THE EFFECTIVENESS OF ZEEL AND NEW RESEARCH METHODS IN RHEUMATOLOGY

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SUMMARY

Purpose of this work is to prove the reliability of two instrument methods - normally used in studying crystalline structures - on the assumption that they could help in evaluating the structure of the cartilage, considering that the cartilage itself has a crystalline-type of spatial arrangement.

We worked with Zeel - a homotoxicologic remedy of proved therapeutical effectiveness in rheumatology, checking its effects on a progressively destructuring pathology. The pathology was artificially induced by the use of proteolytic enzymes, in order to obtain a series of samples at various degrees of destructuring.

Each sample was then accurately examined with x-rays, difractometric instruments and the results were reported on a set of predetermined scales.

The final results of this study have demonstrated that consolidated analytical methods can be successfully used in the bio-pathological field and the therapeutical effectiveness of Zeel as an anti-destructuring drug.

The experiments were conducted on a variety of samples, ranging from pathological condition to perfect health, in order to establish a precise methodical approach.

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The human locomotive system depends on a specific part of the connective tissue - called the hyaline cartilage - which covers the articular heads and has two main functions:

* To absorb the gravity load exerted on the surface of the cartilage
* To allow a smooth sliding movement of the articular heads (WOO et al,1987)

Both these functions can be performed thanks to the specific three-dimensional internal structure of the cartilage itself.

If we project the cartilage's structure on a perpendicular field, we can see that it is composed of the following different layers of tissues:

- Lamina splendens

- Superficial or tangential area

- Intermediate or reticular area where most of the chondrocytary lacunes and chondrocytes (specific cells of the cartilageneous tissue) are located.

- Deep vertical area with its proteoglycanic=collagenic components

- Tide-mark - or ondulation area - which separates the calcified cartilage area from the rest and where collageneous fibres are mostly cememented by hydroxyapathitis, the fundamental mineral component of the underlying subchondral bone.

Mineral infiltration over this deep area, generally has heavy pathological consequences, even though ipercalcification may occur also in the internal area.

The chondrocytes are the only cells scattered inside the cartilage and represent a tiny fraction of its total volume (usually less than 0.1%)

Despite this and their practically anareobic metabolism notwithstanding, they control the electrolytic homeostase of the fundamental substance, synthetize the collageneous fibres and they are in charqe of their correct structuring, repairing and spatially redistributing, in case of mechanic or bio-meta(cata)bolic injuries, suffered by the cartilage.

The chondrocytes are "plunged" in the fundamental substance, whose specific functions they manage and administrate, granting an effective and - above all - painless movement.

In case of osteoarticular pathology, the cartilage undergoes different stages of destructuring:

- Disruption of the intermolecular crosslinks of the collageneous fibres (constituted by idrossilinic tropocollagen. type II)

- Hydrolysation of the simplest components of the fundamental substance

* Glycosaminoglycans (condroitin-4 or 6 sulphate or cheratansulphate)

* Jarulonic acid which - together with the glycosaminoglycans - constitutes the proteoglycanic microstructure connecting the collageneous fibres(H MUIR, 1983) (2)

Therefore, the impossibility of proteoglycans to establish "soft" ligamentary interactions with H20 molecules and with the electrolytes dissolved in it (these interactions are pressure-dependant), causes pathology.

The articular pathology depends also on a number of other strictly biochemical events:

- Disruption of the established link between the jarulonic acid and the collagenic interlace, by galactose and galattosiglucose residues, on the surface of the collageneous fibres; this link can be easily disrupted by acid or basic pH variations; by an over-concentration of lymphocytokines (Interleukines, Interferon's, primary factors in the inflammatory reaction) and by pharmacological active principles such as FANS and other cortisonic drugs.

- Lack of H20 and electrolytes inside the cartilaginous fundamental substance, which is usually connected to a progressive increase of mineralization of a secondary type.

- Disappearance of disulphide bridges (-S= S -) generally present within the polysaccharides - constituting the jarulonic acrd - which link themselves to the "link-protein", in its turn connected to the above mentioned residues, typical of the collageneous structure; in this case, the destructive process is also due to an oxidative (or inflammatory) condition that succeeds in disconnecting disulphide links, usually stable in anti-inflammatory conditions.

- Death of the chondrocyte; this event sums up all kind of negative events concerning the cartilage.

All the above has been known for a long time and has been documented by an enormous international literature in the course of the past 70 years or more.

The purpose of this work however, is not to give a detailed list of this literature's references and therefore, it will be enough to mention the major work of Benninghoff A. 1925, a study by Schenk R. et al 1986 and finally one of the most interesting publications on chondrocytarv morphology, written by Stockwell R. 1978 (5)

On the other hand it should be pointed out that a general, objective, quantitative work that sums up the various, progressive pathological levels of or the cartilaginous structure, does not exist yet, as of today, in this field, there is nothing more than simple qualitative or at most, semi-quantitative descriptions. That is to say that an overall scale illustrating the various degrees.

of pathology ranging from maximum destructuring to perfect health - does not yet exist.

Such a scale however, would allow us to achieve two major purposes:

- Establish immediately and outside any personal and individual evaluation, the degree of the pathology

- Establish the effectiveness of any therapeutical agent by comparing the pathology degree before and after the treatment - once, of course, a proper scale has been established in the above-mentioned objective way.

We believe we have succeeded in filling this gap by implementing two different criteria of research:

- Determination of the birefrangence (or degree of the birefractive optical power) of the proteoglycanic structure, which can be estimated by an optical microscope with polarised light and crossed nicols; in order to determine this, it's enough to find the wavelength of the so-called interference colours.

- Determination of the intensity of the reaction (which can be estimated in photons "x"/S) of the structure, when submitted to a difractometric x rays investigation.
This is possible because the articular cartilage has a crystalline three-dimensionality which means it's a structure which repeats itself in space and is in many ways similar to mineral and organic (also synthetic) crystals and can therefore be investigated in the same way.

The birefractive values can vary - according to the level of the pathology - from a minimum very close to zero ((: 0,005, corresponding to a wavelength perceived by the human eye as grey of the first order on Michel Levy's table - see Table A), to a maximum of (: 0,022 (orange-red of the second order on Michel Levy's table).

These values are calculated on the degree of the "delay" induced by the cartilage examined under a polarised light spreading between 200 and 1000, max 1100 nm.

To explain it better, we could say that if from a structural point of view the cartilage is not comparable to crystals, when observed under polarised light (and crossed nicols), it would appear completely black (total extinction of the investigating light) and this usually happens to glass and other amorph not opaque substances (that can be penetrated by light) - that is in absence of a spatially repeatable structure, equal to itself.

The presence of any colour brightness during experimental investigation, shows the passage (despite the orthogonal nicols) of one or more wavelength of the polarised white light. This happens because of a displacement of the exit and a delay of such wavelengths, compared to the others which continue spreading themselves and - in exit - are finally extinguished by the perpendicular position of the nicols in orthoscopic investigation.

The delay induced by the investigated substance, allows the observation of such wavelengths, which therefore represent the measurable basis of the birefractive phenomenon correlated to the examined structure. In other words if there is a delay, there is a structure; the first is a consequence of the second and varies according to its variability.

The physicians explain all this by saying that propagation of light's rays in glass becomes larger and larger in time, while in not opaque crystals (In this case not belonging to the monometric cubic system), it forms an ellipsoid of propagation with 2 or 3 axes, whose crushings - as to an ideal sphere - are closely related to the delays of the colours' wavelengths observed under the microscope; these crushings will reach their top along the minor axes (or axe) of the ellipsoid.

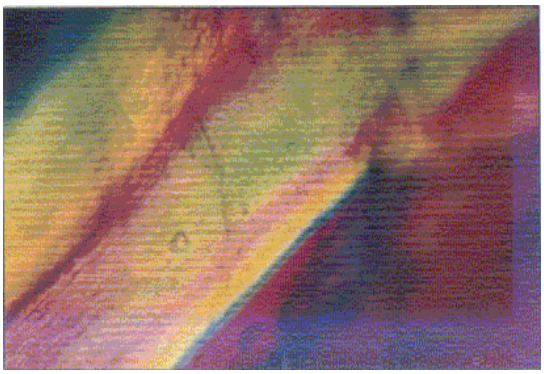
The birefrangence's indexes are related to these crushings
Serious pathologies (destructuring = invalidating) correspond to low birefractive indexes.
High birefrangences indexes instead, indicate that the cartilage is well structured (functional).

Picture 1 shows an example of seriously damaged connective tissue with
residue areas at higher optical value, which means with higher articular functionality.

Picture 1: *The wide white or dark grey area shows birefractive indexes inferior to 0.005. The residual interference colours show the presence of isolated nodules of cartilage containing a sufficient concentration of electrolytes. Notice the fraying of the collagenous interlacing which tends to come out of the fundamental mass, reduced to a semi-incoherent state. These collagenous fibres are more resistant than the latter to structural degeneration. Observation under polarised light with analyser.*

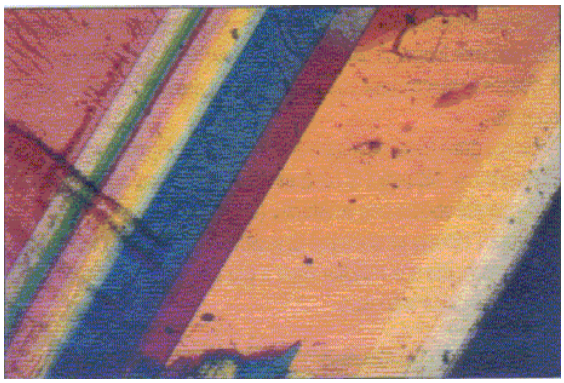
Picture 2 shows a well structured cartilage still capable to respond in the best way to the input received: we notice high birefrangence indexes' values (widespread interference colours, corresponding to high delay indexes in nm.) 8 max 0,020)

Picture 2 *We can see a splendid sequence of emission from the crystalline structure, with all the colours' range of the first and second order of Michel Levy's table. Delays from 400 to over 1100 nm. 8 max=0.022. Observation under polarised light and with crossed nicols.*

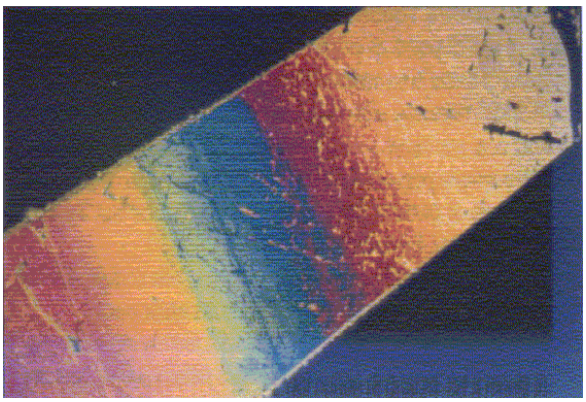


It's interesting to notice the perfect match between the birefractive crystalline structure of the examined anatomical tissue with one of the two different "measurement units" normally used in the course of scientific observation of natural mineral crystals. As we pointed out, the simultaneous presence of correlative phenomena gives us the chance of using the same investigating method. The correlation is quite in evidence in Picture 3 and 4.

Picture 3. *Section of calcium sulphate bihydrate germinated (that is, constituted by a number of small crystals having the same spatial orientation). The special internal constitution of the slant cut multicrystal (as to the observation point) allows the surfacing of all interference colours from the first to the subsequent visible orders of Michel Levy's table. (b max 0.030) Observation under polarised light and with crossed nicols*



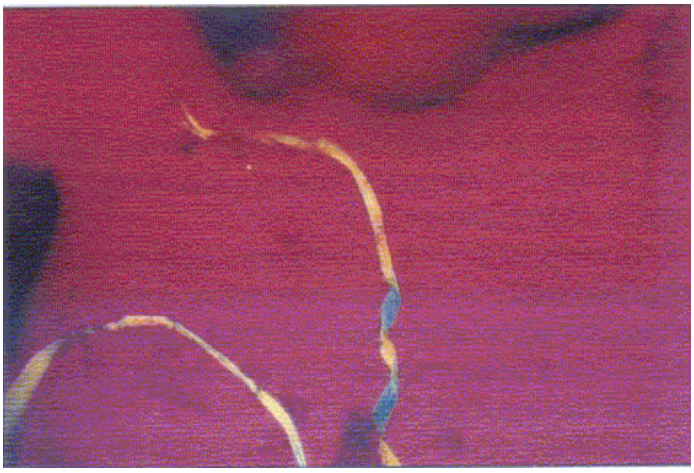
Picture 4. *Observation under polarised light and with crossed nicols, of a quartz wedge. In this case, the different thicknesses from one point to the other, of the same substance, helps the surfacing of the different interference colours. However, we still get the same arrangement of the order of the interference colours, typical of the variability of birefrangence indexes.*

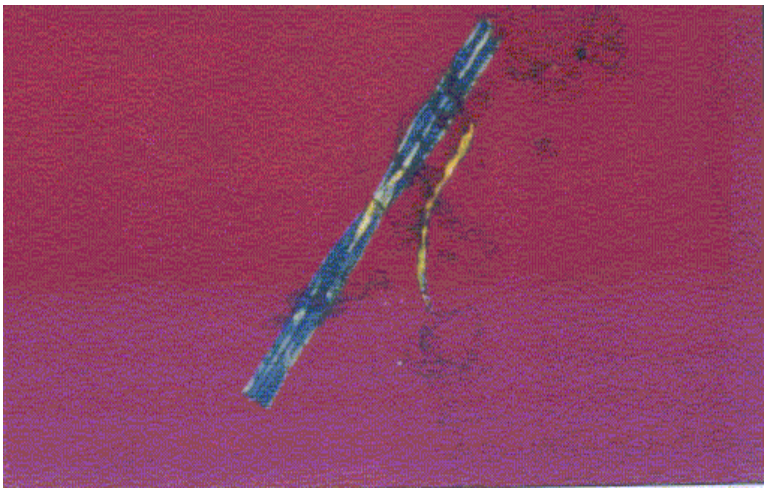


Another considerable discovery made in the course of this research was the observation that even isolated collagenous fibres - as the proteoglycanic fundamental mass inglobing them - usually behave in the way we have already noticed in Pictures 1 and 2 (as to the cartilage as a whole, see Pictures 5 and 6).

It's therefore possible with these analytical methods, to carry out a quantitative objective research also on the specifically collageneous pathologies.

Picture 5. *Enlargement of a collageneous fibre taken from the fundamental mass: the fibre appears crushed and wrapped up in itself. The screwing areas show places of lower resistance where disruption usually happens perpendicular to the fibre. The fibre shows an important optical feature the delay induced by the investigating light, changes depending on whether the light crosses the structure from top to bottom or vice-versa. This indicates an iso-oriented type of structure and therefore an adaptable answer to mechanical stress (distress). Observation with crossed nicols under polarised light with the addition of retarding lamina of 1/4 wave.*

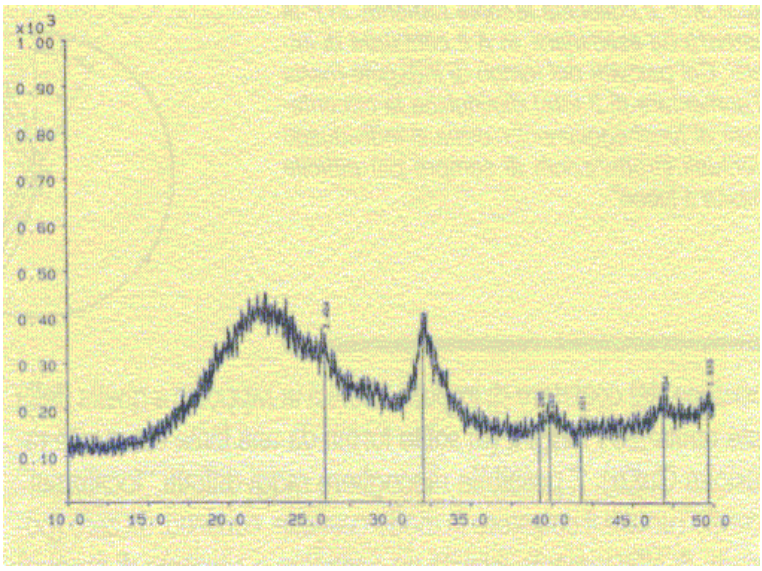




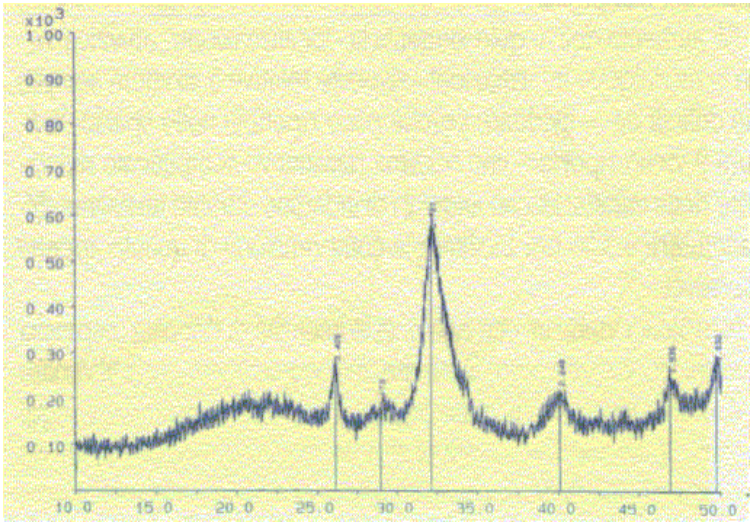
Picture6*Transversal disruption of the collageneous fibre. Fibrils of inferior order come out from the isolated stump and show birefractive indexes considerably different from those of the stump itself. This phenomenon indicates that the inner structure of the collageneous fibre has been disrupted. Observation as in previous Picture.*

The second research method - the difractometric one - has proved to be up to giving even more subtle and interesting results, as it was possible to focus on the structural layers of the elementary cartilaginous asset. We could say that we have been able to investigate the similar elementary cells of crystals either natural or synthetic.
Pictures 7 and 8 show two examples of difractograms obtained from cartilaginous samples taken from different layers of the tissue's differential sequence, as mentioned before.

The first one- calcified - (Picture 7) is from the deep cartilaginous are: the second (Picture 8) from the superficial area and lamina splendens, which is usually de-mineralised.



Picture 7. *From peak of 3,424 value onwards - that is to the right side of the graphic - there is a series of intensity peaks due to the physiological presence of a mineral fraction which permeates the depth of the cartilaginous tissue. The collageneous proteoglycanic component instead, is shown by the Gauss line on the left of the mentioned peak. The Gauss line in itself does not have a great structural meaning, but its intensity index (height of the same) shows a remarkable - yet normal - collagenic presence, with an internal structure which is not casual, since on the right side of the curve, the mentioned 3.424 peak appears. Later on we will refer to this peak as Peak "D" (see Pictures 16 and 17).*



Picture 8. *In this case the mineral component is not only in excess, but "out of place ", since the sample is a superficial one and therefore not in contact with the subcondral bone. It shows, on the left of peak 3.406, a collagenous residue (to be correlated with Peak D). The low density of the related plateau shows its pathological regression. Luckily, there is a certain degree of internal structuring given by the peak on the abscissa 26.5, but we do not believe it could be enough to grant the normal functioning of the cartilage, since it is associated to very high intensity indexes due to a sclerotic cartilage.*

We have been able to illustrate - thanks to the graphics - a first case of good internal structure of the sample and a second case of increasing pathology.

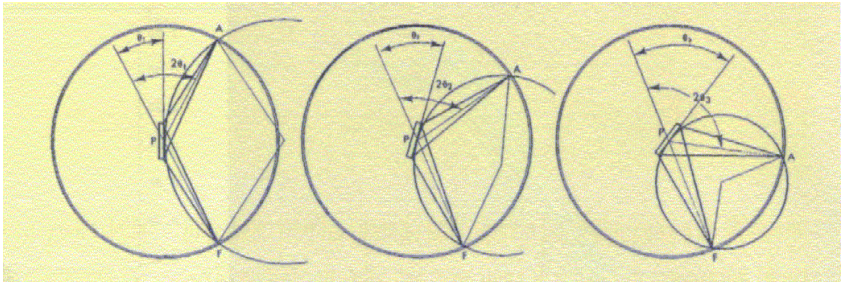
We now have to explain what shows on the two difractometric axes, that is which are the variables that must be taken into account in order to find the position (abscissa) and the intensity (ordered) of the peaks and of the plateau. we have just interpreted.

What we are about to say can be applied also to the following difractograms even if they were obtained with an equipment different from the one used to illustrate Picture 7 and 8: on the abscissa are indicated the angle indexes (called 2 theta), corresponding to the position of the photonic impulse' meter, compared to that of the x-rays source (generally coming from a CuKx radiation source). It's possible to gather "focused" diffracted rays, if we continuously rotate the meter from zero (alignment starting position of meter and x rays source), to a maximum of 2 theta, that is 180° degrees. Practice shows nevertheless that it is sufficient to work on an angular range. going from 1 40°-50°. Simultaneously the sample should be rotated (in the same direction as the meter) at a theta angle (half of the angle of the meter), so that - over time we can get differential information from the structure.

With a theta angle = zero, the x-rays are parallel to the sample that is therefore not hit by them and does not give any information.
With a theta angle = 90° (2 theta = 180°), the x-rays cannot diffract.
With a theta angle between 1 ° and 89°, we can observe the whole structural story of the sample.

Increasing the angle, raises the analytical focus, which means that we can gather structural information and answers increasingly difficult to find because scarcely repeatable in space.

To illustrate more clearly the meaning of the above, in Picture 9 we illustrate a simplified resuming scheme of the variations of the angular investigating features in the course of a difractometric examination.



Picture 9. *The radiating source is located at point F; the substance to be examined at point P; the impulses meter at point A. Over time (and increasing 2 theta), the focus field becomes smaller and we can then observe structures usually quite difficult to focus.*

In the ordinate instead we report the intensity of the reaction (expressed in photons x pro second), to the variation of the theta angle - that is to the variation of the angle with which the sample is hit by x-rays which will in turn - be partially or totally diffracted according to the three-dimensional features of the sample.

The value of theta angle 2, is related to the theta one, which in turn expresses in degrees the angular value between the investigating radiation on a structural cartilaginous surface (on which proteoglycans, jarulonic acid and collagenous fibres are placed) and the diffracted x-ray in exit.

The degree of intensity of the answer - on the ordinate - its expression of the perfection (or imperfection) of a certain architectural structure inside the sample and responsible for the diffraction.

Therefore, a high intensity level (that is a well pronounced peak) is equivalent to a well constituted specific and not casually organised structural surface, since it repeats itself parallely in space a certain number of times for each measurement unit of extension (A or others).

Therefore, a high intensity at a low 2 theta angle indicates the presence of a feature that easily and quickly repeats itself in space. A high intensity at a higher 2 theta angle indicates the presence of a well defined feature which appears only from time to time: it indicates in fact the low structural density of the examined sample

The articular cartilage featuring a high 2 theta angle will clearly resist to the injuries of a pathology much better than one with low angle degree.

But it is also true that such a feature is of little help from the functional point of view. Features that can be noticed at a low theta angle are much more useful but they are also the first to disappear in case of cartilaginous pathology, because they are more ubiquitary.

It is therefore clear that through a great number of difractometric investigations and birefractive measurements (and wave's delays measurements), it is possible to establish a reference scale (even a double one), ranging from the highest pathology level to a state of health (or close to it) of the osteo-articular cartilage.

Such scale would allow to find objectively the exact condition of any examined tissue and - whenever possible to examine a further sample of the same tissue - to evaluate the therapeutical effects of any drug administered between T1 and T2 moment.

To check these two investigating options, we used the following experimental methods:
- Sample of cartilaginous tissue taken from an animal (bovine), with a high structuring value (integral cartilage)

- Difractometric and birefractive investigation of the tissue, establishing its objective condition

- Subdivision of the remaining cartilaginous tissue in twelve samples having exactly the same weight of 50 mg each.

- Dividing the samples into two groups of six samples each, called Group A and group B

- Adding to the samples of group A, a solution containing jarulonidase metalloprotease and collagenase at 0,005% (50 y/ml) in a slightly acid milieu (pH = 6,5) tamponed with 5 ml of a phosphate solution. With regard to enzymatic degradation of the cartilage, we refer to the fundamental work of Barret A.J., 1975 (6)

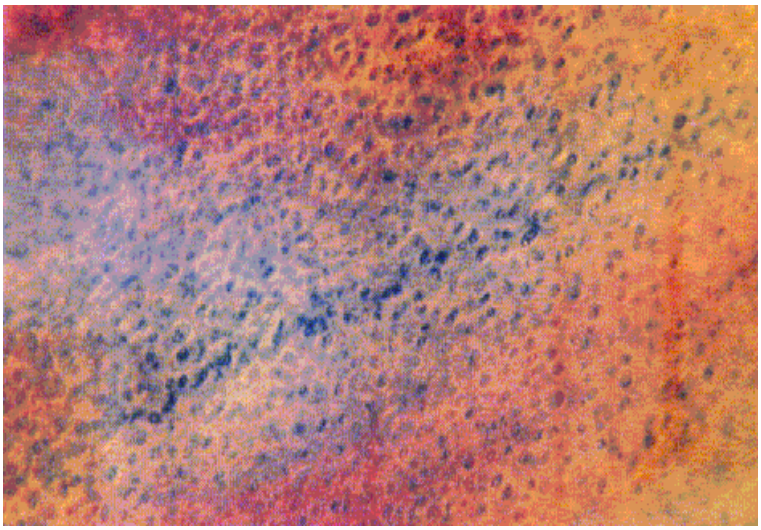
- Adding to the samples of group B 5 ml of desegregating solution at a double concentration and about 5 ml of Zeel, product of the pharmaceutical firm Heel (2 ampoules of 2,2 ml); this way the concentration of proteolytic enzymes was close to that of group A.

- Placing all the twelve samples (group A and group B) in a thermostatically controlled environment at 38 C , every day we took a sample from each group and examined it under an optical microscope at normal light and then eventually we submitted them to the instrumental investigation with the above mentioned methods.

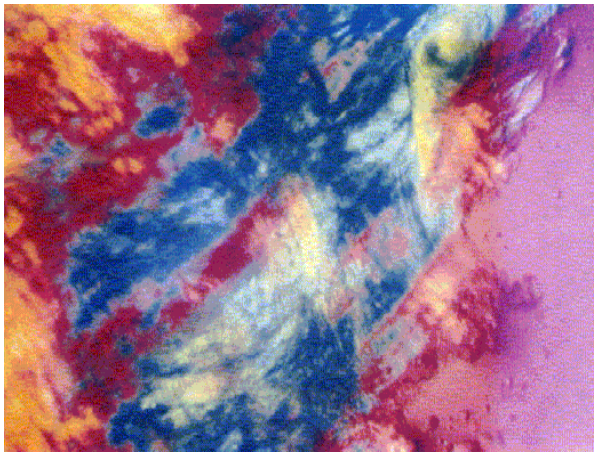
The maximum incubation time was six days.

RESULTS

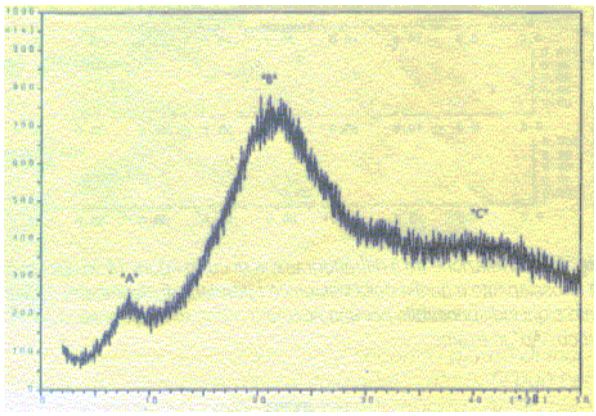
After the first 48 hours of incubation in the desegregating enzymatic solution, the samples of group A presented the features of total destructuring.
This did not happen to the samples of group B: even the last sample of group B on the sixth day of incubation showed signs of structural recovery, if compared to samples of the same group examined on the second day. All the above is supported by the following pictures :



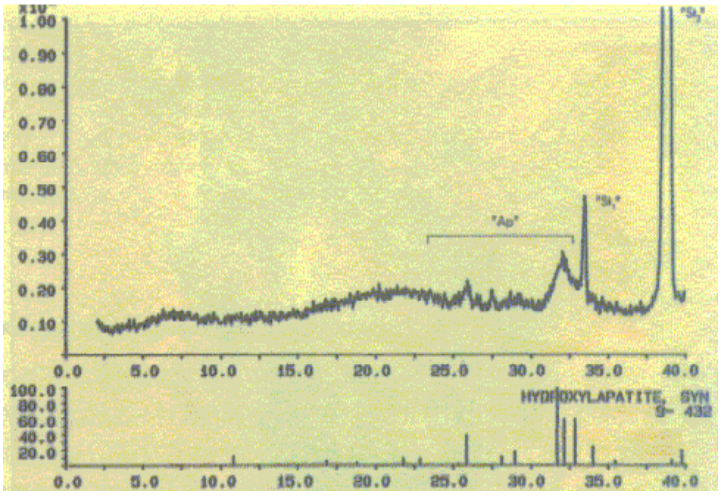
Picture 10. *State of the original cartilaginous tissue. Observation by analyzator. It shows a perfectly integral cartilage, filled by chondrocytary structures.*



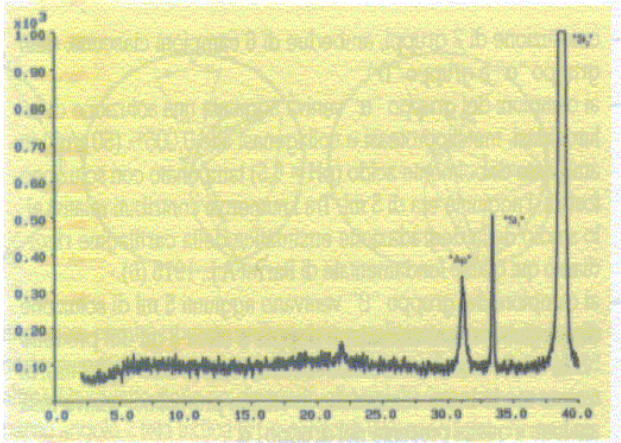
Picture 11: *Observation under polarised light with crossed nicols (with addition of 1/4 wave retarding lamina) of the integral cartilaginous sample before any addition. It shows a wide spreading of interference colours up to the maximum stage of green of the second order of Michel Levy s table equal to a middle-high level of the birefrangence index :*
b = 0, 01 7/0, 018



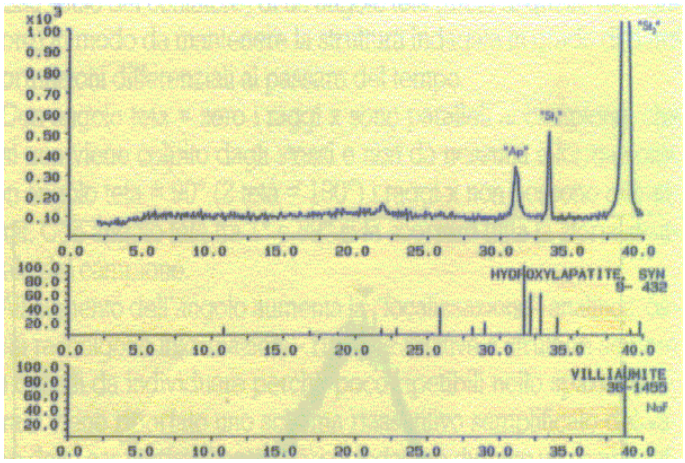
Picture 12: *Difractometric graphic of the cartilaginous sample before any treatment: plateau A, B and C - typical of a cartilaginous tissue with a perfect articular functionality - are well in evidence. Plateau A shows a remarkable structure, repeatable in space at a high frequency; plateau B also shows well marked structural features, but placed at relatively greater distances than those of plateau A; Plateau C is often not noticeable in the course of investigations, since it indicates the presence of a feature that reproduces itself parallelly, at high relative distances, multiple of those defining the intensity of A and B. This is the reason why this is the first feature to disappear, even in case of low pathological destructuring (possibly due also to the normal physiological ageing process of the tissues). Furthermore, notice the total lack of secondary apathitic mineralization peaks, which often concerns the organic component of the cartilage. Compare Picture 12 with Pictures 13, 14, 15, 16, 17, 18 and 19.*



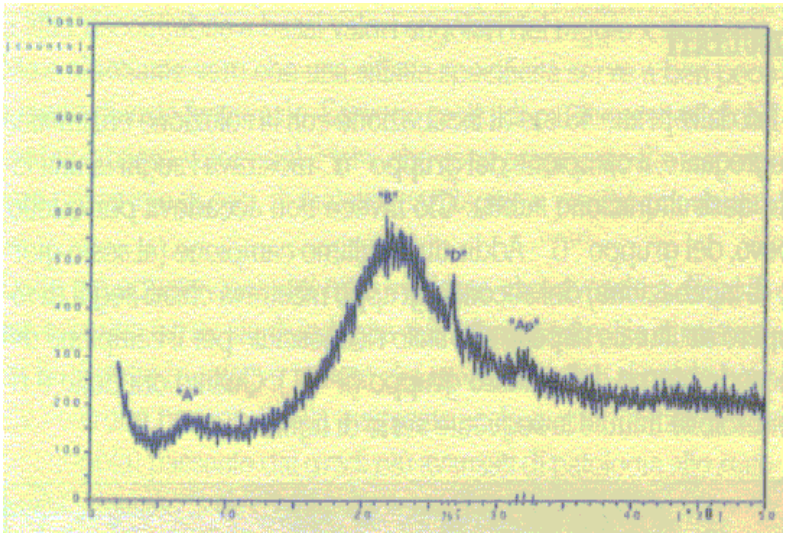
Picture 13: *Example of a difractogram regarding a very compromised sample, showing signs of an additional apathitic mineralization (it's almost flat). The apathitic peaks have been identified through a specific alignment comparison standard (idrossiapathitis).*



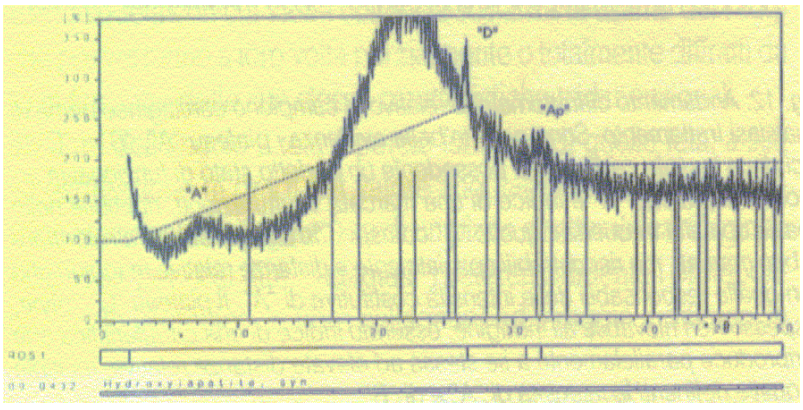
Picture 14: *Practically flat graphic of a difractogram of a completely destructured cartilage sample of group A at the second day of incubation with proteolytic enzymes. In this case, the only remarkable peak - beside the two on the right, called St l and St2, regarding a standard comparison: NaF, viliaumitis - is the one corresponding to a secondary apathization (peak Ap), which is equal to a complete sclerotization of the very low residual functionality. For the optical characteristics of this sample, see Picture 20.*



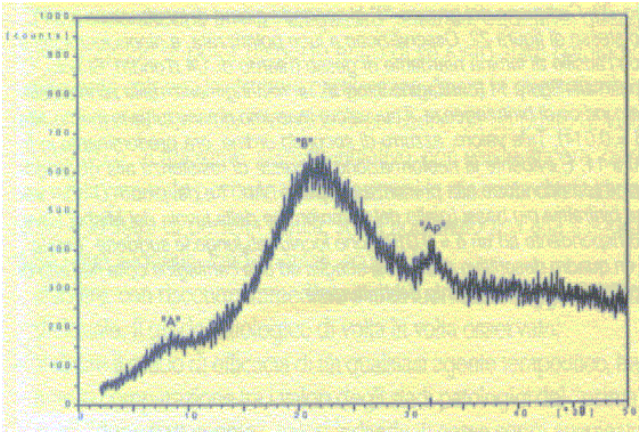
Picture 15: *Comparison between the difractogram of Picture 14, the idrossiapathitis and the viliaumitis spectrum (standard reference). The latter is here indispensable in order to get an accurate identification of the examined Ap peak.*



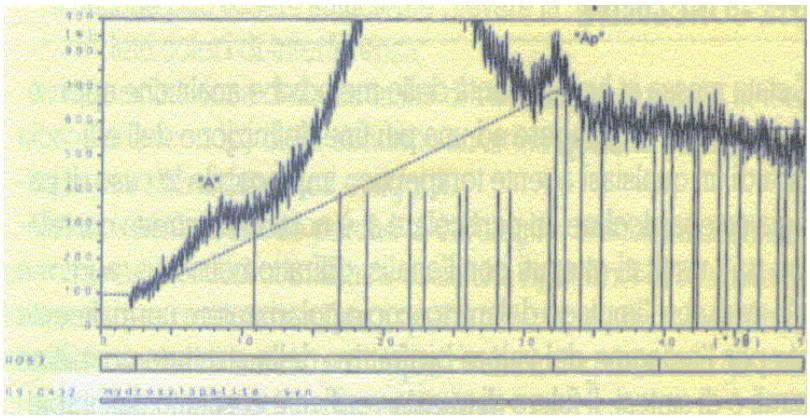
Picture 16: *Difractometric graphic of a cartilageneous sample (belonging to group B). incubated for two days at 38° C. with a disgregating solution and the addition of Zeel. Compared with Picture 12, we can notice an overall reduction of plateau A, B and C. This reduction is high for plateau A (more ubiquitary features), less high for plateau B (fewer ubiquitary features) and very high for plateau C (almost disappeared). The disappearance on plateau C is probably due to the vanished interactions between the structural features corresponding to A and B. Furthermore the presence of peak Ap indicates apathitic mineralization (presence of phosphate tampon). Finally it is of the utmost importance the appearance of a high D peak, which demonstrates the restructuring attempt made by the cartilage submitted to enzymatic distress. The microscopic and birefractive characteristics of the sample observed with the difractometric technique are shown by Pictures 22 and 23.*



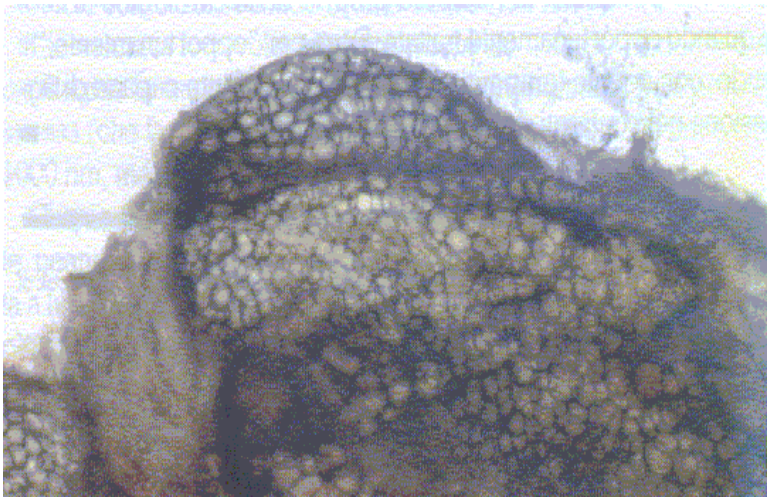
Picture 17: *We are certain of the statement made about Picture 16, because that graphic compared with the apathitic one above shows a full correspondence between peak Ap and the standard characteristics of the mineral and besides - there is an absolute lack of correspondence between the latter and peak D.*



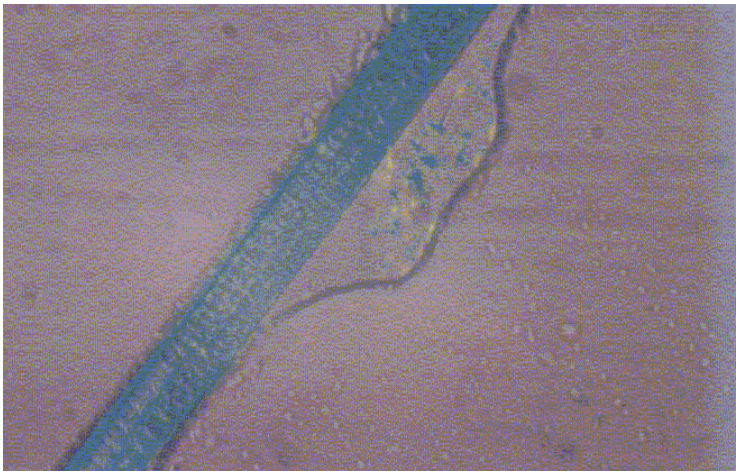
Picture 18: *Difractometric graphic of the cartilaginous sample of group B, on the sixth day of incubation at 38° C. with desegregating enzymes and the addition of Zeel. We can notice a further small decrease of plateau A, a considerable growth of plateau B, the disappearance of plateau C and the growth of peak Ap. That is to demonstrate that there was a resistance and/or a recovery effort against the desegregating input. The optical features of this sample are shown by Pictures 24 and 25.*



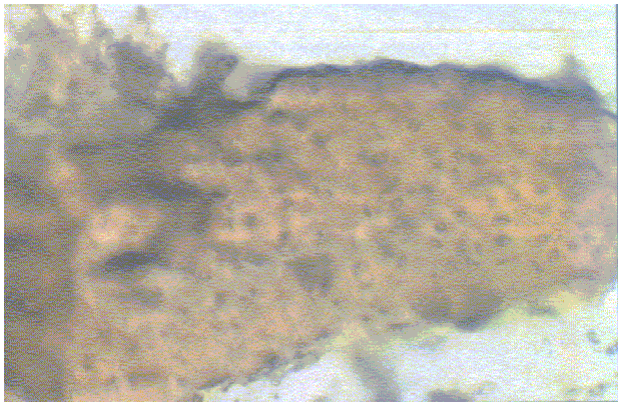
Picture 19: *Comparison between the graphic of Picture 18 and the idrossiapathitis one: peak Ap is clearly in evidence; it is the same peak previously described in Picture 17 and considered to be present because of the phosphate tampon used in the experimental scheme. The disappearance of peak D is due - in our opinion - to the overall growth of plateau B.*



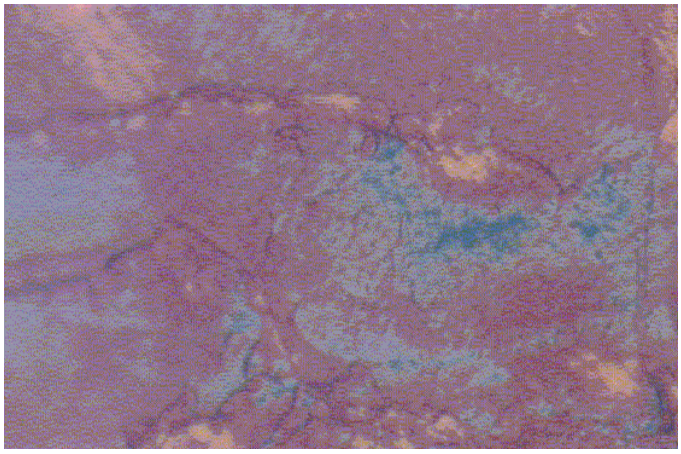
Picture 20: *Observation under microscope of a totally compromised cartilaginous sample whose difractogram (flat) is shown in Picture 14. It has been possible to observe this enlargement under polarised light and with crossed nicols. We can observe the general condition of the collageneous fibres lacking the englobing fundamental substance. The stiffening of these fibres is certainly helped by the presence of encrusting idrossiapathitis, as it can be observed in the difractograms of Pictures 14 and 15.*



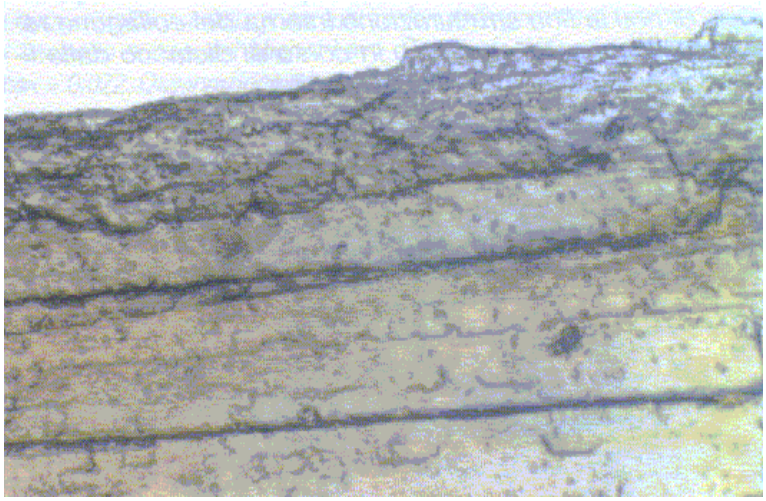
Picture 21: *This is the same sample of Picture 20, still more enlarged and observed under polarised light and crossed nicols, Notice the thin internal structuring of residual collagen and the apathitic "dust" about to detach itself from the fibre, under selective erosion process.*



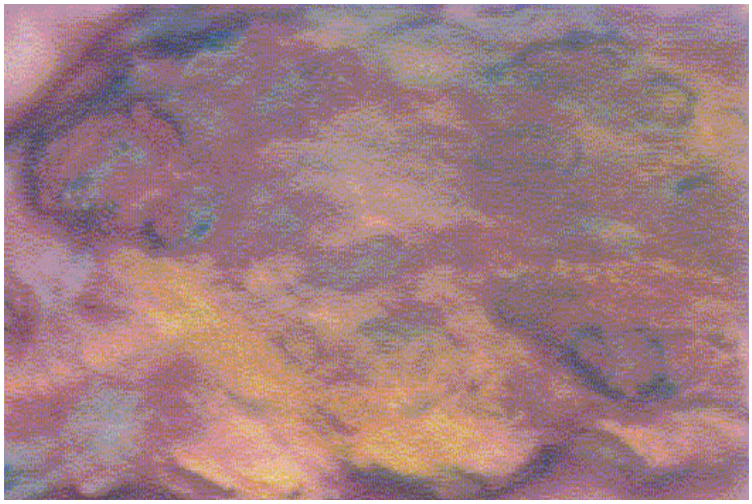
Picture 22: *Sample of group B on the second day of incubation at 38° C, observed only by the analyzator. The beginning of cells and tissues disgregation is quite evident. At the same time there is evidence of areas of stronger resistance, with a granular structure. Compare with Picture 10. For difractometric characteristics see Pictures 16 and 17.*



Picture 23: *Sample of group B on the second day of incubation. It's the same sample of picture 22. Observation under polarised light with crossed nicols and with the help of a chalk retarding lamina (delay of 1/4 wave). Compared to Picture 11 (integral cartilage), we notice the narrowing of areas with high biref2ngence index, whose maximum value however is middle-high (b= 0,0f4). This value, clear blue of the second order, was predominant in Picture 11. The new formation of peaks resisting to disgregation is quite clear (to be related to the presence of peak Ap? Or to peak D?). The lowest birefractive values (yellow of the first order on Michel Levy's table, corresponding to b = 0,010), are located along the so-called peaks. This is a view of the cartilage fighting back a strong destructuring agent.*



Picture 24: *Sample of group B on the sixth day of incubation at 38° C. Observation under an analyzator. Compare with Picture 10 and 22. There is no sign of deliquescence; there is - instead - a considerably iso-oriented destructuring with strong resistance areas in parallel preferential directions and some erosive pits. difractometric characteristics are illustrated by Pictures 18 and 19.*



Picture 25: *Sample of group B on the sixth day of incubation at 38° C. This is the same sample shown in Picture 24, results were observed under polarised light, with crossed nicols and with the addition of a retarding lamina of 1/4 wave. Compare with Pictures 11 and 23. There are a few scattered areas with high birefractive values, "plunged" in an optical milieu with lower interference colours. The increased nodulization of the sample is certainly due to the growth of peak Ap (see Pictures 16 and 18). Notice the presence of circumscribed erosive pits (see also Picture 24) some of which with high birefrangence indexes. All this shows that the cartilage is in a considerable state of suffering but that it has not yet lost its functionality.*

CONCLUSIVE NOTES

In the course of this work we have evidenced the value of the implemented ~ analytical methods in order to obtain a more precise definition of the clinical effectiveness of any therapeutical agent administered in case of osteo-articular pathology.

In particular we have pointed out the close correlation between the results obtained with the use of difractometric x-rays investigation and those obtained by using the polarised microscope, normally implemented to evaluate the Birefractive indices of natural or synthetic crystalline structures.
The main point of this work was the use of Zeel as an anti-destructuring drug for the cartilage.

Zeel has not only proved to have a remarkable effect on limiting the structural damage (experimentally induced by a mixed proteolytic solution), but also to be capable of inducing a partial internal restructuring of the cartilage, making it possible to maximise the natural recovery capacities of the articular cartilage when properly treated.

"Properly" in this case stands for and makes reference to a therapeutical agent intrinsically unable to attack the osteo-articular tissue.

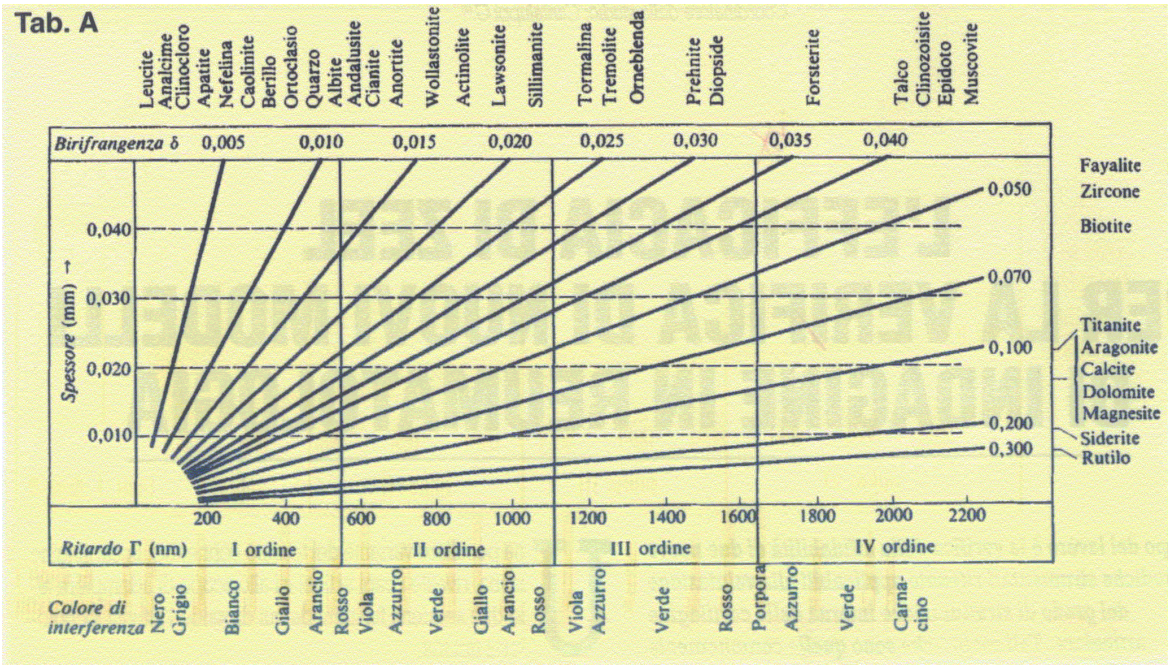


TABLE A

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