultrasound. The mintional cistouretrography was performed after 12 months. The results were statistically evaluated with the "Wilcoxson test", comparing data of patients treated with Deflux and Macroplastique.
Results. Refluxing ureters underwent endoscopic treatment by submeatal injection were 65, 4.6% with VUR grade I, 12.3% grade II, 43% grade III, 29.23% grade IV and 10.87% gradeV. In 19 patients, amounting to 45.2%, this was a unilateral VUR (84.2% left, 15.78% right) and in 23 patients, equal to 54.76%, a bilateral VUR. Thirdy eight per cent of these patients had associated diseases. At the first follow-up, the cure rate was 81.53%. At the second follow-up, the cure rate, including the 9 children re-infiltrated, was 89.23%. Four patients underwent a further infiltration, so as at the third follow-up, the overall cure rate was 93.84%. In only one patient with bilateral VUR grade IV, it was necessary to perform Cohen ureteral reimplantation due to the persistence of VUR after 2 endoscopic infiltration. In another one, due to the clinical severity and the persisting of VUR after two endoscopic infiltration, we decided to plan the bilateral reimplant according to Cohen. We have not been demonstrated significant differences based on gender, age of infiltration and the substance used, were not observed.
Conclusions. At the moment, ours patients have a regular weight-height growth and they don't have urinary tract infection or vescicoureteral reflux. The sub-meatal infiltration is a simple, repeatable and reliable techinique whereby results are immediate and safe in most cases. For this reason, we conclude that the submeatal infiltration represents the first-line treatment in patients with vescicoureteral reflux.

key words: Vescicoureteral reflux (VUR)

Introduction

Vescicoureteral reflux (VUR) is a dynamic event in which there is a retrograde passage of urine from the bladder to the ureters. It is the most common urological disease in childhood and it occurs with recurrent urinary tract infections (UTI).
Management of VUR in children is debated. Since 1970 many studies were elaborated about VUR (1). In particular techniques provide bladder opening and ureters mobilization.
Politano-Leadbetter and Cohen's strategies are most followed surgical techniques. They allowed to augment the length of intra-vescical ureter and to create a strong support by compression exercited by detrusor muscle.
Patients submitted to these invasive surgery had good results but a longer stay in hospital and an higher rate of complications.
In 1984 O'Donnel and Puri first reported the systematic use of injection treatment for reflux in children. The original procedure consisted of endoscopic injection of polytetrafluoroethylene (PTFE) paste suspended in glycerine. Injection was made into the lamina propria just behind the ureteric opening and it was shown to be very effective in a number of clinical studies. Since then many substances were used for sub-ureteral injection like Teflon, bovine collagen, Macroplastique and Deflux .
In particular Deflux and Macroplastique have been used in several studies to correct vesico-ureteric reflux with excellent success rates, but again some concerns

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about the duration of the treatment success has been raised (2).

MATERIALS AND METHODS

A retrospective study was carried out to value the role of endoscopic treatment in patients with VUR.
Since 1° January 2002 to 31 may 2010 42 patients with VUR were treated corresponding to 65 ureters. Male were 20 (47,6%) and female 22 (52,4%).
These data were analyzed:

1. Demographic factors: ratio male/female (M/F), age at the moment of endoscopic injection;
2. Risk factors: associated pathology like duplex system, posterior urethral valves, renal ectopia in patient with malformative syndrome and reflux nephropathy;
3. Unilateral and bilateral VUR, side of VUR presentation (left or right) and grade;
4. mode of presentation and diagnosis of UTI with positive urine exam, urine culture, renal ultrasound and retrograde cystourethrography;
5. Surgical therapy: endoscopic injection with Deflux and Macroplastique; ureteral reimplantation according to Cohen if the previous treatment failed.
6. Complications: VUR relapse, wound dehiscence, hemorrhage and fistula;
7. Mean duration of endoscopic treatment and hospitalization of patients.

For the endoscopic injection the patient was placed in the dorsal lithotomy position and an accurate inspection of two ureters was performed by cystoscopy. Classical technique for STING injection was applied and after introduction of injection needle into subureteral space, infiltration was performed with Macroplastique or Deflux. We excluded all patients spontaneously healed or that were submitted to open surgery.
Diagnostic exams showed ureteral dilatation, presence of renal scars and VUR grade on the basis of International Reflux Study classification (IRS) of 1981, before the treatment.
Urino-culture, renal ultrasound and retrograde cystourethrography were performed on annual follow-up.
Data were expressed by standard deviation and range. Wilcoxon signed test was used for statistical survey. We considered significant p values <0.05.

RESULTS

Patients with VUR were 42 corresponding to 65 refluent ureters. In 4.6% they showed VUR grade I°, in 12.3% grade II°, in 43 % grade III°, in 29.23% grade IV° and in 10.87% grade V (Tab. I).

Table I- VUR grade

VUR grade	n° ureters	% ureters
VUR I°	3	4.6%
VUR II°	8	12.3%
VUR III°	28	43%
VUR IV°	19	29.23%
VUR V°	7	10.87%

Mean age of injection was 5,3 years in male (range 9m-18y) and 5,8 years in female (range 1-18y). The ratio M:F was 1:1.
In 13 cases equal to 38%, there were associated diseases, as show in Table II.

Table II- Associated diseases in patients with VUR

ASSOCIATED DISEASES	PATIENTS	%
Double ureters	2	4,76%
Neurogenic bladder	4	9,52%
Myelomenigoncel	2	4,76%
Reflux nephropaty	5	11.9%
Plurimalformative syndrome	2 (1 with renal ectopia)	4,76%
Posterior Urethral valves	1	2,3%

Nineteen patients (45,2%) out of the 42 analyzed presented unilateral VUR: 16 to the left (84,2%) and 3

	UNILATERAL LEFT	UNILATERAL RIGHT	BILATERAL LEFT	BILATERAL RIGHT
I grade	0	0	0	3
II grade	2	0	2	4
III grade	9	2	12	5
IV grade	3	1	7	8
V grade	2	0	2	3
TOTAL	16	3	23	23

Table III- Ureteral distinction on the basis of grade in patients with unilateral and bilateral VUR

(15,78%) to the right.
Patients with bilateral VUR were 23 (54,76%) corresponding to 46 ureters. Table III shows VUR grades.
All patients come to our observation because of UTI (100%). In fact they had urine exam and urine culture positive.
In all patients with VUR III, IV, V (81,5%) grade renal ultrasound showed an average pelvic dilatation of 19,6 mms. Retrograde cystourethrography revelead the grade of VUR. Thirdteen patients (31%) were treated with Macroplastique since 2002 to 2005; 29 pazients (69%) were treated with Deflux since 2006.
The total cure rates at the first follow-up, after urine exam, urine culture and retrograde cystourethrography at 12 months, was 81.53% corresponding to 33 patients. Figure 1 shows the comparison between successes and failures on the basis of VUR grade to the first follow-up.
Nine patients (21.5%) presented UTI and retrograde cystourethrography revealed the persistence of VUR. For this reason it was necessary a second injection. At the II follow-up after 12 months, the cure percentage was 89.23%. Five patients were recovered after the II injection. Two showed unilateral VUR (1 III left and 1 V left) and 3 bilateral (IV left and I right, the first recovered at the I follow-up; IV bilateral, one of which recovered at the I follow-up; V right and II left the second of which recovered at the I follow-up).
Figure 2 shows the comparison between successes and failures on the basis of VUR grade at the second follow-up.
In 4 patients (11,91%) was necessary to carry out a third injection. They presented bilateral VUR:

- 1 patient with grade V right and III left (VUR grade III was recovered at the I follow-up)
- 1 patient with bilateral grade III (one of which recovered at the I follow-up)
- 1 patient with left grade III and right grade I (VUR grade I recovered at the I follow-up)
- 1 patient with bilateral VUR with grade IV

In the first 3 patient a third injection was performed and this strategy was resolutive. In 4 patients with bilateral VUR with grade IV it was necessary to perform the bilateral ureteral reimplantation according to Cohen after failure of the third injection.
In one patient because of the gravity of VUR and for persisted symptoms, we decided to program Bilateral reimplantation according to Cohen.
At the III follow-up the cure percentage was 93.84%, equal to 40 patients.
Figure 3 shows the comparison between successes and failures on the basis of VUR grade the third follow-up.
Mean duration of endoscopic treatment was 20 minutes.
The hospitalization of patient was 3 days.
Finally we applied Wilcoxon's test comparing results obtained with two substances (Deflux and Macroplastique).
This test did not reveal any difference between the substances used (P was inferior to 0,05).

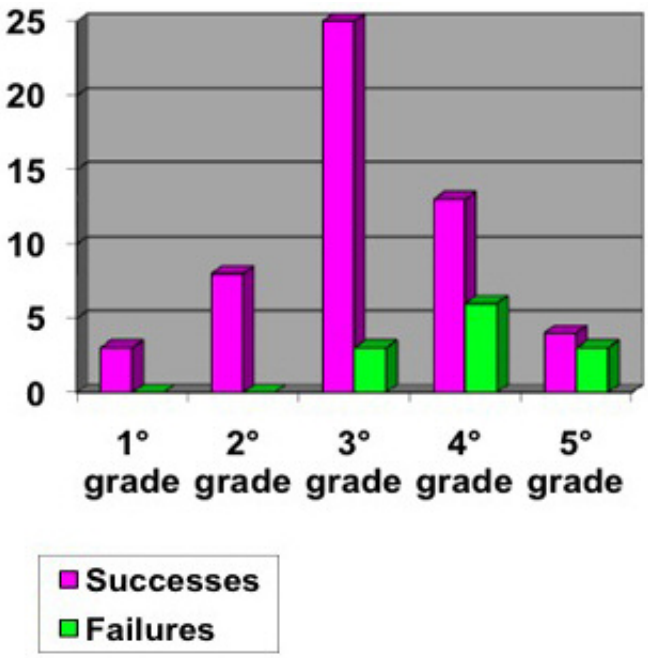
Discussion

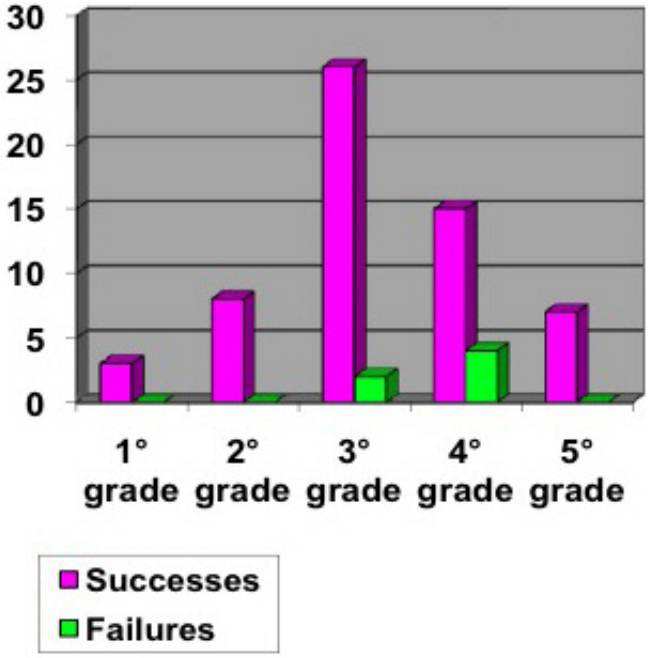
Many Autors, in agreement to the International Literature, propose antibiotic prophylaxis (up to 1 year) in patient with VUR. This strategy rappresents the first therapeutic approach to allow the spontaneous resolution or the reduction of VUR grade. In fact in this period it's possible the development of the vesico-ureteral junction (GUV) (4, 5).
The purpose of antibiotic prophylaxis is to make urine sterile to avoid the onset of UTI and possible evolution in renal insufficiency (2, 3). This strategy presents some difficulties: therapeutic compliance, continue monitoring, possibility to develop bacterial resistances and finally reflux nephropaty (6). American Urological Association Education and Research in 2010 also confirmed the value of prophylaxis in the first year of life (7). In fact the meta-analysis of 21 studies raccomended that:

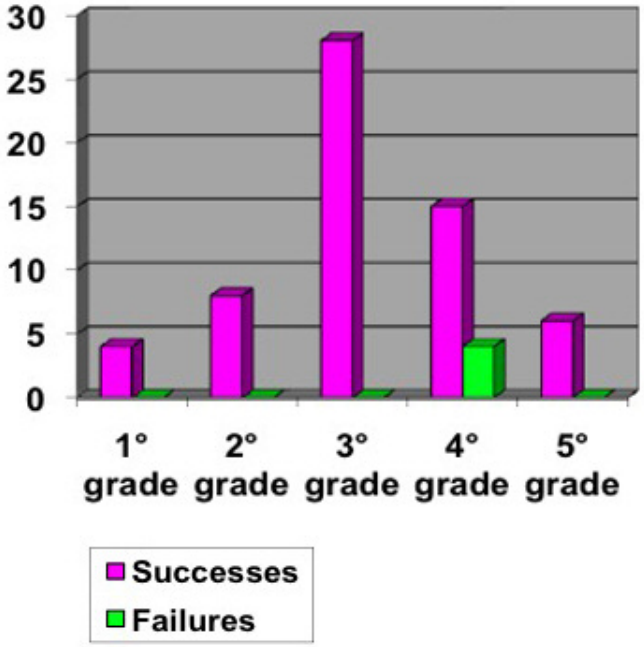
- The continue antibiotic prophylaxis (CAP) is indicated in all children with VUR and symptoms.
- If there are not UTI the continue antibiotic prophylaxis (CAP) is also recommended in all children with high grade (III-V) VUR.
- If there are not UTI in children with VUR low grade (I-II), CAP may be given on the basis of preferences of doctor.
- Male children with VUR and UTI may be subjected to circumcision.

There are also 84 studies that analyzed children above one year of life.
CAP may be given to children with asymptomatic UTI, VUR and without bladder/gut disorders.
A recent article published on Seminars in Nephrology, showed the role of antibiotic prophylaxis in patients (age 0-24 months) with UTI and VUR. On urine culture the most frequent pathogens were Escherichia coli (54-67%), Klebsiella (6-17%), Proteus (5-12%), Enterococcus (3-9%) and Pseudomonas (2-6%). In patients with age below 3 months and with UTI the antibiotic prophylaxis is racommended. Patients older than 3 months were treated with cefixim or amoxicillin / clavulinic acid. In conclusion the Autors argue that the therapeutic management should include antibiotic prophylaxis in the first year of life (8).
American Urology Association recommends endoscopic injection in children with VUR (10). Reccomandations for Endoscopic treatment in patients with VUR are:

1. Prevention of reflux nephropathy;
2. VUR grade III-V;
3. Age older than one year;
4. No controindications;







Figures (clockwise):

Figure 1(above):
The comparison between successes and failures on the basis of VUR grade to the first follow-up.

Figure 2:
The comparison between successes and failures on the basis of VUR grade to the second follow-up.

Figure 3:
The comparison between successes and failures on the basis of VUR grade to the second follow-up.

5. Poor compliance of parents who choose the endoscopic injection instead of prolonged antibiotic prophylaxis also for VUR low grade (9).

The substance actually utilized for endoscopic injection is Deflux, that was introduced in 2001. Before 2001 the silicone (Macroplastique) was utilized. The Macroplastique is constituted by solid particles of polimetilsilossano and no-iodized povidone gel; it is not toxic, biocompatible, no migrant, no antigenic. It causes a local flogistic reaction; moreover it is possibile to utilize a small quantity of the substance. Numerous studies demonstred that Macroplastique isn't effective for possibile migration in the bladder side.
Food and Drug Administration (FDA) in 2001 establish that Deflux is the only substance that can be used for the treatment of VUR. Deflux is dextranomer/hyaluronic acid copolymer and it consists of microspheres in 1% hight-molecular-weight sodium hyaluronan solution. Each milliliter of Deflux contains 0.5 ml sodium hyaluronam and 0.5 ml dextranomer. The molecule is non-toxic and non-immunogenic, and its pseudoplastic properties facilitate the injection. No risk of implant migration and adverse reactions were reported. The repetition of the implant is possible and there were no problem if the expected results are not reached after the first endoscopic injection.
Its effectiveness has been demonstrated for over 7 years. In addition, endoscopic infiltration with Deflux is a minimally invasive procedure without long-term complications and does not require hospitalization. The endoscopic technique, as we can verify from the

literature and our experience, does not preclude the possibility to open surgery in cases of endoscopic treatment failure.

A "review" of the current International Literature shows that, despite the treatment options proposed by various authors, the sub-meatal endoscopic infiltration is now the treatment of "first -line. "

A single centre retrospective performed in Sweden, published in 2006 in the Journal of Pediatrics Urology Company, assessed the long-term follow-up of patients with III-V grade of VUR. Seventy two per cent of these patients received one infiltration, 20,1% and 3,9% two and three infiltrations. In conclusion, this study demonstrates that endoscopic infiltration offers significant advantages over antibiotic prophylaxis and ureteral reimplantation. Therefore the endoscopic treatment can be considered the treatment of choice in patients with VUR (10).

Another multicentric study performed in America with the participation of two centers, published in 2006 in Pediatric Urology Company, has reviewed the main option for treatment of VUR, with particular attention to the endoscopic technique. The conclusion of this review was that endoscopic infiltration is the first-line treatment of patients with VUR (11).

According to the International Literature since 2005 the treatment of choice is endoscopic injection of Deflux.

Our cure rate was 93.84%.Only one patient (bilateral VUR with grade IV) was submitted to ureteral reimplantation according to Cohen. We will program the ureteral reimplantation for VUR high grade for the other patient.

“Open surgery” must be the last choice. In fact complications like urinary disease, wound deiescence, hemorrhage and long hospitalization are more frequent with this tecnique.

The parents are satisfied too for this treatment.

Conclusion

In agreement with International Literature our results are good in patients with VUR treated by endoscopic injection. We may observe that our patients present a good growth, no UTI and no VUR. The results reveal that the sub-meatal infiltration is a simple, repeatable and reliable techinique and the results are immediate and safe in most cases. It is a mini-invasive technique, and it is caracterized by a short surgical time and reduced hospitalization. The results are immediate and safe in most cases. According with International Literature, since 2005, we utilized Deflux for all endoscopic injection. For this reason, we conclude that the sub-meatal infiltration represents the first-line treatment in patients with vescicoureteral reflux.

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Female epispadias: a case report

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Female epispadias without bladder exstrophy is an extremely rare anomaly occurring in 1:480.000 girls. It presents typical features and can be diagnosed immediately at birth. Early surgical reconstruction of the bladder neck, urethra, and external genitalia within the physiological phase for the development for continence, is relevant towards establishing urinary continence and to reduce the psychological impact on the parents and the child. In this case report we present a 3-years-old girl with isolated female epispadias who underwent total reconstruction at a single procedure with a follow-up of 6 months.
Key words: female epispadias, one-stage epispadias reconstruction, bladder exstrophy.

Introduction

Isolated female epispadias without exstrophy is a rare anomaly that forms part of the extrophy-epispadias-complex occurring in 1:480,000 girls. The condition is often missed at first examination but should be diagnosed immediately at birth. At informed routine examination of the external genitalia the wider central space between the labia majora, the associated diastasis of the pubic symphysis, the anomalous 'open' aspect to the dorsal urethra, and the divided hemiclitori lying to each side are obvious telltale indicators. Most cases are associated with an incomplete urethral sphincter, an open bladder neck, and a small bladder capacity with virtually absent bladder filling. Careful observation will confirm constant dribbling of urine and the absence of an intermittent urinary stream. Early diagnosis and surgical reconstruction of the bladder neck, urethra and external genitalia (1,2) within the physiological time frame are relevant to improve the chance for urinary continence and to reduce the psychological and psychosocial problems for the parents and the child.

In this case report we present a 3-year old girl with isolated female epispadias with total urinary incontinence, who underwent total reconstruction at a single procedure, with a followup of 6 months. The child is now 4 years old and is continent, voiding at-will with no interim wetting, with a good aesthetic appearance to the vulva and easy access to the urethra and vagina.

Case report

A 3-year old female, born by normal vaginal delivery at 40 weeks gestation, was admitted to our clinic for evaluation of 'genitalia abnormalities' and constant wetting in the context of otherwise normal developmental milestones. She was the product of a non-consanguineous marriage with no family history of a similar problem. A younger male sibling was normal. There was no significant past medical history except for occasional 'pyrexia of unknown origin' for which a urinary origin had been excluded by urine culture and analysis. Although the correct diagnosis was suspected when she was 1 year old, she was referred to us at 3 years of age when her weight at 10 kg and her height at 84 cm were under the 5th percentile. Informed examination of the external genitalia revealed dribbling incontinence with typical features of complete epispadias without exstrophy. There was a depressed mons pubis with a palpable wide diastasis of the pubic symphysis (confirmed at x-ray) that was covered by thin skin extending in the midline between the labia majora. Separation of the labia revealed a hemiclitoris and a small labium minor on either side. The central urethra lying above the vagina, was short and widely open dorsally, communicating with an open bladder neck that was incontinent of urine (Fig. 1). Renal function studies, routine blood evaluations, urinalysis and urine cultures were normal. Renal ultrasound revealed two normal kidneys with no ureteropelvic dilatation and an empty bladder. A micturating cystourethrogram showed a small bladder capacity and left grade I vesico ureteric reflux. Urodynamic studies were not performed because of the age of the child. X-ray confirmed a 2 cm symphyseal diastasis. Cystourethroscopy confirmed the short wide open urethra and the wide in-

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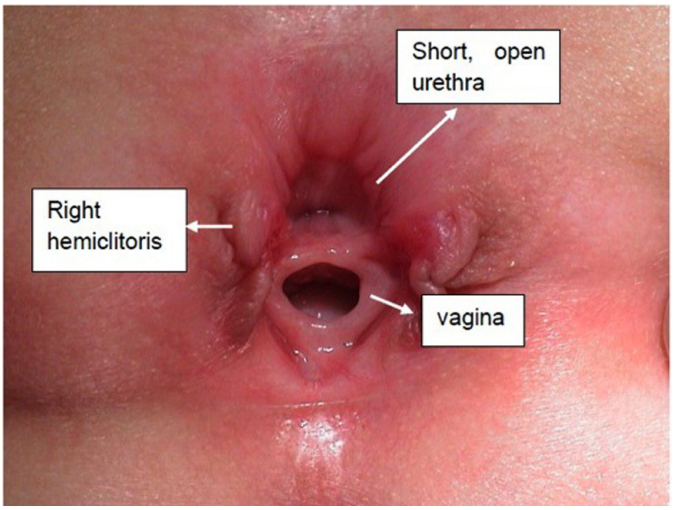
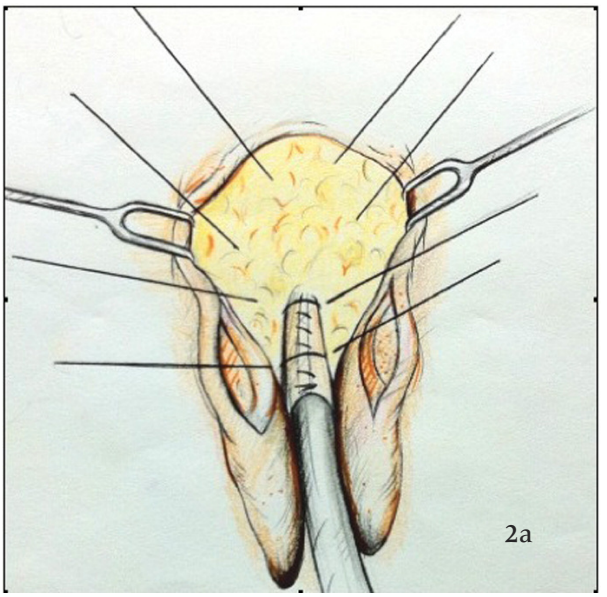


Fig.1: preoperative appareance of external genitalia shows female epispadias without bladder exstrophy



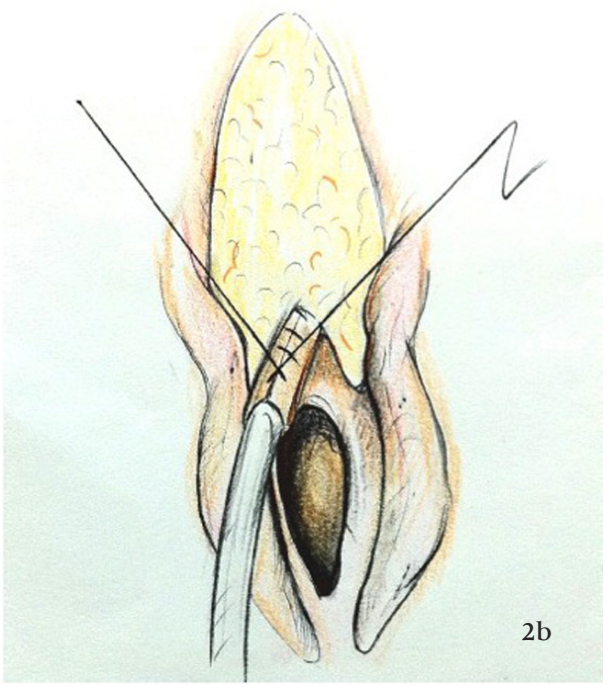


Fig.2(a-b): classical surgery

competent bladder neck. The small capacity (60-70 cc) non-inflamed bladder demonstrated a single normally placed ureteric orifice on each side. There were no other anomalies and all other systems were normal

At a single stage reconstructive procedure, the urethral plate, bladder neck and bladder were carefully dissected free and mobilized at a natural plane close to the pubic bones, liberating all available possible sphincteric muscle anteriorly and laterally. The urethral plate and bladder neck were tubularized with interrupted 5.0 Vicryl absorbable sutures around an 8 Fr silicone catheter, and the bladder neck was further reinforced by approximating the available 'sphincteric muscle' in the midline (Fig 2 a,b). The hemiclitori (glans and corpora) were freshened medially and approximated in the midline at the normal site above the urethral orifice. The procedure was completed with a cosmetic reconstruction of the external genitalia. A supra-pubic cystostomy was placed, and maintained with antibiotic cover for 3 weeks. A pelvic osteotomy was not performed.

The child healed well without any postoperative complications. Assessment under anaesthesia 2 weeks postoperatively confirmed easy passage of a 6 and 8 F Foley catheter through a good length patent urethra and bladder neck with no stenosis or dehiscence. On the 20th postoperative day an US study with a clamped suprapubic catheter showed bladder filling and voluntary micturition with no recordable residual volume and no uretero-pelvicalyceal dilatation. The suprapubic catheter was removed 24 hrs later. The child was discharged one month after operation, at which time she was observed to be completely dry for periods of up to 70 mins. Her mother reported that the child would express a sensation of a full bladder and a desire to micturate, with an ability to hold her urine and to initiate micturition voluntarily with complete bladder emptying and no residual urine. At 6 months follow-up her continence had improved and she could be dry for at least 2 hours. She had been potty trained and was using knickers by day and the nappy by night. Her bladder capacity was assessed by US and clinically at about 100 ccs. There had been no urinary infections and there was no upper tract dilatation. The cosmetic appearance of the vulva was considered to be acceptably normal.

Discussion/Conlusions

Although epispadias lies at the less severe end of the exstrophy-epispadias complex, it is nonetheless a fundamental and complicated developmental anomaly often associated with complete urinary incontinence. The condition is commoner in the male, and female epispadias is indeed rare, with an incidence of 1:480,000 girls. The aetiology of this developmental anomaly of the urethra and

bladder neck remains uncertain and is considered possibly to be the result of a combination of several genetic and environmental factors (3). Associated anomalies are commonly confined to the urinary tract with an incidence of vesico-ureteral reflux at 30%-75% (3), to the pelvis, the pelvic floor, and the abdominal wall but it is relevant also to assess the spine and the anus (4).

The physical findings in female epispadias are characteristic and well described such that the anomaly can and should be diagnosed immediately at birth on routine informed clinical genital examination, but is often missed presenting at a later age with failure to potty train and constant wetting. Early diagnosis allows early parental counselling and the option of a planned surgical reconstructive procedure preferably within the natural time for the physiological development of urinary continence. The issue and relevance of pelvic osteotomy for isolated epispadias remains controversial however early diagnosis allows easier and more manageable pelvic surgery that is possible within the first months of life but becomes more complex at an older age and once the child is attempting to stand.

The main objective behind surgery for epispadias is voluntary urinary continence, and to this end it is particularly relevant to mobilize carefully all available tissue anteriorly and laterally, minimizing injury to nerve plexi and potential sphincteric muscle in the area. The urethral plate and muscle, and the bladder neck and sphincteric muscles are tubularized and united without tension in the midline anteriorly to create a normal length urethra and bladder neck of normal diameter. Effective suprapubic diversion is relevant to avoid pressure disruption of healing tissue in the first days after surgery. Although multistage procedures were favoured historically, it is now considered preferable to attempt correction of the associated vulval and pubic anomalies at the same time within a single reconstructive event. At first operation there is the advantage of clean surgical dissection planes without scarring from previous surgery and therefore a greater likelihood of avoiding nerve and sphincteric muscle injury. The hemiclitori can be carefully mobilized and brought closely together, also reconstructing a prepucial hood and achieving closer apposition of the labia majora and the tissues of the mons pubis towards a more normal appearance of the vulva. Indeed the literature suggests that single stage reconstruction is safe and aesthetically acceptable, with a 60% and 87.5% (4,5) chance for urinary continence.

In our management of this child we have attempted to replicate the best practice of the day, reconstructing the urethra and bladder neck with the least possible injury to surrounding structures, and with vulval reconstruction for a good aesthetic appearance. We were gratified by good primary healing without complications, and particularly with the early suggestion of good bladder sensation and urinary continence. Interestingly within the first weeks of surgery the mother reported that the child expressed a sensation of bladder fullness and a desire to pass urine. She demonstrated from early on an ability to hold her urine, to initiate micturition and to void without leaving any residual urine. Although this is only a single case report with a relatively short follow-up, the immediate result, together with the absence of urinary infection or uretero-pelvicalyceal dilatation, supports the concept of a careful single stage reconstruction with mobilization and least possible trauma to crucial nerves and sphincteric muscle around the urethra and bladder neck. The child's response with early bladder sensation and the ability to hold and voluntarily void completely, have been gratifying and would suggest the likelihood of long term effective urinary control and normal continence.

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Lymphangioma of the tongue: case report

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Introduction. Lymphangiomas are uncommon congenital hamartomas of the lymphatic system, usually diagnosed in infancy and early childhood. Commonly located at head, neck extremities and genitals, they are rarely situated in the oral cavity. Preferred site of oral involvement is the tongue. The authors present a case of lymphangioma of the tongue treated with laser therapy.
Case report. The patient was a 7 years old female, that came to us for right upper quadrant abdominal pain. On examination we found the median lesion of the tongue in absence of symptoms. Thyroid scan was performed to exclude the presence of ectopic thyroid. Surgery was performed by excision of the lesion with CO2 20 W by trans-oral laser therapy. The anatamopathological report posed diagnosis of lymphangioma. The follow-up to 8 months is in the norm: the aesthetic results are excellent and the patient doesn't report any symptoms.
Conclusion. This case had a very rare site of occurrence, the tongue, and was successfully managed with laser therapy. This surgical technique is very unusual among the various types of interventions but it allows good aesthetic results and good radical surgical excision, preserving vital structure.
Keywords: Lymphangioma of the tongue, laser terapy

Introduction

Lymphangioma is a localised malformation of the lymphatic system frequently located in the head and neck. Many of these lesions are congenital. They can be evident after surgery or trauma. Most of them are diagnosed before two years of age (1-2). The lymphangiomas represent about 6% of benign tumors of the smooth tissues (3).
It has been described for the first time by Redenbacher in 1828, and it is classified as malformation and not as neoplasm (4).
Two theories have been advanced to describe the formation of the lymphatic system (5). The first is the centrifugal theory, whereby two endothelial buds grow from the jugular sacs and develop into lymph vessels. The second is the centripetal theory whereby an anastomotically developing lymphatic system eventually joins up to the central venous system.
Lymphangioma circumscriptum is a lymphatic malformation that is localised to the skin, subcutaneous tissue or sometimes muscle. The commonest sites are axillary folds, shoulders, flanks, proximal parts of the limbs and the perineum (6). There are few cases of lymphangioma circumscriptum of the oral cavity, but the most common site in this case is the tongue, especially the anterior part (7).
The authors present a case of lymphangioma of the tongue treated with laser terapy.

Case reports

The patient was a 7 years old female, who came to us for abdominal pain in right upper quadrant, because of gallstones.
On examination of the oral cavity, we found a median lesion of the tongue (Fig.1). The patient had no respiratory symptoms and no problems to swallow.
In suspicion of ectopic thyroid we did a scan. This examination excluded the possibility of ectopic thyroid.
It was decided to perform surgical excision of the lesion. The excision was performed under general anesthesia with 20 W CO2 by trans-oral laser therapy. The microscopic report made diagnosis of lymphangioma.
At the follow-up at 8 months everything is normal: the aesthetic results are excellent and the patient does not report any symptoms.

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Discussion

Lymphangiomas are uncommon, congenital tumours of the lymphatic system which mostly appears in children under five years as lobulated masses or cysts, usually in the head and neck. Involvement of the tongue, however, is rare. Lymphangioma of the tongue was first described by Virchow in 1854 (8). A lymphangioma is thought to be a contiguous mass of dilated lymphatics, and the cause is believed to be a developmental defect or primary malformation of the lymphatic channels.

The management of a child with lingual lynphangioma involves various clinical features: respiratory obstruction, drooling for tongue protrusion, difficulty in chewing and swallowing and orthodontic abnormalities8. Sometime, the combined effects of these can make the child a social outcast requiring psychological support. Management is therefore complex and requires a team approach involving different specialities (9).

Lingual localisation presents specific therapeutic problems because of the microcystic character of the lesions and the marked functional problems (10). Various treatments have been attempted and proposed. Complete surgical excision is not possible because of the unacceptable degree of mutilation it would entail. Partial glossectomy, performed regularly, is a serious operation with a high rate of relapse or secondary growth and often results in a morphological modification of the tongue. Post-operative healing is painful and relatively long (2). The injection of sclerosing agents can be useful for macrocystic lesions but is not appropriate for microcystic lesions (11).

In our case the formation of the tongue was very small (2 cm). The patient did not show symptoms. For this reason, in our opinion, the best treatment was been the 20W CO2 Laser Therapy. This type of treatment allows a good control of bleeding, a good resection of lesion, with low the chances of recurrence, and preserves the structures of tongue, avoiding functional deficits.

Fig.1
Lesion of the tongue

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Bilateral vesico-ureteral reflux in patient with crossed renal ectopia and fusion type A

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Introduction. Crossed renal ectopia with fusion is a very rare congenital anomaly and the reported incidence varies between 1:1000 and 1:7000. The kidney is located on the opposite site of the midline from where the ureter enters the bladder. Eighty-five percent of crossed renal ectopia kidneys are fused from below to the normally located kidney. This anomaly is more frequent for left kidney and it's associated with vesico-ureteral reflux (VUR) in 25-70% of cases. We report the management of a six-years-old patient with Plurimalformative Syndrome, trisomy p16 and monosomy q2, crossed renal ectopia with fusion type A and bilateral vesico-ureteral reflux (grade IV in the right kidney and grade III in the left).
Materials and Methods. A 6-year-old boy was ammitted to our hospital for UTI in plurimalformative syndrome characterized at birth by cleft palate, macrocephaly, congenital clubfeet, twisted right arm, congenital dysplasia of the hip, balanic hypospadias, bilateral inguinal hernia, right renal agenesis and epilepsy tonic-clonic. MRI revealed a fusion of the ectopic kidney with the left orthotopic kidney (crossed renal ectopia with fusion type A). Voiding cystography showed a dilatated ureter of the crossed ectopic kidney passing across the midline and of the left ureter, and a bilateral vesico-ureteral reflux (grade IV VUR in the right kidney and grade III VUR in the left). For this reason bilaterally endoscopic subureteral infiltration was performed with Deflux (0.3 cc for side).
Results. Patient was discharged in third day and he took antibiotic for one week. There weren't complications like fever, obstruction or UTI. Follow-up after 1 month is normal and there weren't UTI.
Conclusion. Generally the outcome of patients with fused crossed renal ectopia is good. Presence of associated pathology likeVUR, could lead to a progressive deterioration of renal function. Therefore, in patient with uninhabited kidney area and UTI, it's very important a carefull radiological investigation to exclude a renal ectopy complicated by RVU and especially to realize an appropriate treatment strategy before the patient develops a chronic renal failure. Endoscopic infiltration with Deflux, in our case, was detected a viable surgical technique for its minimally invasiveness and also for its efficacy with a relatively short hospital stay.

Female epispadias: a case report and review of the literature

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Female epispadias without bladder exstrophy is an extremely rare anomaly, occurring in 1 in 480.000 female population. It's the mildest form of the extrophy-epispadias-complex (EEC), that is the most serious form of abdominal midline malformation (incidence of EEC can be estimated at 1 in 10.000 births). It's imperative to diagnose this abnormalities at birth, because that's enough a complete local examination and a good patient's past medical history, when the patient is greater, about congenital urinary incontinence and recurrent urinary tract infection. The early diagnosis, so the early treatment, is very important to reduce the psychological and psychosocial problems and prevent the urinary incontinence, that's a real problem for the social life of the patient. The pathology can be corrected by surgical reconstruction of bladder neck, urethra and external genitalia. Epispadias surgery is a reconstructive surgery and it has mainly two aims: the correction of the urinary incontinence and the reconstruct of the external genitalia with good aesthetic appearance. In this case report we present a 3-years-old girl with isolated female epispadias, who underwent just one operation to correct her anomaly. In this patient the epispadias was unrecognized until 1 years-old. We reviewed, also, the Literature about cases of female epispadias that confirmed the rarity of the disease.

Treatment of keratoconus, doubts about the mechanism of action and impediments to discussion

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In an experimental study on keratoconus (G. Wollensak et al., "Stress-strain measurements of human and porcine corneas after riboflavin-ultraviolet-A-induced cross-linking", J Cataract Refract Surg 29 (2003) 1780-1785), the authors observed that when strips of corneal tissue were stretched at constant elongation velocity, they responded with strain that increased according to an exponential fitting curve. This behaviour was qualified as "the typical exponential increase of a bio-viscoelastic solid". This claim is wrong and misleading, because typical viscoelastic behaviour is asymptotic, not exponential, as we have already pointed out (A. Albanese et al., "Keratoconus, cross-link-induction, comparison between fitting exponential function and a fitting equation obtained by a mathematical model", Biomedicine & Pharmacotherapy 63 (2009) 693-696). Other doubts are raised by the unjustified choice of fitting function, the surprising agreement of the experimental points with the curve (in contrast to the wide margins of error indicated) and finally the original criterion used for data processing. The fact that treatment is clearly effective does not prove that the mechanism of action is the one described in the above paper. We therefore consider it advisable to conduct further research into the real biomechanical properties of corneal tissue, because erroneous interpretation could make it more difficult to develop and direct therapies. This summarizes the contents of a paper that we submitted to the journal in November 2009, but which fails to be accepted on the pretext of formal minutiae. We believe that in specialist journals scientific disputes should be solved by an exchange of arguments. Shilly-shallying to deny space for critical observations does not favour the advance of science.
Keywords: cornea, keratoconus, viscoelasticity

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A Sienese academic woman of the eighteenth century: Aretafila Savini de' Rossi.

Eleonora Spinosa - Siena Academy of Siences

This research aims to show the correlation between Tuscan literary and scientific academies and the discourse on women's education at the dawn of the eighteenth century, considering Aretafila Savini de' Rossi, Sienese poetess and painter, famous for her Apologia in favore degli Studj delle donne, written in 1723. For the first time the circumstances that prompted to the publication in 1729 of her modern work in favor of women's studies are clarified. The link was doctor Antonio Vallisneri, naturalist philosopher, who wrote to Aretafila in 1726, asking a copy of her work, known to him thanks to the mediation of his son Antonio junior. The Savini was in contact with eminent scientific-cultural circles of that time. Among these were: Anton Maria Salvini, Greek Literature professor at the Florentine Studio, Crescenzio Vaselli, a Sienese member of the Fisiocritici Academy and Princess Violante of Bavaria's personal doctor, Anton Francesco Marmi, Florentine colleague of the famous librarian Magliabechi, Pier Jacopo Martello, well-known Bolognese playwright, and Antonio Vallisneri, chair of Medicine at the University of Padua and member of the Fisiocritici Academy. These cultural contacts were possible thanks to academies, the only cultural institutions that granted women's participation in the age of Enlightenment. The Arcadia Academy, of which Aretafila became a member in 1712 under the name of Larinda Alagonia, as well as the peculiar Sienese combination between the Arcadians and the Fisiocritici (1699-1733), allowed her to contact and dialogue with the custodians of the scientific culture from which ladies were traditionally excluded.

Key words. Aretafila Savini Rossi, Academy of Arcadia, women's education, Fisiocritici Academy, Antonio Vallisneri, Larinda Alagonia.

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The evolution of management of breast after mastectomy

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Mammary carcinoma is the most common malignant pathology in women, and the plastic surgeon is daily in the frontline in treating it. Our task, and our aim, is to offer patients the most valid solutions in order to reach the most effective oncological outcome together with the best aesthethic result. The surgical treatment of mammary tumors has undergone profound and continuous changes in the last thirty years: the tendency towards completely destructive operations has given way to evermore preserving surgery thanks to the possibility of combining general surgery with plastic surgery techniques. This has enabled the conflict between surgical removal and aesthetic results to be overcome, thus improving the patients' quality of life. This possibility has given rise to the need of a thorough study of the evolution which has taken place in the last years in the field of post-mastectomy mammary reconstruction, taking into exam all available techniques and evaluating their usefulness at present. This study has been carried out with the collaboration of Bryant A. Toth, a world-famous plastic and reconstructive surgeon with a vast experience in numerous aspects of this field and who has taken us through this ambitious project.

Key Words: mammary carcinoma, evolution, post-mastectomy mammary reconstruction

Pandemic flu A/H1N1v: Virological surveillance in South Tuscany

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On April 24, 2004, the World Health Organization confirmed a number of cases of contagion of the new Influenza virus A/HIN1 in Mexico and the United States. On June 11 2009, the rapid spread of infection compelled the WHO to raise the pandemic phase to 6, which corresponds to the highest state of alert. The virus probably originated from a recent reassortment between a swine virus previously reassorted with three different viral strains (swine, avian, human) and a new viral strain similar to the Euroasiatic avian virus[1]. This unprecedented circulation of the new influenza virus was facilitated by travel and international exchanges and has reached, in the period of little more than six weeks, the same extent that had been present in previous pandemics in a period of six months, therefore making necessary the implementation of various strategies of Epidemiological and Virological Surveillance. During the pandemic season, 866 pharyngeal swab samples of persons who presented influenza symptoms were gathered. The patients were then divided into different age groups: 0-4, 5-24, 35-54, 55-64 and ≥65 years of age. The virus' RNA was extracted from each swab by using a specific kit. Afterwards the RNA was reverse transcribed in cDNA and amplified in a single reaction of real-time PCR using the one-step kit recommended by CDC protocol. The analysis of the 866 pharyngeal swabs has shown the presence of 262 positive sample results for the new variant of the A/H1N1virus. Several parameters of this study have been taken in consideration: age groups, geographical distribution of infection in the three cities studied, the weekly trend of positive results which had shown up after a trip abroad, the incidence in local cases and the measure of infection of the virus among patients who came in contact with infected persons. Among the examined age groups, those majorly affected are: ages 0-4, 5-14, 15-24, the least affected age group was ≥65. In conclusion, the data demonstrates that the age group mainly affected is that between ages 0-24. Presumably such data is justified by considering that the immune system of younger people has never come in contact previously with the variants similar to the A/H1N1, whereas older adults would seem to be less susceptible, probably because they have already come in contact with similar viruses. The obtained data illustrates an elevated level of contagiousness among individuals, since the new influenza virus A/H1N1 of 2009, represents a variant of completely different from other H1N1 viruses that had previously circulated in the human species. It should be pointed out that the hemagglutinin differs by 27.2% and the neuraminidase by 18.2% compared to the amino acid sequence of the 2008 H1N1 influenza virus and by the variant of the viral strain used for the production of the vaccine, this lent to a significant pandemic potential. The trend of positivity in local cases from the 29th week was kept at medium-low levels until the 38th week with a great increase, probably due to the reopening of schools and offices after the summer break.

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A stellar representation in the Paleolithic?

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Introduction

In the early 70's, an extended archaeological campaign was carried out in Maremma, Tuscany, by researchers of the University of Florence. There, to the south of the Colline Metallifere, it lies a vast region, which is very rich in prehistoric remains and extends as far as the Tyrrhenian coast, westward, and Grosseto city, southward. Most archaeological finds date back to Iron Age, some thousand years b. C., and are due to the Villanovan culture and to the Etrurian civilization. The names of some among such sites are very well known, like Baratti, Vetulonia or Roselle. In the present case, the place of interest is almost unknown and it is situated in a country featured by Karst formations. At the mentioned time, one of the authors (FM), as a member of archaeological team, was exploring the territory around a spot called Vado all'Arancio and discovered a natural cavity which turned out to have been inhabited in prehistoric times. Luckily, the small cave has remained sealed until then because of a landslide and therefore a large stone was, and still is, almost completely blocking the entrance. The survey work could be performed only by slithering along the ground. The following excavations conducted by FM revealed very interesting findings. First of all, there were two human burials, one adult male and one child, with the skeleton well preserved and almost complete. The graves were at different depths, not far below ground level, however. It is important to stress this aspect, because the skeletons were still composed and therefore, very probably, there had been no intrusions in the inner part of the cave ever since. Moreover they were also found various worked items, mainly stone tools and several animal bones dressed with wildlife engravings. Among these are the typical depictions of symbolic male and female, namely horse and bison, respectively. It is believed that all the worked stuff in the cave was intended to represent something observed in nature.

A pretty odd stone

In fact, some of the stones, taken from the site at the time, were recently examined again, more carefully. In particular, it was noticed a clear nice pebble having a rather regular geometry: thin, with axial symmetry and an elliptical profile. Quite flat on one side, whereas the other surface is slightly convex. Of course, all this is very probably just a result of natural phenomena, but close to the border there is a small area with a few tiny holes in it, close each other. They are seven or maybe eight. Just to give some figures, dimensions of the pebble are 6x8.3x0.6 cm and that particular area is only 1.2x2 cm. On the average the wideness of the holes is about one millimetre, or less, and the depth a few tenths of millimetre. The hypothesis that they were produced by some animal like the so-called stone-eater molluscs, actually there are hundreds of species, does not seem justified: those holes are much wider and then the rock should be completely covered by them. So, we strongly suppose those holes to be made on purpose by a man, who might have chosen that stone thanks to its regular shape. Of course, now an open question is understanding what tool was used to realize those holes. Although a work of limited extension, their not random layout suggested the possibility that they have a meaning and may display a stellar configuration even. Or maybe it could be just the beginning of a job, because all the holes occupy only a lateral area on the surface of the pebble. We decided to investigate the astronomical interpretation, looking for a first possible interpretation of these signs. One has to bear in mind, however, that it is not to be expected an accurate agreement like in cartography. In addition, it has to be considered that the starry sky observed on Earth is not immutable, as centuries go by.

Estimates, obtained on the basis of radiocarbon dating of organic material found in the burials, indicate that it dates back to the end of the Palaeolithic, specifically the age of about 11,500 years by our times. Actually, this was the shift applied while taking into account the precession of the equinoxes, when calculating the early position of stars. The corresponding configuration of constellations was then compared with marks on stone.

A first guess

Attempts based on 'easy' constellations like the Great Bear did not succeed: simply, they do not match. Now it is here suggested a possible interpretation of that work based on the constellation of the Southern Cross, standing next to two main stars of the Centaur, i.e. Alpha and Beta. These two constellations nowadays are visible only in the austral sky, but, as is well known, in the past they were visible from our hemisphere as well, at the latitude of the site. However it is in progress a more extensive verification of correspondences to other stellar identifications alternatives: surely, the Pleiades are worth to be considered next. Moreover, the effect of stellar proper motions has to be taken into account with a grater accuracy.

It has to be pointed out that, regardless of association with some specific constellation, if this work is really a representation of stars, it is one of the oldest such artefacts: indeed, thousands of years older than any other known prehistoric sky representation.

Key Words: Constellations – Stellar map – Palaeolithic – Maremma

Validation of Neurotensin tetra-branched peptides as tumor targeting agents in pancreas, colon and bladder carcinoma

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The identification of new tumor targeting agents, which might allow either cancer cell tracing or therapy, is a crucial issue in cancer research. Membrane receptors for endogenous peptides such as neurotensin, somatostatin, bombesin and many others are over-expressed in different human cancers and could therefore be targeted as tumor-specific antigens. In the meantime the extremely short half-life of peptides impeded their development for effective peptide-based tumor targeting strategies.
We synthesized tetra-branched neurotensin peptides (NT4), which ensure extremely long half-life maintaining peptide specificity and increasing avidity through multimeric binding. Moreover this bio-synthetical strategy allows a considerable modularity of peptides through the conjugation of different functional unit, such as fluorophore, radioactive moieties or chemotherapeutic drugs.
Aim of our studies is to validate NT4 for cancer cell tracing in different human tumors. In this view we use fluorophore-conjugated NT4 to discriminate between tumor and healthy tissue obtained by surgical samples from pancreas, colon and bladder carcinoma. Peptide binding on tumor and healthy biopsies was measured in each patient by quantitative analysis of confocal microscopy images. These results show a considerable difference in fluorescence emission between healthy and tumor samples in colon, pancreas and bladder cancer, opening the way to the development of NT4 as selective diagnostic tools for these pathologies. Moreover our peptides can be conjugated with different chemotherapeutic moieties in order to allow the selective killing of tumor cells.

Tumor selective drug delivery by Neurotensin branched peptides

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Detection of new tumor-selective targets, which allow either cancer cell tracing or therapy, is a crucial issue in cancer research. Membrane receptors for endogenous peptides such as Neurotensin are over-expressed in many human cancers and could therefore be used as tumor-specific antigen, while peptide ligands might act as targeting agents.
The development of peptides as drug has always been limited by their short half-life, due to degradation by peptidases and proteases. Chemical modification, which can stabilize the molecules, may modify peptide affinity or specificity. Moreover, coupling of peptides to effector units for imaging or therapy, may interfere with biological activity. We demonstrated that peptide sequences, when synthesized in an oligo-branched form, become resistant to proteolysis and thank to their multimericity are more efficient than corresponding monomers in binding cellular antigens1. Moreover, the branched core allow coupling of effector units without affecting peptide activity. Drug-armed tetra-branched neurotensin peptides (NT4) were synthesized with different conjugation methods, resulting either in uncleavable adducts or drug-releasing molecules2-4. Recently we developed DOPC liposomes filled with the cytotoxic drug Doxorubicin (Doxo) and functionalized with NT4. Armed DOPC liposomes showed a clear advantage with respect to nude liposomes in drug internalization and their cytotoxicity is fourfold increased with respect to the same nude liposomes.
Conjugation to NT4 switches drug internalization to a peptide-receptor mediated mechanism, which greatly increases drug selectivity and also might allow by-passing drug cell resistance. In vitro and in vivo results indicated that branched NT peptides are valuable tools for tumor selective targeting.

Trans-epithelial cross-linking

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Purpose. To evaluate safety and efficacy of Trans Epithelial Cross linking trough functional and histological corneal induced modifications.
Materials and Methods. 8 patients affected from progressive keratoconus and candidated for lamellar corneal transplantation because of corneal thickness under 400 microns, were previously treated by Trans Epithelial Corneal Cross Linking with Ricrolin TE solution (Sooft). Patients were evaluated before and after treatment with a 6 months of maximum follow-up, monitoring the following parameters: UCVA, BSCVA, topographic and in vivo confocal analysis. All crosslinked corneas were explanted during corneal transplantation (DALK, PK) and histologically analysed by optical and electron microscopy.
Results. After follow-up we recorded an improvement of 1 Snellen line in BSCVA, Sim K max reduction of 0.63 D and SAI reduction of 1.18 D. Histopathological results showed inhomogeneous micromorphological changes immediately below lamina of Bowman and anterior stroma until 80 microns of maximum depth.
Conclusion. The variation of corrected visual acuity is likely attributable to the cross linking action concentrated in the most anterior stromal portion as confirmed by histological and confocal study. An increase of corneal surface regularity is described by reduction of Surface Asymmetry Index (SAI), K MAX and Comatic aberration values. Present study confirm safety of TE CXL and If long-term results will confirm also the efficacy of this new method, it will become a new way for the treatment of corneal ectasia (keratoconus, post-LASIK ectasia) with thickness under 400 microns.
KeyWords: Cross linking, keratoconus, riboflavina, trans-epithelial.

Introduzione

Il Cross-linking trans-epiteliale o TEXL rappresenta un importante novità tra le strategie terapeutiche per trattare le forme di Cheratocono non avanzato. Lo Studio Siena C.L.E.S. (Cross Linking Evolution Study) sul TEXL è iniziato dopo l'approvazione unanime del Comitato Etico della Azienda Ospedaliero-Universitaria di Siena nel luglio 2009. Si tratta di nuovo approccio terapeutico, che affianca e non sostituire il Cross-Linking standardizzato con rimozione dell'epitelio1,2,3,4,5. I vantaggi che questo nuovo trattamento può dare sono rivolti sia al chirurgo oftalmologo che al paziente (tabella 1).
Occorre determinare se tale procedura può garantire la stessa efficacia, durata e sicurezza che sono stati dimostrati negli studi condotti con la tecnica classica senza rimozione dell'epitelio corneale.
La tecnica di esecuzione del TEXL prevede gli stessi step del CXL tradizionale (tabella 2), ad eccezione della prima fase (fase 0 di imbibizione) che in questa nuova procedura può essere effettuata al di fuori dell'intervento senza blefarostato con notevole vantaggio sia di tempo che di comfort per il paziente. La novità più importante è rappresentata dalla sostanza foto-sensibilizzante utilizzata, ovvero il Ricrolin TE. Tale soluzione favorisce il passaggio della Riboflavina idrofila attraverso l'epitelio corneale idrofobo integro appositamente modificato da bio-enhancers Trometamolo o TRIS (atossico, non irritante né ipersensibilizzante).
Il lavoro descritto di seguito mira a valutare l'efficacia funzionale e istologica del TEXL eseguito con soluzione di Ricrolin TE (Sooft. Italia) su pazienti candidati e sottoposti a trapianto di cornea per cheratocono.
In particolare gli end-points della Ricerca sono stati:

1. la valutazione clinico-topografica durante il periodo pre-trapianto.
2. l' analisi istopatologica ex vivo sulle cornee trattate con TEXL e successivamente espiantate.

Materiali e metodi

Si tratta di uno studio pilota aperto prospettico non randomizzato, in cui abbiamo arruolato 8 pazienti affetti da cheratocono già in lista di attesa per intervento chirurgico di cheratoplastica lamellare (DALK) o perforante (PK). Ciascun paziente, prima del trattamento di CXL TE è stato sottoposto ad una visita preliminare con valutazione del visus naturale e corretto e topografia corneale. I dati di tale studio sono stati confrontati con i risultati ottenuti con il trattamento CXL standard con rimozione dell'epitelio (CXL epi-off), su un campione di 44 pazienti11.
La procedura di CXL è stata eseguita previa somministrazione di 2 gocce di Ricrolin TE ogni 5 minuti a partire da 2 ore prima del trattamento seguita dalla instillazione di 1 goccia ogni 2 minuti nei 10 minuti

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precedenti e per tutta la durata dell'esposizione alla sorgente UV A applicata per 30 minuti con CBM (Caporossi-Baiocchi-Mazzotta) Xlinker Vega-CSO.
Il follow-up è stato eseguito a 1, 3 e 6 mesi dal trattamento con rivalutazione dei parametri pre-operatori (UCVA, BSCVA, Analisi Topografica). I dati relativi al visus, non parametrici, sono stati studiati con l'analisi statistica Test U di Mann-Whitney, mentre i dati topografici, parametrici, con il test T di student.
Tutti i nostri pazienti sono poi stati sottoposti a cheratoplastica lamellare, DALK, (4 pazienti) o perforante, PK, (2 pazienti) come da programma e le cornee espiantate sono state inviate presso il Dipartimento di Anatomia Patologica dell'Università di Siena per l'analisi Istopatologica mediante analisi istologica ottica e microscopica elettronica.

STUDIO SIENA CLES 2009-2010 VANTAGGI ATTESI DEL CXL TRANS-EPITELIALE
• Riduzione/eliminazione della fase 0 (fase di imbibizione intra-operatoria)
• Eliminazione del dolore post-operatorio
• Recupero post-operatorio più rapido
• Riduzione/eliminazione del rischio infettivo
• Eliminazione della necessità di sala operatoria e riduzione dei costi della procedura
• Conferma della sicurezza e della efficacia vs la procedura standard

Tab.1: Vantaggi attesi dal Cross-Linking trans-epiteliale (TEXL). Studio Siena CLES 2009-10.

TRANS EPITHELIAL CROSS LINKING: TEXL STEPS
Pilocarpina 1%: 30 min prima.
Ricrolin T.E. imbibizione: 1-2 gocce ogni 10 min per 2 ore di imbibizione.
Lidocaina 4% (10 min. prima)
UVA Power Meter Check (3 mW/cm²)
Focalizzazione (CBM X Linker Vega aiming beam)
Irradiazione UVA (6 steps di 5 minuti)
Lente a contatto terapeutica: 3 giorni
Ciprofloxacina coll. (1 sett.), lacrime artificiale e fluorometolone (2 sett.)

Tab.2 (sopra): Step del cross linking trans epiteliale (TEXL). Studio Siena CLES 2009-10.

Tab.3: Dati funzionali (UCVA, BSCVA) e analisi topografica (K MAX, SAI, SI) del cross linking trans epiteliale (TEXL). Studio Siena CLES 2009-10.

		PRE-CXL	1° MESE	3°MESE	6°MESE
DATI FUNZIONALI	UCVA	0,08	0,07	0,05	0,07
	BSCVA	0,43	0,51	0,52	0,53
ANALISI TOPOGRAFICA	K MAX	55,51	55,71	54,74	54,88
	SAI	9,81	9,46	8,58	8,63
	SI	7,95	8,64	8,22	8,04

RISULTATI

Con il trattamento CXL TE i risultati funzionali refrattivi medi preliminari dimostrano una variazione della UCVA non statisticamente significativa a fine folow up, mentre con il trattamento standard, con rimozione dell'epitelio (CXL epi-off), si registrano cambiamenti significativi dell'acuità visiva naturale. Infatti si passa da 0.08 ±0.017 linee di Snellen del pre-CXL TE a 0.07 ±0.039 linee di Snellen 6 mesi post-CXL TE (p=0.089) e da 0.33 ±0.12 linee di Snellen del pre-CXL epi-off a 0.49 ±0.13 linee di Snellen 6 mesi post-CXL epi-off (p=0.0031). La BSCVA varia, in maniera statisticamente significativa sia con il trattamento CXL TE che con quello standard epi-off. Infatti si passa da 0.43 ±0.09 linee di Snellen del pre-CXL TE a 0.53 ±0.09 linee di Snellen 6 mesi post-CXL TE6 (p=0.0045) (Tabella 3) e da 0.58 ±0.09 linee di Snellen del pre-CXL epi-off a 0.69 ±0.08 linee di Snellen 6 mesi post-CXL epi-off (p=0.00311).
All'analisi topografica, eseguita con topografo corneale CSO, si evidenziano, tra il pre-CXL TE e 6 mesi post-CXL TE, le seguenti variazioni medie dei valori dei K readings e degli indici di asimmetria (SAI-SI):
Sim K Max: 55.51D ±2.6 (pre-CXL TE), 54.88D ±2.6 (6 mesi post-CXL TE), variazione statisticamente significativa (p=0.0042); SAI: 9.81D ±1.3 (pre-CXL TE), 8.63D ±1.0 (6 mesi post-CXL TE) variazione statisticamente significativa (p=0.0032); SI: 7.95D ±2.25 (pre-CXL TE), 8.04D ±1.6 (6 mesi post-CXL TE) variazione statisticamente non significativa (p=0.0789), Tabella 3.
All'analisi comparativa topografica, eseguita con topografo corneale CSO dopo trattamento CXL Standard epi-off, si evidenziano invece i seguenti dati:
Sim K Max: 52.59D ±2.1(pre-CXL epi-off), 51.42D ±1.9 (6 mesi post-CXL epi-off), variazione statisticamente significativa (p=0.0041); SAI: 7.67D ±1.8 (pre-CXL epi-off), 7.13D ±1.6 (6 mesi post-CXL epi-off) variazione statisticamente significativa (p=0.046); SI: 5.66D ±0.98 (pre-CXL epi-off), 4.17D ±0.72 (6 mesi post-CXL epi-off) variazione statisticamente significativa (p=0.043).

I risultati dell'analisi istopatologia hanno evidenziato che:

- *Dopo procedura TEXL l'epitelio mostra modificazioni micromorfologiche con cellule apoptotiche e vacuolizzate anche dopo 3-4 mesi ad una profondità media di 50 µm ±20, di stroma anteriore con una stratificazione di cellule basali molto compattate e allungate7,8,9.. Nel trattamento con rimozione dell'epitelio (CXL epi-off) l'apoptosi si presenta ad una profondità compresa tra 280 e 300 µm di stroma anteriore con scomparsa dei cheratinociti.*
- *Dopo procedura di TEXL l'organizzazione delle lamelle dello stroma anteriore mostra una compattamento medio di 20-50 µm Fig.1, contro i 250 µm dimostrati con il trattamento CXL standard epi-off..*
- *Lo stroma medio e profondo dopo trattamento trans epiteliale non mostra modificazioni microstrutturali rispetto alle cornee cheratoconiche non trattate. Fig.2-3. Con il trattamento standard (CXL epi-off) sono state dimostrate modificazioni fino allo stroma medio.*

Discussione

La procedura TEXL si è dimostrata efficace in quanto induce alcuni cambiamenti microstrutturali in maniera disomogenea al di sotto della lamina di Bowmann e nello stroma corneale anteriore.

I risultati refrattivi preliminari, dopo trattamento CXL TE, hanno dimostrato a fine follow up una variazione della UCVA (visus naturale) non statisticamente significativa, contrariamente a quanto riscontrato con il trattamento CXL standard epi-off. Si misura, sia dopo CXL TE che dopo trattamento CXL standard epi-off, un miglioramento della BSCVA (visus corretto) di una linea di Snellen a fine follow up, verosimilmente dovuto all'effetto cross linkante concentrato nella porzione stromale più anteriore (responsabile della forma corneale). Si evidenzia, inoltre, un aumento della regolarità della superficie corneale come suggerisce la diminuzione del valore dell' indice di asimmetria della superficie corneale (SAI) e del K readings MAX 10. Variazioni topografiche statisticamente significative sono state registrate anche dopo trattamento CXL standard epi-off. Se i risultati a lungo termine, su congruo campione, confermeranno l'efficacia della metodica con rimozione dell'epitelio, si aprirà una nuova strada al trattamento di cornee ectasiche sottili con spessore al di sotto di 400 µm. Già oggi gli studi preliminari concordano sul rispetto dei parametri di sicurezza per l'endotelio, il cristallino e la macula11,12,13,14.

In conclusione, allo stato attuale, ogni qualvolta ci troviamo di fronte ad un paziente con ectasia corneale (cheratocono, ectasia post-LASIK) in evoluzione, specie se giovane sotto i 26 anni, che ha lo spessore corneale sopra i 400 µm, raccomandiamo sempre l'utilizzo del Cross-Linking standardizzato con rimozione dell'epitelio al fine di ottenere il massimo effetto stabilizzante cross-linkando almeno 2/3 dello spessore stromale. Per ora riserviamo la tecnica TEXL ai casi di ectasia evolutiva (indicazione assoluta) o stazionaria (indicazione relativa) con thinnest point < di 400 µm in attesa della conclusione degli studi sperimentali in corso14.

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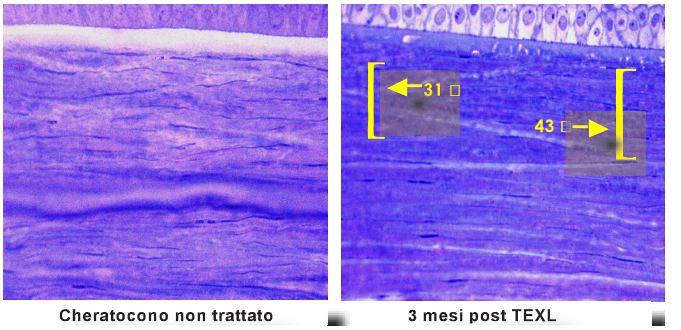

Fig.1: Analisi istologica al microscopio ottico. Sezione ultrasottile – Toluidine Blue Staining 40x

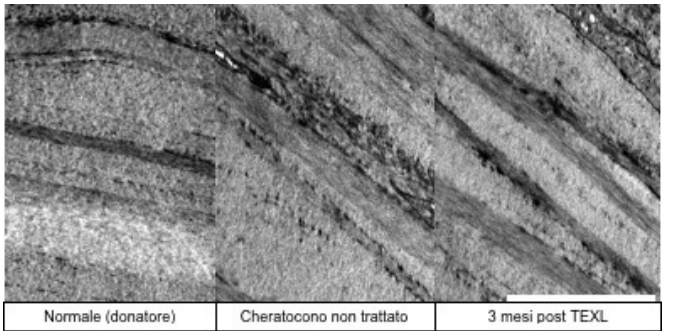

Fig.2: Analisi istologica al TEM. Organizzazione lamelle stroma intermedio (180-260 micron).

Fig.3: Analisi istologica al TEM. Organizzazione lamelle stroma profondo.

Keratoconus and epi-off corneal cross-linking by riboflavin-ultraviolet type a: indications and rational of employment

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Keratoconus is the most common dystrophic corneal ectasia, characterized by the presence of irregular astigmatism associated with a reduction of corneal thickness. It is the leading cause of corneal transplant in Italy and Europe. Recently a new therapeutic opportunity is offered by Riboflavin + UV A Corneal Cross-linking, first introduced in Italy in 2004 by Professor Aldo Caporossi at the Department of Ophthalmology of Siena.
This treatment requires early diagnosis to prevent corneal ectatic modifications related to pathology. The modern treatment of keratoconus is directed into three "directories": 1) prevention of its progression; 2) reduction of the related refractive defect and induced corneal aberrations; 3) replacement of ectatic corneal in advanced phase not subjected to conservative approach and HRGP lens intolerance. Riboflavin + UV A Collagen Cross-linking is mostly indicated in patients between 10 and 26 years old with progressive keratoconus (stage 1 and 2) with strict adherence to the recommended inclusion thickness (thinnest point > 400 microns).
Keywords: Keratoconus; Cross-linking; Riboflavin

Introduzione

Il Cheratocono(1) rappresenta la più comune distrofia "ectasica" della cornea a carattere degenerativo. Generalmente la malattia ha esordio in età puberale, è bilaterale, asimmetrica ed è contraddistinta dalla presenza di un astigmatismo irregolare associato a riduzione dello spessore corneale. Si tratta di una malattia generalmente progressiva ad andamento capriccioso, prevalentemente sporadica la cui componente genetica appare evidente in circa il 20% dei casi con penetranza ed espressività clinica variabile. In circa il 20% dei casi il cheratocono evolutivo necessita di una cheratoplastica lamellare e/o perforante1 a seconda dello stadio e della compliance del paziente alle lenti corneali(4).
I progressi tecnologici a servizio dell'oftalmologia e l'uso quasi routinario della topografia e della pachimetria corneale in chirurgia rifrattiva hanno messo in luce una grande quantità di cheratoconi non ancora diagnosticati pertanto, nella nostra esperienza, l'incidenza della malattia è realmente ben superiore (1 caso su 420 pazienti nel nostro database)(1) a quella riportata in letteratura (1 caso su 2000)(2). Il cheratocono in Italia ed in Europea è la prima causa di trapianto corneale e ciò desta non poche preoccupazioni per l'impatto sociale e sanitario dovuto al coinvolgimento di pazienti molto giovani2. Una recente opportunità terapeutica ad "indirizzo patogenetico" è offerta dal Cross-linking Corneale Riboflavina UVA, per la prima volta introdotto e sviluppato(3) in Italia nel 2004 dal professor Aldo Caporossi presso la Clinica Oculistica di Siena. Questo nuovo trattamento impone la necessità di una diagnosi precoce al fine di prevenire e possibilmente bloccare le modificazioni corneali correlate alla patologia. La scelta terapeutica nel cheratocono deve essere basata sulla corretta stadiazione clinico-strumentale della malattia, sulla sua evolutività, sulla compliance alle lenti a contatto, sull'età del paziente e sulla sua qualità di vita. Pertanto, a nostro avviso, la moderna terapia del cheratocono è diretta in tre "directory" principali:

1) prevenzione o rallentamento della sua progressione in "fase rifrattiva";
2) riduzione del difetto rifrattivo correlato e delle aberrazioni corneali indotte;
3) sostituzione della cornea ectasica in stadio avanzato (III-IV) in pazienti non suscettibili di miglioramento contattologico(4) .

Nella maggior parte dei pazienti affetti da cheratocono le relative modifiche della curvatura, il progressivo assottigliamento e il decadimento delle qualità ottiche della cornea tendono a concentrarsi fra l'età puberale (tra i 10 e i 15 anni) ed i 25-30 anni per esaurirsi in

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modo pressoché totale dopo i 35-40 anni anche per l'insorgenza di un fisiologico processo di Cross-linkaggio indotto dalla glicosilazione proteica (reazione di Maillard), dall'effetto della lisil-ossidasi (LOX) su tutti i connettivi(1).
Dal punto di vista ottico-fisiopatologico il cheratocono è generalmente caratterizzato da una dislocazione infero-temporale dell'apice dell'ectasia corneale e dalla riduzione del raggio di curvatura i quali producono una superficie corneale "asimmetrica" ed "iper-refrattiva" sebbene quest'ultimo parametro è parzialmente compensato dall'assottigliamento del tessuto6. Tali modifiche producono i noti "effetti ottici" responsabili del degrado della funzionalità visiva caratteristica della malattia.
Fino ad oggi, eccetto il Cross Linking Corneale Riboflavina + UVA (5), nessun altra terapia si era dimostrata in grado di prevenire e rallentare la progressione del cheratocono. Le proposte terapeutiche cosiddette "mini-invasive" come gli anelli intrastromali sono infatti rivolte esclusivamente alla cura degli effetti rifrattivi (astigmatismo e miopia) secondari alla malattia ed hanno dimostrato la loro inefficacia in tema di stabilizzazione. L'individuazione di una "terapia eziopatogenetica" come il cross-linking del collagene corneale si sta dimostrando effettivamente utile fino ad oggi nel ridurre la percentuale dei pazienti che arriva alla chirurgia, avvalorando ancora di più l'importanza della diagnosi precoce che obbliga ad una revisione critica della "strategia attendista" per spostare l'obbiettivo terapeutico dalla correzione degli effetti (decadimento delle proprietà ottiche), alla prevenzione dei più importanti meccanismi fisiopatologici alla base della malattia (rinforzo biomeccanico e biochimico della cornea ectasica)(4).

Indicazioni e Razionale del Cross Linking Riboflavina + UVA

Il Cross-linking del collagene mediante Riboflavina 0,1% + UVA è oggi elettivamente indicato(4) nel cheratocono in fase rifrattiva (stadio 1 e 2) in progressione, il cui peggioramento negli ultimi 6 mesi sia documentabile clinicamente (riduzione del visus, microstrie alla biomicroscopia), topograficamente (incremento degli indici di simmetria e cheratorefrattivi), pachimetricamente (peggioramento del thinnest point e dello spessore centrale medio), topo-aberrometricamente (peggioramento delle componenti comatica e di alto ordine)(4).
A nostro avviso sono da escludere dal trattamento soggetti di età inferiore ai 10 anni e superiore ai 40 anni (salvo casi particolari di scarsa compliance alle lenti a contatto o a scopo rifrattivo in casi selezionati), pazienti con spessore corneale inferiore a 400 micron in thinnest point1, in fase di stabilità clinico-strumentale acclarata negli ultimi 6-12 mesi, pazienti con storia di pregressa cheratite erpetica, presenza di opacità cicatriziali corneali e strie di Vogt marcatamente evidenti alla lampada a fessura e verificate anche con microscopia confocale per la tipizzazione dell'orientamento; in tal caso sono da escludere dal trattamento soggetti con strie di Vogt a distribuzione o pattern reticolare o intrecciate evidenti all'esame della cornea mediante microscopia confocale(6). Questa ultima rappresenta una controindicazione relativa da noi evidenziata per la prima volta a livello internazionale(6), per il potenziale sviluppo di haze corneale post-operatorio; sono da escludere dal trattamento anche pazienti con grave occhio secco, infezioni corneali in atto e malattie autoimmuni concomitanti(4).
I parametri da tenere in considerazione per valutare la progressione del cheratocono sono clinici e strumentali, in particolare quelli topografici (occorre valutare con attenzione le mappe topografiche differenziali, gli indici cheratorefrattivi con particolare riguardo all'indice di simmetria supero-inferiore – SI e all'indice di asimmetria di superficie SAI), aberrometrici di superficie anteriore (l'aberrazione comatica in particolare si colloca tra i dati più sensibili e precocemente variabili) e posteriore della cornea e pachimetrico-topografici (variazione dello spessore corneale centrale e del thinnest point). Inoltre, data la possibile evoluzione del dato biometrico (lunghezza assiale), vanno valutate, con molta attenzione, le modificazioni refrattive che possono insorgere con il normale accrescimento del paziente. Una condizione irrinunciabile è rappresentata dallo spessore minimo corneale "thinnest point", rilevabile mediante mappe pachimetriche ottiche dettagliate (Orbscan™, Pentacam™, Visante OCT™) che permettono rilevazioni precise anche in cornee non perfettamente trasparenti. Lo spessore corneale minimo, per la tecnica di cross linking standard con rimozione dell'epitelio (epi-off), non deve mai essere inferiore ai 400 µm. Questo valore è dettato dal mantenimento dei parametri di sicurezza (emersi dagli studi ex-vivo di Dresda(9,10), valutato e confermato, per la prima volta a livello internzionale presso la Clinica Oculistica di Siena, dal Prof Aldo Caporossi, attraverso gli studi effettuati, con la microscopia confocale e la tele-termometria, in vivo (11)). I risultati di tali studi hanno dimostrato la sicurezza del trattamento e confermano l'estensione del trattamento ai due terzi anteriori della cornea, risparmiando le cellule endoteliali, la lente e la retina.
Un ultimo, sofisticato, parametro in grado di fornirci indicazioni sul cambiamento del cheratocono in esame è l'analisi tissutale ottenuta mediante la microscopia confocale che ci fornisce informazioni micromorfologiche in vivo confrontabili nel tempo e soprattutto utili a chiarire la presenza-assenza di fattori di rischio per lo sviluppo di haze post-operatorio (presenza di strie reticolari, cheratociti iperattivati, edema subclinico, etc)(3,5,6,10,15).
Un discorso a parte va fatto per le Ectasie post-LASIK in rapida evoluzione in cui biomeccanicamente l'effetto cross-linkante può risultare particolarmente utile(13). Infatti, laddove il dato pachimetrico lo consenta, un precoce cross-linking può evitare o dilazionare il crollo refrattivo spesso presente in tali condizioni iatrogene. La mancanza di uno spessore minimo di 400 micron

risulta un limite a questa applicazione la cui tenuta nel tempo è in corso di investigazione data la ridotta importanza del lembo in termini biomeccanici. Altra indicazione emergente per il crosslinking riboflavina UVA è l'ectasia post Cheratotomia Radiale, attualmente in corso di investigazione, così come l'impiego di questa metodica nelle ulcere corneali e nel melting.

In conclusione, di fronte alla diagnosi di cheratocono, l'età del paziente assume un ruolo rilevante nella decisione terapeutica in relazione alla diversa prognosi. L'obiettivo primario del cross-linking è la stabilizzazione della cornea possibilmente prima che si instaurino le modificazioni refrattive e pachimetriche da evoluzione che rendono insoddisfacente il risultato del trattamento. Peraltro non esistono controindicazioni assolute legate alla forma della cornea, tuttavia l'indicazione nasce dal miglior risultato funzionale-anatomico ottenibile rispettando tali parametri. Oltre all'età il parametro più importante, per il cross linking standard epi-off, è dato dal rispetto rigoroso dello spessore raccomandato (thinnest point > 400 micron) che è fondamentale per preservare l'endotelio corneale dalla radiazione UVA a seguito degli studi di base e alle valutazioni confocali in vivo da noi eseguite(3,5,8). Gli studi più recenti di microscopia confocale da noi eseguiti e pubblicati(5,14) stanno fornendo indicazioni su alcuni possibili meccanismi biologici di funzionamento del crosslinking, attraverso l'individuazione di bande collagene più compatte ed iper-riflettenti che suggeriscono la presenza di un collagene crosslinkato di nuova sintesi, diversamente strutturato e più compatto. Studi biochimici di gel elettroforesi10 hanno evidenziato nella cornea crosslinkata la presenza di una banda collagene ad alto peso molecolare, resistente al calore e alla digestione enzimatica, non evidenziabile nelle cornee non trattate. Tali evidenze scientifiche(5,12) sono state messe in correlazione alla stabilità a lungo termine del crosslinking registrata fino ad oggi(13).

Tra le novità e le prospettive future c'è il Cross linking trans-epiteliale o TEXL, che rappresenta un importante passo avanti nella terapia della malattia cheratoconica. La tecnica è in fase di sperimentazione (studio Siena C.L.E.S.: Cross Linking Evolution Study), presso la UOC di Oculistica di Siena diretta dal Prof. Aldo Caporossi e si propone di valutare i vantaggi offerti da questa nuova tecnica, che vuole affiancare ma non sostituire il crosslinking standardizzato con rimozione dell'epitelio.

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Aesthetics: from nature to engineering and back

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Aesthetics rises in XVIII Century involving the inner and the senses of the subject. The bipolar couple beautiful/ugly is no more enough to define new emerging aesthetic experiences of agreeable, charming, interesting, sublime...
Traditional models of Perfection (based on metron, nomos, logos, eurhythmy, symmetry, once centrepieces of the metaphysics of beauty) are soon overruled: irrational, disgusting, ugliness, darkness, mystery, shadow, troubled, dreadful, perturbing gain wide and independent spaces. Time, body, proximity senses, geographic, naturalistic and technological discoveries and inventions are among the factors of this turning point that go with the development of aesthetics. Rising and growing modern science, new technology and engineering determine a deep crisis in the aesthetic vision of nature, art, world, but also offer the references to build a new cultural and existential frame. In particular, actual engineering contributes to quality of life and offer explanations and demonstrations about how and because a thing or an action can satisfy our aesthetic sense, meant in a wide acceptation. As example we use the geometry of road and its curves and we compare this with structures we find in Nature. We use curves whose curvature changes along their development. They are very suitable to show how science and technology have dispelled the classic idea of perfection (based on quite, simple, exactly defined shapes as circle or square) and have contributed to build a new kind of aesthetic taste that understands and appreciates less defined and more problematic ones, coming from different laws.
Keywords. Aesthetics – Nature – Time – Science – Art – Engineering

ESTETICA E SCIENZA: DEBITORI RECIPROCI

"Aeasthetica (artium liberalium Theoria,
gnoseologia inferiore, ars pulchre cogitandi, ars analogi rationis)
sensitivae cognitionis est scientia." (Baumgarten, 1750)

Il bello esiste nella nostra cultura fin dalle sue origini (il concetto di *tò kalòn*, nell'antica Grecia, era carico di profondi significati ed era associato a quelli di vero e di giusto, ma l'estetica (una disciplina con un suo corpus epistemico autonomo) nasce nella modernità inoltrata, nel XVIII secolo: noi consideriamo il suo atto di nascita l'"Aesthetica" di Baumgarten (1750), ma il termine è un po' più vecchio: lo stesso autore lo usa già nella sua tesi di laurea. In altri autori sono presenti molti concetti connessi con l'estetica moderna. Addison, per esempio, in alcuni articoli per la rivista "The Spectator", nel 1712, parla di "piacere dell'immaginazione".
Con l'estetica l'attenzione non è sul bello in sé, ma sull'esperienza che provoca nel soggetto che con esso ha a che fare. Il soggetto, il suo pensiero, il suo centro di **soggettività**, guadagnano il primo piano nella concezione della conoscenza e della sua costruzione. Ma il soggetto non è solo pensiero, egli "ha" un corpo, anche…
La cultura occidentale ha sempre o quasi sempre diviso il corpo dalla mente, ha sempre identificato questi due aspetti come due cose reciprocamente estranee, e l'uomo è stato considerato un'entità composta, la cui natura è doppia.
Non solo corpo e mente sono divisi, ma sono anche disposti gerarchicamente, con la mente in posizione preminente.
Quelli delle concezioni materialistiche possono complessivamente essere considerati tentativi che non hanno ottenuto successi duraturi o convincenti nell'invertire questa gerarchia. Sono stati costruiti equilibri instabili che di fatto non hanno cancellato il dualismo mente-corpo.
Sebbene tale posizione diviene talvolta un monismo eliminativo nei riguardi della controparte del corpo (la mente), comunque queste dovevano essere confrontate con gli aspetti più profondi della nostra cultura. E la nostra cultura non può rinunciare alla mente che, se viene eliminata, presto trova il modo di riguadagnare le posizioni perdute.
L'ineliminabile esperienza del sé, l'autocoscienza, la soggettività acquistano importanza proprio nel XVII-XVIII secolo, quando la rivoluzione scientifica colloca la necessità di oggettivare la realtà in primo piano.
La soggettività rimane nel regno dell'anima o della mente e diviene sempre più forte, opponendosi al mondo esterno oggettivo.
Descartes dà un contributo decisivo alla svolta della cultura occidentale in direzione dell'introspezione e del sog-

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gettivismo. Sulla stessa linea troviamo anche il metodo e la teoria della logica di Port Royal. Descartes di rendere la "*res extensa*" e la "*res cogitans*" due cose distinte ma confrontabili. Tuttavia la loro simmetria non è durevole, perché il corpo è pensato e non pensante, è passivo e oggettivo, è misurabile e non "misurante". Esso è irrimediabilmente lontano dalla mente. Al momento non c'erano le condizioni perché divenisse pienamente soggetto.
L'uomo e cosciente di pensare ma anche di percepire (nonostante i dubbi di cui Descartes investe quest'ultima attività). In ogni caso, insieme con il pensiero individuale, i sensi guadagnano sempre più importanza in molti contesti. Soprattutto a partire dal XVII secolo, notiamo un uso sistematico del metodo osservativo e l'affermarsi del sensismo.
Il primo che ha dato un riconoscimento a questo modo di acquisire conoscenza è stato Aristotele (anche se ne troviamo traccia già in Protagora), e, attraverso l'aristotelismo rinascimentale, giunge a confluire nella moderna teoria che ne fa un vero e proprio metodo scientifico: quella di Locke, Hume, Berkeley, Condillac, James.
Fondamentalmente il sensismo è basato sul concetto che "nulla esiste nella mente che prima non sia stato nei sensi" (Locke nel 1690 scrive «*Nihil est in intellectu, quod non prius fuerit in sensu*»).
Non a caso Leibniz poco dopo puntualizzerà:«*excipe: nisi intellectus ipse*» (Leibniz, 1765), come dire: il principio primo di conoscenza, astratto dal mondo e autoreferenziale, non può essere tagliato fuori o messo nell'angolo, il dominio dei sensi non copre tutto il conoscibile. Il sensismo non coincide affatto con il materialismo: il corpo è materiale e accede al mondo in quanto materiale, ma questo non esclude un'ulteriorità dove si svolgono i giochi della conoscenza da una parte e, un secolo più tardi, quelli del giudizio (estetico).
Come dire che per adesso il sentire "esterno" (tramite i recettori fisiologici) è costitutivo del sentire "interno" (gradimento, piacere, gratificazione, compiacimento, attrazione, emozioni e sentimenti in genere).
Corollario di questo è stato lo sviluppo di studi e ricerche focalizzati sugli aspetti concreti, di uso, di interazione materiale con oggetti, contesti, processi…
Per adesso il corpo solo è punto di passaggio, è rilevatore di dati nel mondo, ma la sua detenzione di questi dati è transitoria. Consegnati alla mente, è qui che saranno usati per generare contenuti cognitivi o giudizi estetici. Ma i sensi, con l'esercizio del rapporto estetico con il mondo, non possono essere a lungo costretti entro lo stretto limite di strumento. Sono stati tirati in ballo ed è questione di tempo perché sviluppino le conseguenze della loro implicazione.
Fondare l'estetica ha messo in moto più di quanto era nell'intenzione dei suoi iniziatori, pertanto possiamo dire che l'estetica moderna (o, *tout-court*, l'estetica) nasce con un doppio seme, di straordinari sviluppi culturali e di crisi (implosione, frammentazione, dissolvimento dei significati originari) che culminerà nel XX secolo.
Ma nella seconda metà del Novecento viene anche scoperto tutto un sistema di "effetti crociati" tra la funzione fisiologica, il vissuto corporeo e quello interiore del pensiero e dei sentimenti.
Si apre un orizzonte di ampiezza straordinaria, con implicazioni spesso inattese e sorprendenti.
Tornando al XVIII secolo, fin da allora il *focus* si sposta dalla bellezza "oggettiva", "esterna", all'interiorità del soggetto. Ed è qui che già da Addison, Burke, Hume, etc., l'estetica incontra le prime difficoltà, nei limiti del soggettivismo, avvisaglia della crisi che deve svilupparsi.
La coppia bipolare "bello/brutto" non riesce a racchiudere la gamma di sensazioni di cui il soggetto ha coscienza. Tale coppia non basta più per definire le nuove esperienze estetiche di piacere, gradevolezza, fascino, interesse, carattere pittoresco, sublime…
L'atto del guardare (dal XVII secolo) acquista consapevolezza e trova il suo scopo in se stesso. Il soggetto che guarda trova piacere e soddisfazione nella sua attività di scoperta. Va a caccia di immagini, visioni che devono essere sempre più belle, sorprendenti, spettacolari, difficili da trovare, persino proibite. Tale soggetto diviene a poco a poco un **voyeur**, un curioso, un invadente, un inopportuno, un "affamato" di immagini.
Guarda l'artista (la realtà) e guarda lo spettatore dell'opera. L'occhio di entrambi è testimone della medesima scena. Paesaggi, scene di genere, situazioni intime, scabrose, soggetti inusuali, tutto può soddisfare questo desiderio, e quando qualcosa viene scoperto il voyeur ha bisogno di qualcos'altro di più spettacolare, più sorprendente. È la stessa molla che talvolta spinge alla ricerca delle stranezze della natura, di esemplari o fenomeni bizzarri da inventariare, annotare, illustrare, classificare.
Tale tendenza porterà, nel tempo, all'attuale inflazione di immagini (anche grazie alla possibilità tecnica della loro (ri)produzione, cfr. Benjamin, 1966), alla ricerca (anche da parte di cinema e TV) dell'estremo, dell'iperbolico, dello shock, arte e media spesso propongono il "kitch".
Valore, significato e funzione dell'immagine cambiano: l'immagine non è più imitazione che offre un doppio di livello inferiore, una deriva peggiorativa (cfr. Platone), ma comunque capace di filtrare e abbellire ciò che rappresenta (cfr. Aristotele). Ora essa è la creazione di un nuovo e differente mondo, capace di offrire *hybris* non del vero, ma del verosimile, di far dimenticare il mondo reale, confondendolo con quello fittizio…
Fondare l'approccio estetico con il mondo coincide con la scoperta che possiamo fare più che conoscere il mondo, più che darci una legge morale, possiamo giudicare, scegliere, decidere cosa siamo e come, avere il nostro autonomo spazio di discrezionalità.
Il giudizio si accorda al proprio gusto e orientamento, alla ricerca del piacere, della soddisfazione, e l'uomo è l'unico soggetto di queste decisioni, a conferma del suo potere individuale. Scivolare verso un individualismo egocentrico, nell'Estetismo (XIX-XX secolo) del Dandy, nel solipsismo, perdere il senso di appartenenza alla comunità e l'identità culturale e sociale, questi sono i rischi e i pericoli spesso attuatisi nella nostra società, quando l'originaria libertà e soggettività è stata esasperata e ha degenerato.
La **perfezione**, cifra della bellezza, una volta modello e riferimento, perno della tradizionale metafisica della bellezza, nella Modernità (e in particolare nel secolo scorso) si è capovolta, divenendo persino negativa, l'opposto della vita, della libertà e del dinamismo.
Logos, *metron*, *nomos*, euritmia, simmetria, una volta principali riferimenti della teoria della bellezza (cfr. Policleto, Vitruvio…), sono perduti. Già con Beethoven e Mozart vengono usate le dissonanze. Nella tarda letteratura romantica la malattia, l'anomalia, la mostruosità,

il vizio, la degenerazione, la turpitudine sono esaltate. A partire dal Manierismo, e dopo con Caravaggio, Goya… ma principalmente con il romanticismo, l'irrazionale, il disgustoso, il brutto, il tenebroso, il mistero, l'ombra, il terrificante, il perturbante conquistano spazi ampi e indipendenti, al di là dell'opposizione dialettica alla bellezza che tutto ciò ha in Rosenkranz ("*Aesthetik des Hässlichen*", 1853).

Il **tempo** come dimensione si stabilisce ovunque: tutto è soggetto al cambiamento, dai corpi celesti dell'astronomia galileiana alla teoria geologica delle catastrofi, all'entropia, al darwinismo. Ma questo è un tempo diverso da quello hegeliano, che mirava a una conclusione perfetta, capace di ripristinare l'armonia, l'unità, di condurre il mondo e l'uomo a un livello gerarchicamente superiore.

La storia geologica mostra che esiste un tempo oscuro e profondo, un lungo passato senza l'uomo. Questo è terrificante.

Il principale riferimento parmenideo, la distinzione tra ciò che è (l'ente, l'essere) e ciò che non è (il non-essere), garanzia di verità, ha prevalso nella cultura occidentale. L'eracliteo "tutto scorre" (così come l'unità degli opposti) rimane sullo sfondo, ma adesso la pervasiva dimensione del tempo ritorna, e per di più caricata dell'incertezza del caso.

Determinare entità e isolate, la loro identità, localizzare il loro nucleo, segnare i loro confini, è stata la tendenza dominante della scienza e della filosofia occidentale.

Sulle vecchie certezze adesso prevale un differente quadro: il flusso, il processo, la mutevolezza, la variabilità, la metamorfosi… emergono in primo piano negli studi naturali e umanistici.

Molte illustrazioni (a partire dal XVII secolo) ricercano l'"attimo fuggente", colgono un'"istantanea di transizioni più o meno rapide, movimenti, iniziando, tra l'altro, a fare attenzione alle trasformazioni patognomiche del volto e del corpo, all'espressività gestuale e non soltanto alle caratteristiche fisiognomiche, strutturali.

Il **corpo**, inizialmente condizione di percezione, più tardi (XX secolo) diviene un soggetto che non può essere subordinato o ridotto alla mente o all'anima (Irigaray, Derrida…). I sensi di prossimità (cfr. la *Madalaine* di Proust in *Alla ricerca del tempo perduto*) acquistano importanza: il gusto (termine che designa sia il senso che ci fa apprezzare il cibo sia la facoltà di giudicare e scegliere!...) e l'olfatto (dal XVII secolo l'arte del profumo è molto apprezzata, e sia il fascino che il disgusto dell'odore acquistano interesse.

La nudità non richiede più giustificazioni mitologiche o religiose. L'eros e la carne sono in primo piano («*L'anima nel Barocco intrattiene con il corpo un rapporto complesso: sempre inseparabile dal corpo, trova in quest'ultimo un'animalità che la stordisce, che la impastoia nei ripiegamenti della materia, ma pure un'umanità organica o cerebrale che le permette di innalzarsi, e la farà salire su tutt'altre pieghe.*», Deleuze, 2004).

Il dinamismo corporeo diviene fonte di piacere e occasione per produrre/esibire prestazioni.

La **natura** è un campo di indagine, una sorgente di meraviglia (la ricerca e l'ossessiva attenzione per i vari *mirabilia* è sintomatica) da collezionare e studiare. Le cose scientificamente studiate sono anche giudicate esteticamente e viceversa, con reciproca influenza tra le due attività. Ma, per adesso, senza armoniosa integrazione. Eccezioni e stranezze sono preferite alle cose usuali, regolari.

L'esplorazione di continenti esotici e lontani amplia gli orizzonti della Modernità. La natura mostra anche il suo lato oscuro e selvaggio, il pericolo, il pauroso, le terribili profondità, offre brividi (*hybris*) e richiede coraggio.

La dimensione prometeica dell'uomo che affronta tale natura può essere trovata sia nella sua anima sia nel suo corpo (cfr. Burke, 1757).

Nel XX secolo il giudizio estetico si estende a campi fino ad allora inesplorati, oltre il visuale (delle arti e della natura) e l'uditivo (della musica), fino ad essere riferito alla vita (anche nell'accezione politica del termine), alla forma (della comunicazione e quindi ai media), alla conoscenza (in chiave criticistica e scettica), al sentire (fin nelle sue implicazioni fisiologiche).

La scienza e la metodologia hanno gran peso in molti di questi campi, soprattutto nello studio della forma (scienza, tecnologia e ingegneria dell'informazione), della conoscenza (metodologia), del sentire (lo studio della fisiologia e della neurofisiologia, i cui dati sono sempre di più interfacciati e correlati con quelli mentali, cognitivi, emotivi, culturali). In particolare lo studio della sensorialità, che ha rivelato un numero di sensi (se di sensi si può ancora parlare) ben superiore ai cinque aristotelici. La sensorialità legata all'esperienza corporea di movimento, di auto percezione, di interazione con il mondo, può essere inscritta nei paradigmi di apprezzamento (estetico) al pari di ciò che vediamo o udiamo. Sensazioni (o insiemi di sensazioni) come la propriocezione, la cinestesi, la vezione possono offrirci un vissuto gradevole o sgradevole, intenso o scialbo, stimolante o demotivante.

Ciononostante la caratteristica distintiva dell'estetica era originariamente definita (cfr. Kant) come altro dall'utile (al contrario di filosofie orientali) e dalla conoscenza. La **scienza** e la **tecnologia** che si affermano costruiscono una nuova visione del mondo, ci danno un'accezione diversa del senso estetico.

Quando Galileo mostra che persino le stelle muoiono, che i corpi celesti non sono perfetti e sono omogenei al mondo sublunare, il vecchio mito della perfezione celeste è infranto. Quando Keplero dimostra che il moto dei pianeti non è uniforme, che le orbite degli stessi non sono circolari, le vecchie regolarità geometriche sono dissolte.

I dispositivi ottici (microscopio e telescopio) spostano i limiti dimensionali e di distanza (micro e macro) oltre il naturalmente visibile, l'uomo non è più copula mundi, di un mondo i cui estremi erano comunque commensurabilmente vicini. Il senso della misura e dell'equilibrio è perduto.

Il tempo rende tutto transitorio, dalle stelle ai viventi, alla materia, nella quale vuoto e discontinuità divengono prevalenti (teoria atomica), inoltre, con la fisica quantistica, "*Natura facit saltus*"!

La teleologia e i principi ordinatori in senso tradizionale sono persi.

Solo nella seconda metà del XX secolo si ha un vero punto di svolta.

Per esempio, quando la scienza ci mostra la similarità fra le strutture di una colonia batterica, di una galassia, una mappa di router di internet e una pittura di Pollock, tutte riconducibili al modello di un frattale, noi non possiamo ignorarlo e ciò ha inevitabilmente influenza sui nostri gusti, sul nostro modo di giudicare la natura, l'arte, il mondo.

La teoria della complessità e i modelli olistici ci mo-

strano un altro genere di bellezza.
La **psicoanalisi** rompe la originaria unitarietà del soggetto. Cento anni fa o poco più Freud (1998) mostrava come le pulsioni di vita (libido/*Eros*, sopravvivenza, propagazione, fame, sete, sesso) e le pulsioni di morte (*Tanathos*) influenzano le nostre preferenza e guidano il nostro comportamento, non di rado in conflitto una contro l'altra, come l'A. mostra nel suo lavoro "Oltre il principio del piacere". L'inconscio ha il suo ruolo insospettato, ciascuno di noi è teatro di conflitti tra forze in parte sconosciute…
La medicina, d'altro canto offre una ben articolata visione di malattie, patologie e difetti, ma mostra anche come la salute non sia assoluta assenza di essi, non sia perfezione.
La condizione **postumana** rende il mondo naturale lontano e i significati tradizionali incerti. Le realtà vicarie, fittizie, virtuali, ci rendono "sdoppiati" e capovolgono il mondo: il reale e la finzione si scambiano, i sensi divengono "nudi sensi" se non integrati con dispositivi tecnologici (il *cyborg* non è più solo fantascienza) che rimpiazzano facoltà mancanti, amplificano o modificano quelle naturali. Per esempio la fotografia nel XIX secolo e il cinema o le tecnologie di *imaging* animata nel XX. Occhio e obiettivo divengono un sistema unico. Dacché usiamo obiettivi fotografici e cinematografici, non guardiamo più il mondo nello stesso modo, anche se non li stiamo usando concretamente. Siamo così abituati a guardare in uno schermo (apparecchi di ripresa sono ovunque) che persino il mondo reale può sembrare un film…
Le tecnologie di *imaging* rendono visibile l'invisibile, e possibile l'impossibile, sciolgono la cifra di realtà indefinite, ci danno la certezza strumentale: è sempre più difficile rinunciare a questa amplificazione dei nostri poteri.
L'occhio biologico "vedeva il mondo", ma adesso l'"occhio nudo" è inevitabilmente superato dai dispositivi ottici. Riassumendo, questa possibilità di andare lontano, oltre la natura è attuata in ogni senso dal cinema, dalla TV e dalle tecnologie di sintesi grafica digitale:
In senso geometrico: con la rappresentazione di parti interne, lontane dalla superficie, misurando la loro dimensione, la loro profondità…
In senso **dimensionale**: amplificando l'infinitamente piccolo con il microscopio, e non solo quello ottico.
In senso **psichico**: oggettivando gli aspetti invisibili o elusivi come le emozioni e la cognizione.
In senso **cronologico**: una ripresa video è una memoria artificiale che può essere differita nel tempo.
In senso **spaziale**: posso osservare un evento molto lontano da me nello istante in cui succede.
L'integrazione tra il sistema sensoriale (che include sia il recettore periferico che la componente cerebrale) e il dispositivo tecnologico è implementato da modificazioni neurali e cognitive, così che nell'esperienza è molto difficile distinguere tra i due.
L'interazione sensomotoria con dispositivi artificiali è sempre più estesa e articolata, espandendo il nostro dominio ma confondendoci, dandoci un senso di estraneità.
Come esito di ciò, nel XX secolo i principali assi dell'esperienza estetica sono dissolti:
Poiesis: l'uomo non si sente più autore (creatore) di nulla che abbia un'aura (cfr. Benjamin, 1966). La frammentazione della produzione (post)industriale, così come il linguaggio cinematografico, richiedono di essere diretti dall'esterno, con un lavoro di montaggio affinché abbiano un senso.
Aisthesis: i sensi hanno bisogno degli ausili tecnologici, che però ci fanno perdere la diretta connessione al mondo.
Katarsis: la trascendenza dell'identità, della relazione con se stessi, portano a confusione esistenziale, al disorientamento, a obiettivi indecifrabili.
Ricordando la famosa frase di Rimbaud"Io è un altro", il nostro pensiero va a molte terribili esperienze, dalla schizofrenia all'anoressia (non a caso tipica della società occidentale contemporanea) dove il corpo, la sua consistenza materiale è l'estraneo, il nemico.
L'**anoressia** è tutt'altro che un edonistico desiderio di essere agili, snelli e desiderabili. È ben di più che un semplice disturbo del comportamento alimentare: è un problema globale, esistenziale, profondo, ha capovolto l'approccio estetico al corpo, l'aspirazione erotica di essere desiderati e desideranti. *Thanatos* ha vinto su *Eros*…
Distruggere, dissolvere, annientare il corpo è il perverso "contropiacere", principale scopo dell'anoressia.
Solo dopo la metà del XX secolo il **corpo** guadagna un ruolo primario: superando i pesanti limiti sia dell'idealismo che del determinismo materialista, il corpo è un essenziale aspetto della soggettività e del soggetto. Nei processi cognitivi, così come in quelli emozionali, relazionali o estetici, la modalità corporea non può essere esclusa: non possiamo vivere né capire simili esperienze a prescindere dal corpo.
Oggi parliamo di neuro estetica, sensologia, estesiologia comprendendo l'accezione scientifico-medica di tali termini), il corpo ha pienamente acquisito un ruolo primario. Il S.N., l'informazione biologica, l'interazione fisica con l'ambiente (inteso come *umwelt*) sono i termini per parlare di una reciproca inclusione (introdotta dalla fenomenologia) tra il soggetto e il mondo, tra la mente e il corpo, secondo le nuove teorie dell'Esternalismo, la Conoscenza Incarnata e Radicata (EEC, dall'inglese *Embodied Embedded Cognition*), il Comportamento Interattivo Immediato (IIB, *Immediate Interactive Behaviour*) e così via, che dischiudono nuovi orizzonti culturali ed esistenziali.
Il nostro cervello è il nostro corpo (esattamente una sua parte), ma non solo. Il cervello è una parte molto particolare, grazie alla quale percepiamo, conosciamo e gestiamo coscientemente il nostro corpo, viviamo il nostro corpo, *siamo* il nostro corpo. vivere il corpo attraverso la mente-cervello è l'unico modo per viverlo…
Non c'è dualismo tra il cervello e il corpo (somatico), tra controllore e controllato. Al più essi sono differenti aspetti della stessa cosa. Possiamo considerarli come indistinguibilmente integrati uno con l'altro.
I processi evolutivi, adattivi, di sviluppo, di apprendimento, e persino culturali non possono essere concepiti senza il corpo, le sue percezioni, le sue interazioni fisiche, la sua collocazione nel mondo (*umwelt*). Il nostro corpo sarebbe una semplice "cosa" se non fosse pensante/pensato (pensiamo anche con il corpo!...), se non gestisse il/fosse gestito dal cervello, se non fosse immerso nella cultura.
Il corpo è sempre impegnato in uno scambio con il mondo che è insieme fisico e informazionale/semiotico.
La retro propagazione dell'errore (cfr. il connessionismo) ha una natura corporea ed è condizione essenziale per rendere il cervello capace di interagire con il mondo. La micro- e macro-architettura del cervello acquista specificità per ottimizzare le interazioni future,

così come è atteso che si verifichino.
Ciò è possibile grazie all'inclusione del mondo (esterno) nella funzionalità cerebrale.
Dobbiamo superare la teoria puramente proiettiva dell'*homunculus*. Anche se esiste un importante sistema di connessioni (cervello-corpo e corpo-cervello), ciò non è né l'unico né il principale modo di unire "centro" e "periferia".
Possiamo notare che il corpo insieme con i dispositivi esterni è un sistema nuovo e differente, che emerge dalla reciproca interconnessione. Tale connessione è molto stretta, non è una semplice giustapposizione, e può essere definita "accoppiamento strutturale" o in modo simile.
Il nuovo sistema che in virtù di essa emerge non è "autocentrato", talvolta nemmeno "neurocentrato". I processi di apprendimento trasformano l'interazione occasionale e casuale in una inclusione del mondo esterno nel corpo.
Persino processi tipicamente cerebrali come il rievocare sono basati solo su risorse interne. Il cervello "fa conto" nei riferimenti offerti da opportuni oggetti/eventi esterni. I dati del mondo esterno sono processati insieme e nello stesso modo dei dati del corpo. È molto difficile distinguere l'oggetto esterno come tale: lo percepiamo in continuità e omogeneità con il nostro corpo. l'apprendimento è la confluenza dei dati interni ed esterni in un unico sistema computazionale. Tramite l'apprendimento acquistiamo familiarità con nuove situazioni, correlando opportunamente l'input con l'output (cfr. P.M. Churchland, 1992).
Siamo inestricabilmente connessi con il mondo; i trasduttori sensoriali e l'apparato esecutivo possono essere considerati solo un differente tipo di sinapsi, attraversate da un flusso di informazioni provenienti da e dirette al mondo: i "Memi" non abitano nel chiuso della nostra scatola cranica, essi fluiscono, tra la mente/cervello e il corpo, tra il corpo e il mondo in ambo i sensi.
Oggi non possiamo più separare concezioni scientifiche ed esperienza estetica. La scienza ci dà le basi per una rivoluzione tutt'altro che conclusa. Alcune frasi possono riassumere questa svolta epocale:
Il **sistema** diviene prevalente sul **singolo elemento**.
L'**intero** diviene prevalente sulla **parte**.
La **connessione** diviene prevalente sul **nodo isolato**.
L'**oikos** diviene prevalente sull'**ontos**.
L'**emergere** diviene prevalente sulla **causazione deterministica**.
La **codeterminazione** diviene prevalente sul **determinismo lineare**.
La **complessità** diviene prevalente sull'**ordine rigido** e sul **caos stocastico**.
L'**autorganizzazione** diviene prevalente sulla **catena causa-effetto**.
L'**omeorresi** diviene prevalente sull'**omeostasi**.
La **commistione sfumata** diviene prevalente sul **rigido confine**.
L'estetica attuale deve procedere in questa direzione: i vecchi dualismi sono superati. Coppie come bello/brutto, soggetto/oggetto, esterno/interno divengono sempre più ricche e articolate fino a perdere i significati originari, grazie a ulteriori e differenti concetti che configurano un nuovo e complesso quadro teoretico, euristico ed estetico.
Nel XX secolo la dimensione corporea dell'estetica raggiunge la sua completa crisi, ma anche fa intravedere nuove prospettive future.
Come Sean B. Carrol suggerisce nel titolo del suo libro sulla biologia Evo-Devo "Infinite forme bellissime" noi possiamo insieme conoscere (scientificamente) e godere (esteticamente) nuovi mondi.

L'INGEGNERIA TRA COMPRENSIONE DELLA NATURA E PROGETTAZIONE

Tale nuova direzione è indicata da molti esempi. Uno di essi può essere considerato la nascita della ergonomia contemporanea a Oxford, nel 1949, che inaugura un filone di ricerca su base medico-ingegneristica finalizzato a orientare la progettazione (compreso il *design*) al soddisfacimento di esigenze psico-fisico-relazionali.
L'ingegneria estende il suo campo, non di rado interferendo in quelli che tradizionalmente erano i domini di azione dell'architettura e delle "belle arti" in genere.
La contemplazione e l'"*aisthesis*" non sono più l'unico e nemmeno il principale modo di rapportarsi con l'oggetto.
La staticità lascia il posto al dinamismo, il prodotto al processo, la contemplazione all'azione, la struttura alla funzione, e via dicendo.
In particolare l'aspetto processuale e la sua valorizzazione ha radici che potremmo far risalire addirittura al tardo Medioevo: si pensi alla tecnica di costruzione della cupola del Duomo di Firenze senza ricorrere a centine. Oltre che progettare il prodotto l'ingegneria progetta anche e sempre di più il processo, processo che ha un contenuto tecnologico e teoretico sempre più elevato. Nella modernità avanzata, e soprattutto dal XIX secolo, la conoscenza diviene sempre più fonte di piacere e gratificazione. Un'altra barriera che dovrebbe isolare l'estetica nella sua torre d'avorio (separandola in questo caso dalla conoscenza) è infranta.
In tutto questo trova spazio l'ingegneria che costruisce e offre gli strumenti di formalizzazione, controllo e gestione di produzione e uso degli oggetti.
Dall'ingegneria vengono le spiegazioni e le indicazioni di come e perché un oggetto è o può essere piacevole, gradevole, elegante…
Ingegnerizzare i rapporti con la realtà non significa avere la garanzia dell'infallibilità, anzi significa proprio lasciare all'errore spazio e assegnargli una funzione informativa, farne un elemento di confronto.
Il vissuto estetico oggi non può prescindere dalla semplicità di uso dell'oggetto, dalla qualità (anche fisica, corporea) dell'esperienza di interazione, dalle implicazioni funzionali e di utilità che tale interazione mette in gioco. Semplicità come eleganza, come pulizia, come purezza, come assenza di disturbi, come economia di tempo e risorse attentive per le caratteristiche intrinseche dell'oggetto e quindi come possibilità di concentrarsi su aspetti esterni e sovraordinati, quindi come libertà e apertura del pensiero.
Un esempio paradigmatico è il mouse prodotto dalla Apple ("*Magic Mouse*") nel 2009, che presenta una superficie compatta, intera, senza alcun elemento di azionamento meccanico, come erano i tradizionali tasti (sx e dx), e la rotellina di scorrimento. Il lembo di superficie superiore è di fatto un "touch-pad" interattivo, con ulteriori funzioni. Il tipo di contatto (ampiezza, direzione, velocità…) calibra e determina le azioni "sfoglia pagina", "zoom" di pagina o di schermo, "scorri pagina", e in genere tutte le funzioni tradizionalmente affidate ai tasti dx e sx. Le vere novità sono che tutta la

superficie è recettiva in tal senso (e quindi non ci obbliga a azionare in modo mirato questo o quel comando), inoltre i gesti di "touch" sono molto più intuitivi e accettano un margine di approssimazione maggiore, senza perdere efficacia e precisione, infine la gamma di comandi è molto più articolata e consente di evitare in molti casi lo spostamento fisico del mouse sul pad.
Questo è ottenuto grazie a una tecnologia sempre più evoluta e che è presente in sempre maggior quantità all'interno dell'oggetto, ma per offrire un'interfaccia sempre più conformata sulle esigenze e i gusti del soggetto.
È pur vero che l'oggetto diventa sempre più una "scatola nera" e si segna un divario sempre maggiore tra la conoscenza del fruitore (relativa all'uso) e la natura dell'oggetto, sempre più inaccessibile, ma il vissuto è sempre più soddisfacente in termini che possiamo definire insieme "estetici" e "funzionali".
La vera innovazione è che questa nuova estetica si interessa di oggetti che tradizionalmente sono stati non solo ignorati, ma anche ritenuti tutt'altro che degni di considerazioni di questo tipo. Cosa c'è di "bello" o esteticamente apprezzabile in un mouse? In una curva di un tracciato stradale?
La prima cosa che notiamo (che l'ingegneria ci fa notare) è la stupefacente (dobbiamo davvero stupirci?) analogia tra strutture naturali (del mondo biologico o minerale) e strutture ingegneristiche, la convergenza tra le soluzioni cui è giunta l'evoluzione biologica o a cui portano i processi entropici da una parte e quelle messe a punto dall'ingegneria dall'altra. Tale convergenza viene notata spesso a posteriori, come dire che l'ingegneria giunge a tali soluzioni, analoghe a quelle naturali, indipendentemente da esse.
Questa può essere se non una prova almeno un indizio a sostegno del fatto che le soluzioni in questione [es. l'impacchettamento esagonale delle schiume, dell'alveare, le reti metalliche di contenimento degli argini, o briglie] rappresentano un optimum a prescindere. In seguito alla constatazione di tali convergenze, oggi l'ingegneria sempre di più cerca zone di intersezione con le scienze naturali. Sembra evidente che questo interloquire tra l'ingegneria (progettuale e prescrittive) e le scienze naturali (descrittive ed esplicative) sia solo all'inizio e molto resti ancora da fare su questa strada.
Nell'attualità la demarcazione tra utilità, modo di fruizione, funzionalità, convenienza da una parte e bellezza dall'altra non è più sostenibile nei termini tradizionali che facevano della mancanza di implicazioni pratiche l'elemento definitorio della bellezza: "un oggetto è bello se prescinde dall'utile".
Per dimostrare quanto ormai bellezza e utilità siano indissolubilmente legate, segnando un decisivo salto di qualità rispetto alla visione dell'estetica del XVIII secolo, possiamo usare innumerevoli esempi, che ci offre l'ingegneria.
Uno di questi esempi significativi di convergenza di utilità e bellezza lo possiamo trovare nello sviluppo plano-altimetrico di una sovrastruttura stradale. L'aspetto altimetrico sarà qui solo accennato per brevità di esposizione.
Il percorso della strada, per molti e differenti motivi, presenta uno sviluppo non rettilineo.
In natura troviamo molti esempi di curvatura che possono offrire significativi termini di confronto.
La matematica, la fisica e l'ingegneria hanno costruito un interessante paradigma concettuale che consente di superare la vecchia dicotomia "naturale-artificiale" non con un semplice sincretismo o con una banale analogia o con un rimando semantico, bensì dimostrando come si possa riconoscere e quindi ricondurre a un modello matematico una struttura fisica o biologica e una antropica.
Il problema della curvatura lo troviamo, è l'esempio comparativo che scegliamo, nell'anatomia dei molluschi, in particolare dei gasteropodi. Probabilmente per ragioni di "compattamento dimensionale" forse per essere meno esposti ai predatori, l'evoluzione ha favorito massicciamente l'avvolgimento elico-spirale delle parti molli e del guscio da esse formato e che le protegge.
Infatti, la torsione viscerale e la conseguente torsione conchiliare dei molluschi gasteropodi, di alcuni cefalopodi e, anche se in modo meno evidente, dei bivalvi segue un andamento particolare che, guarda caso, è "imparentato" con la tracciatura delle nostre strade.
La conchiglia soddisfa esigenze biologico-adattive del mollusco, ma attira anche la nostra meravigliata ammirazione. La conformazione della strada non ha forse lo stesso tipo di attrattiva, ma ugualmente ha dei pregi estetici, come vedremo, che, al di là dell'eleganza della formulazione matematica, offre piaceri percettivi e motori, in accordo con quelle che sono oggi le concezioni dell'estetica.
Cominciando a porre il problema della tracciatura di una strada, il primo elemento che intuitivamente (e rozzamente) possiamo usare è un insieme di segmenti variamente dimensionati e variamente angolati.
Per la schematizzazione di una sovrastruttura stradale, scegliendo di rappresentarla con il suo asse (all'interno di un piano cartesiano georeferenziato), possiamo infatti utilizzare una linea poligonale irregolare che tenga conto, questo sì, della morfologia del territorio indicata dalle curve di livello. Questo tipo di tracciatura richiede solo l'uso del righello. La strada così fatta sarebbe impercorribile!...
Un secondo approccio, tutt'oggi utilizzato nelle strade a basso scorrimento e nei piani di zonizzazione primaria, per ragioni di semplicità ed economia (anche se nulla vieterebbe di ricorrere a modelli più evoluti, come quello che si va ad esporre), che affina quello suesposto, è quello di raccordare gli angoli della poligonale tramite archi di circonferenze tangenti ai segmenti, il cui centro è ovviamente collocato nell'angolo convesso che a due a due, disposti contiguamente, essi formano. Ma anche questa combinazione di segmenti e parti di circonferenza non ottimizza la percorrenza, lasciando un ampio margine di pericolo e di disagio all'utente, soprattutto perché si passa istantaneamente da una curvatura zero a una curvatura determinata e quindi con una derivata dell'accelerazione (contraccolpo) infinita. A basse velocità il fatto è pressoché trascurabile. I problemi iniziano con velocità sostenute. Pertanto occorre andare oltre!... ed è qui che le curve conciliari fanno il loro significativo ingresso, a sostegno di quella visione olistica degli oggetti naturali e artificiali cui si accennava.
Nella progettazione di sovrastrutture stradali (tipologia "A", cioè autostrade, superstrade…), l'elevata velocità di percorrenza dei veicoli richiede un modello più evoluto di quello segmento-arco.
In tale modello si sceglie di realizzare il raccordo tra l'elemento geometrico rettifilo (segmento di retta) e lo sviluppo di una curva a raggio fisso (arco di circonferenza) attraverso l'impiego di una curva a raggio varia-

bile, appartenente alla famiglia delle spirali logaritmiche, di equazione

$r \cdot s^n = A^{n+1}$, dove

r è il raggio della curva nel punto considerato,
s non è altro che lo sviluppo della curva nel punto considerato, ovvero l'ascissa curvilinea,
n è il coefficiente di forma che rappresenta di quanto varia la curvatura (1/*r*) al variare di *s*, ovvero dello sviluppo.

I tre casi limite sono

1)n=0 ⟹ r=a, che è una curva circolare ($x^2+y^2+ax+by+c=0$)

2) n=∞ ⟹ r=∞, che è una retta (y=mx+q)

3) n=1 ⟹ $r \cdot s = A^2$, che è una clotoide, ovvero un caso particolare di spirale logaritmica.

Di questi tre la scelta cade sul terzo perché rappresenta l'entità fisico-geometrica adatta a realizzare il raccordo tra elementi a curvatura diversa, grazie alla variazione punto-punto del suo centro di istantanea rotazione (e quindi anche del raggio) lungo l'intero sviluppo dell'asse stradale. In coerenza a tutto ciò, anche la rotazione della piattaforma stradale viene effettuata all'interno della curva di transizione in modo che la transizione dell'asse stradale corrisponda anche la transizione della piattaforma. La piattaforma, infatti, dotata di ingombro, richiede uno studio sul comportamento ed evoluzione dei cigli (margine sx e dx), e questo anche per evitare fenomeni di ristagno dell'acqua con conseguente acqua-planning (effetto Orn).

Tutto ciò è necessario perché il semplice inserimento di un arco di circonferenza tangente al rettifilo non tiene conto del fatto che su un tracciato stradale ci sono veicoli in movimento a velocità elevata e per questo sottoposti a diverse considerevoli forze fisiche, in particolar modo all'accelerazione centrifuga il cui modulo è espresso dalla relazione $a_c = \frac{v^2}{r} \left[\frac{m}{\sec^2}\right]$.

Un rettifilo può essere pensato come una porzione di circonferenza il cui centro è posto all'infinito, per cui, come riscontrabile nella pratica, il veicolo è sottoposto solo ad accelerazione longitudinale, poiché $\vec{a}_c = 0$.

Una circonferenza dal canto suo presenta un raggio definito e quindi un centro identificabile fisicamente, ad es. con coordinate cartesiane assolute o parziali (x;y), il quale fa sì che $\vec{a}_c \neq 0$ e, nell'ipotesi che la velocità si mantenga costante, l'accelerazione faccia altrettanto.

Tralasciando l'importanza del contributo dato dalla ovra pendenza della carreggiata stradale e dal fenomeno dell'aderenza trasversale lungo la percorrenza di un tratto di curva, utilizzate per compensare la spinta della forza centrifuga, possiamo esaminare il ruolo del **contraccolpo** [*c*].

La grandezza del contraccolpo nell'ipotesi di velocità costante, di essere così definito:

$$c = \frac{\partial a_c}{\partial t} = \frac{\partial\left(\frac{v^2}{r}\right)}{\partial\left(\frac{s}{v}\right)} = v^2 \frac{\partial\left(\frac{1}{r}\right)}{\frac{1}{v}\partial s} = v^3 \frac{\partial\left(\frac{s}{A^2}\right)}{\partial s} = \frac{v^3}{A^2}$$

L'unico elemento geometrico che consenta un passaggio graduale da accelerazione nulla ad accelerazione definita, è rappresentato dalla figura geometrica della clotoide in cui, per ogni punto appartenente al suo sviluppo, il centro di rotazione cambia consentendo una variazione graduale e controllata della forza dell'accelerazione centrifuga.

Per cui il contraccolpo, applicato puntualmente negli ipotetici punti di attacco retta-cerchio e cerchio-retta, può essere "spalmato" con una funzione ad andamento lineare lungo lo sviluppo dell'elemento di transizione, rappresentato proprio dalla clotoide, in cui il centro di istantanea rotazione cambia punto per punto, facendo variare gradualmente la accelerazione centrifuga, sia nella fase di ingresso in curva che nella fase di uscita.

Come si traduce questo nella fase di progetto? Dettate delle limitazioni sul c_{max} e sulla v_{max} di percorrenza della sovrastruttura, ne derivano le limitazioni sul parametro A e quindi, per la $r \cdot s = A^2$, sullo sviluppo della clotoide stessa in base alla sua equazione reologica.

La questione sembrerebbe semplice, ma in realtà il vero problema dell'inserimento è costituito dallo scostamento iniziale tra cerchio e rettifilo.

Tale problema può essere risolto:

1) esprimendo l'equazione parametrica della clotoide in funzione dell'angolo al vertice τ, da cui

$$\tau = \frac{1}{2}\frac{s^2}{A^2} = \frac{A^2}{2r^2};$$

2) ricavando l'espressione della curva di transizione in coordinate cartesiane.

Si rende necessario definire *dx* e *dy* in funzione dell'angolo al vertice τ, risolvendo il sistema algebrico di coordinate del punto generico P appartenente alla clotoide con uno sviluppo in serie di Taylor ed arrestandolo al primo ordine che corrisponde ad un errore accettabile (ingegneristicamente parlando) dell'ordine del mm;

3) si arriva alla determinazione della grandezza fisica cercata, ovvero lo scostamento indicato con Δr.

Questo parametro è stimato attraverso la relazione

$$\Delta r \cong \frac{A^4}{24r^3}$$

arrestando lo sviluppo in serie di Taylor al primo termine.

Tutto questo, che pare un'elucubrazione astratta e poco pertinente, ha invece delle importanti ricadute nell'esperienza di uso della strada, quando la percorriamo guidando un veicolo qualunque.

Oltre al maggior margine di sicurezza che viene garantito sul singolo elemento (ma che tuttavia è già contemplato dall'esame del diagramma delle velocità del tracciato nel suo insieme), si nota che vengono soddisfatti dei parametri che possiamo porre in relazione con il lato "estetico" dell'esperienza di guida.

Si tratta della piacevolezza e della facilità di conduzione del veicolo, della tempistica dell'azionamento dei comandi, oltre che dell'assenza di ambiguità o disorientamento. Quest'ultimo aspetto è legato alla percezione e ai processi cognitivi che consentono di anticipare, programmare, eseguire e supervisionare tali operazioni nel loro insieme.

Il nostro S.N, i nostri recettori e analizzatori, il nostro apparato locomotore hanno delle loro caratteristiche strutturali e funzionali, irriducibili ad aspetti puramente concettuali. Se tali caratteristiche vengono rispettate e sollecitate in modo ottimale (anche tramite un'opportuna configurazione della sede stradale, appunto) questo corrisponde ad una esperienza piacevole

e gratificante.
Pur se tale aspetto estetico dell'esperienza conserva un *quid* difficilmente definibile, tuttavia possiamo trovare dei modelli matematici che lo esprimono.
Il primo è percettivo (ottico: visione della curva) e il secondo è motorio (velocità di sterzatura).
Viene definito "criterio di guida ottica", in cui per una corretta percezione della prospettiva stradale la curva di transizione deve possedere uno sviluppo minimo oltre il quale non si provochi disagio ottico, ovvero se la clotoide ha una lunghezza piccola, l'angolo al centro è piccolo e quindi è piccolo anche l'angolo di deviazione. Affinché una clotoide sia otticamente individuabile, l'angolo τ deve essere ≥ 30° nel punto di tangenza con la curva circolare. Questo implica che A≥(1/3)r.
Dall'altro canto se la clotoide è molto lunga non si riesce a percepire la presenza dell'arco di circonferenza. Vuol dire che si è portati ad arrivare su tale arco con velocità troppo elevata, da cui, limitazione superiore, A≤r.
Indicando con P il passo del veicolo (distanza tra asse anteriore e posteriore), definito con θ l'angolo di sterzo e con *r* il raggio della curva, si ha che

$$P=r\theta \Rightarrow \theta=\frac{P}{r},\ dove\ r=\frac{A^2}{s},$$

la variazione di θ con il tempo porta a definire

$$P = r \cdot \theta$$

$$P = \frac{A^2}{s} \cdot \theta$$

$$\theta = \frac{P \cdot s}{A^2} \Rightarrow \frac{\partial \theta}{\partial t} = \frac{\partial}{\partial t}\left(\frac{P \cdot s}{A^2}\right) = \frac{P}{A^2}\frac{\partial s}{\partial t} = \frac{P}{A^2} v$$

$$\dot{\theta} = \frac{P}{A^2} v$$

Questo ci dice la velocità angolare con cui muovo lo sterzo è proporzionale alla velocità lineare del veicolo e inversamente proporzionale al quadrato del parametro *A* della clotoide.
Questo modo di concepire, realizzare e usare un tracciato stradale trova una sua generalizzazione in alcuni casi più complessi.
Oltre che raccordare un rettilineo ad una curva o viceversa (da cui il nome curva "di transizione") si assiste a necessità ulteriori che la realtà ci presenta:
a) Raccordo tra due curve (con centri distinti) nello stesso senso e consecutive, oppure
b) Raccordo tra due curve con verso di sterzata opposto senza lo spazio sufficiente per interporre un rettifilo tra esse.
Nel primo caso si ha una "clotoide di continuità", mentre nel secondo si ha una "clotoide di flesso". Tra le due la più interessante è la seconda perché presenta un cambio di convessità e una schematizzazione geometrica affascinante per la disposizione dei centri di istantanea rotazione.
Questa nomenclatura ci permette di rileggere in chiave ingegneristica le strutture conchiliari. Possiamo prendere come esempi alcuni gusci di conchiglia. Sono particolarmente interessanti alcuni cefalopodi del tardo Cretaceo, il cui avvolgimento piano-spirale presenta molte e bizzarre variazioni di curvatura. Tali gusci, denominati scaficono, amiticono, baculicono ed appartenenti a diversi generi, hanno andamenti che "fanno spreco" di tali curve di raccordo.
Se poi si osserva la sezione di una valva dorsale di un Lamellibranco, genere Pectinidae, con guscio piano-convesso, si nota che la valva dorsale, approssimativamente piana, in realtà è concava nei pressi dell'umbone, in prossimità cioè della cerniera, mente è leggermente convessa nella parte distale. È presente quindi un flesso a tangente orizzontale, nella parte intermedia della valva.
Altro esempio di clotoide è dato dalla curvatura in proiezione sagittale dei condili femorali. Il raggio di tale curvatura varia e varia il centro di rotazione, in relazione alla fisiologia dell'articolazione, in particolar modo della tensione dei legamenti e della combinazione mobilità-stabilità del ginocchio stesso.
Se un approccio matematico basato sull'esame degli "stress pattern" sembrerebbe di maggior pertinenza, si è preferito l'esame strutturale perché il paragone con la progettazione ingegneristica (in questo caso stradale) consente pienamente di analizzare, per es., lo sviluppo elico-spirale di una conchiglia con altri strumenti che ci fanno meglio comprendere gli atti di moto assiali (rotazione, traslazione, espansione) infinitesimi che la generano e ce la fanno meglio rappresentare in 3D.
Ciò è possibile perché da un punto di vista matematico una figura non perfetta, la clotoide, può essere scelta come elemento di raccordo per lo sviluppo di due elementi perfetti (il cerchio e la retta), in quanto può essere intesa come un insieme infinito di cerchi con l'errore di approssimazione che tende a zero.
La riflessione ingegneristica, che l'esperienza e l'utilizzo ci porta a verificare, non aspetta più di constatare coincidenze a posteriori, ma si dà come criterio euristico primario l'esame della natura, in questo viaggio andata e ritorno che unisce il naturale e l'artificiale.
La stasi del mondo classico è definitivamente infranta, ma la scienza e, con essa la tecnologia, hanno nello stesso tempo lavorato per costruire un altro tipo di bellezza. Con i dovuti tempi si è capito che l'ellisse kepleriana, per es., non era solo una negazione delle sfere e dei cerchi, ma anche l'affermazione di forme nuove, diverse, animate da dinamismi fino ad allora sconosciuti, aperte verso orizzonti che in un primo tempo avrebbero spaventato. Oggi consideriamo "bello" un frattale, o la sensazione tattile e motoria offerte da un oggetto tecnologico ben realizzato, o un procedimento cognitivo e/o costruttivo: le frontiere dell'estetica hanno traslato quantitativamente e qualitativamente in modo e misura tali da essere impensabili nel XVIII secolo.

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