Crystallisation workshop

By MARCEL COCUDE (Paris, 06/22/1998)

SOMMAIRE

French Ministry of the Economy, Finance and Industry

Secretary of State for Industry

Commission for Scientific and Technical Research on Safety and Health in the Extractive Industries

OVERVIEW (Marcel COCUDE[1])

Mr. Cocude, President of the CORSS (Commission for Scientific and Technical Research on Safety and Health in the Mining Industries - *Commission des Recherches Scientifiques et techniques sur la Sécurité et la santé dans les industries extractives*) **.**

Can the crystallisation of Copper (II) Chloride in the presence of additives be used for early diagnosis of illnesses and evaluation of food quality?

It is well-known that the crystallisation of salt solutions, the solidification of molten metal baths and the electrolytic deposits of metallic salts are strongly affected in terms of shape and consistency by the presence of impurities — even in extremely small quantities. In the same manner, crystallisation of CuCl2 solutions is highly sensitive to the presence of additives, even in small quantities.

The elimination of impurities can be an important goal inasmuch as they affect the quality of the final product. On the other side their presence, which is often difficult to detect and even more difficult to measure, could be determined by examining the result obtained at the end of the crystallisation process.

The particular sensitivity of Copper (II) Chloride to the presence of impurities has been used through the “sensitive” crystallisation method to demonstrate the specific effects of additives of many types on crystallisation.

We can assume that **biological extracts contain information concerning the state of health of humans and plants and that, through their influence on crystallisation, we can evaluate this status and consequently** identify recognised or latent pathologies.

This was the approach used by the founders of the sensitive crystallisation method such as Pfeiffer in the 1930’s in Switzerland and Germany. Since that time, researchers, physicists, biologists, have been

studying and trying to codify the crystallisation of CuCl2 solutions containing biological additives such as vegetable juices or human blood.

The medical approach

The German-language authors presented the correlation between certain diseases and the patterns of crystallisation and went on to establish a semiology by which certain shapes were labelled hepatic, pulmonary, etc.

This approach was used in France to determine whether crystallisation could be used to predict the further appearance of pneumoconiosis while still at a pre-clinical stage. Once this disease becomes apparent, a process which is irreversible with the currently-available treatments, has already begun. Detection before this stage would therefore make it possible to take preventive measures. A study carried out with coal miners yielded encouraging results. Other similar experiments are now underway.

Agro-industrial application:

The aim is to determine a product's "quality" or its biological compatibility with the human organism. This goal is very ambitious and more for the future than for today. We suggest a more limited one which is to determine whether crystallisation can be used as an indicator of a product's origin and of the treatments it has undergone driving processing and storage up until its purchase by the end consumer. Experiments now being carried out in various areas will allow us to go further with this idea which still remains a hypothesis at the moment.

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Crystallisation research was initially confined to the German-speaking countries where it began.

It spread further within Europe and then to Japan and other countries.

The time has come to compare the results of these experiments and to see whether CuCl2 crystallisation can be used:

- in medicine, as an indicator of health risks.
- in the agro-food industry, as an indicator for classification of food products with no, or very little, risk of error.

This is the subject of the conference organised by the Commission for Scientific and Technical Research on Safety and Health in the Mining Industries.

Editor's note: The various articles briefly summarise the papers presented at the conference.

I. SCIENTIFIC BASIS

I ² SCIENTIFIC BASIS

From germ to tree, growth and form of out-of-equilibrium ramified patterns

(Vincent FLEURY)[1]

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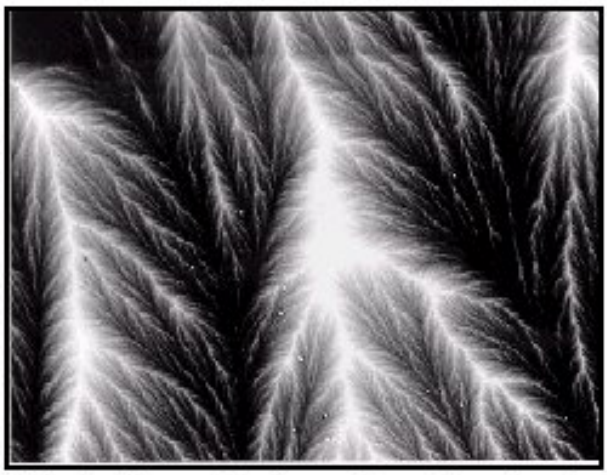
Abstract

It is well known that the growth of crystals results, when the growth is slow, in well definite macro-crystalline forms (e.g. Quartz). These forms may, in some cases, permit the identification of the basic crystalline grids. During crystallisation the microscopic information can be transmitted to the macroscopic scale. Rapid growth, out of equilibrium, leads also to particular patterns : dendrites, fractal trees, spherulitic form. When the growth is out of equilibrium, the concept of crystalline form makes no longer sense ; a better term would be «texture». The texture represents the crystallisation geometry as a result of polycrystalline rapid growth which can show a scale organisation (e.g. tree-pattern).



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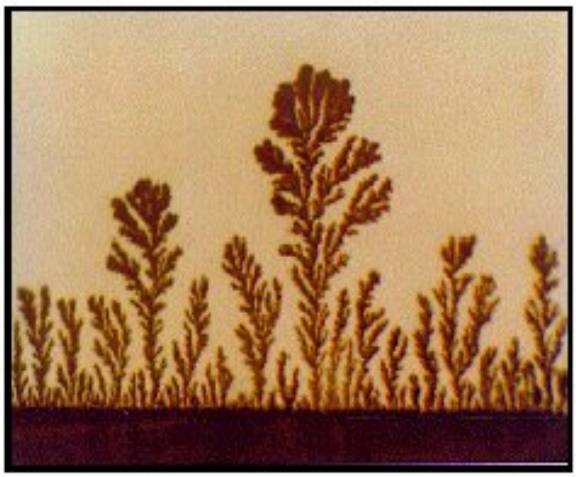

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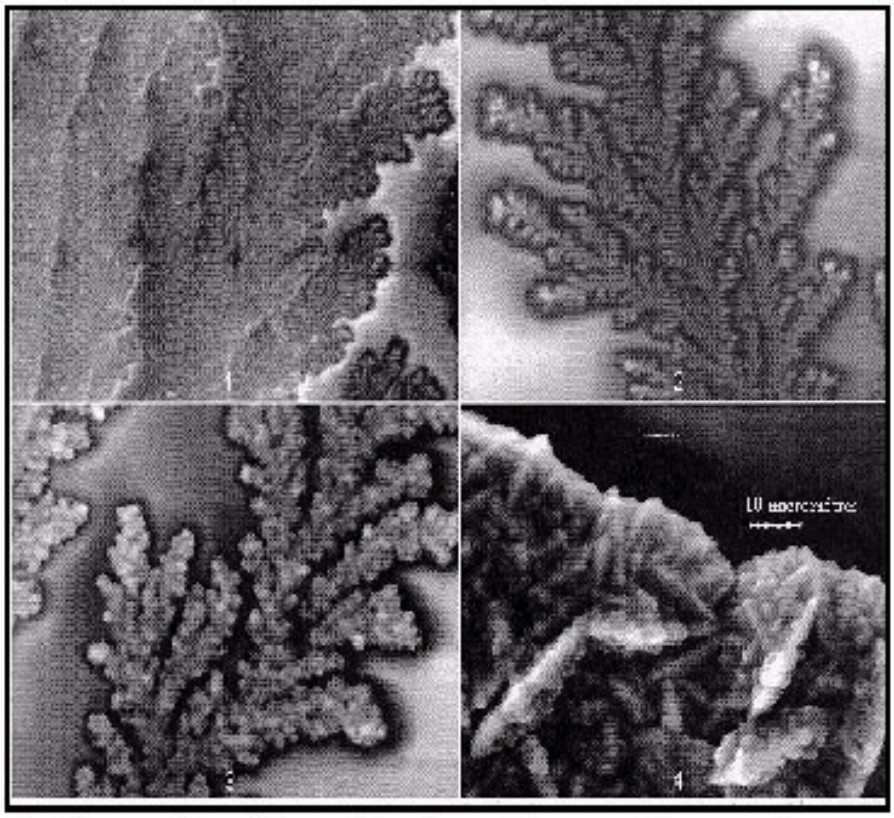
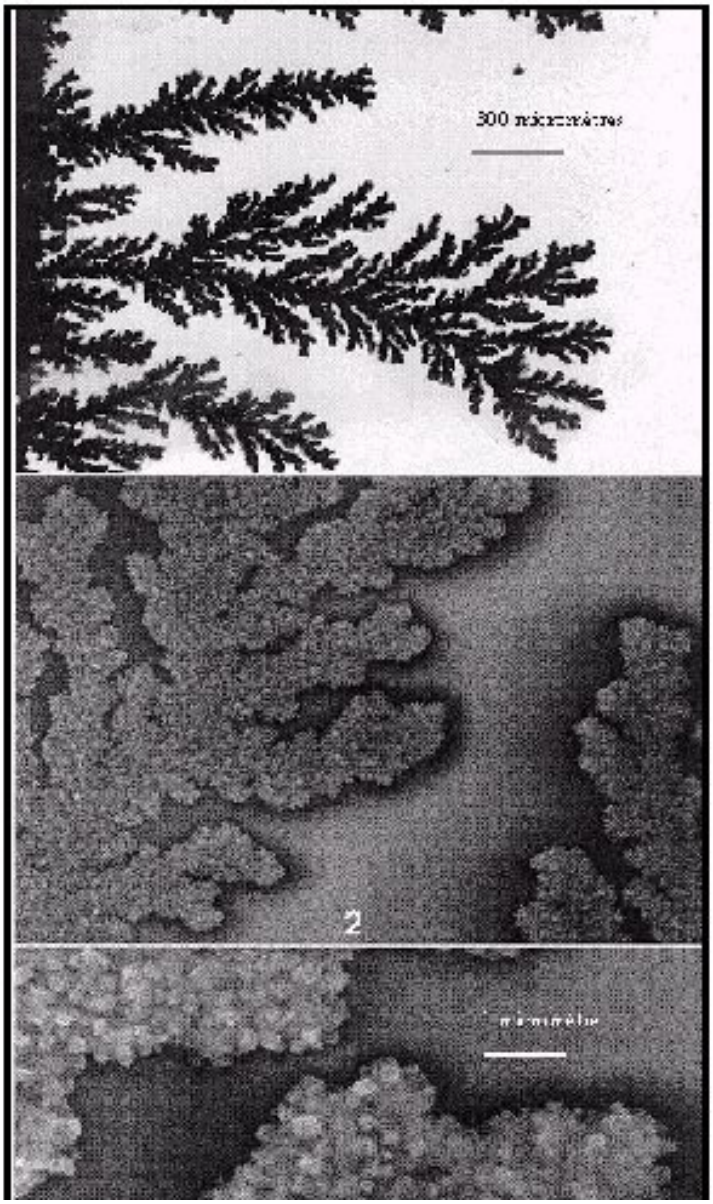
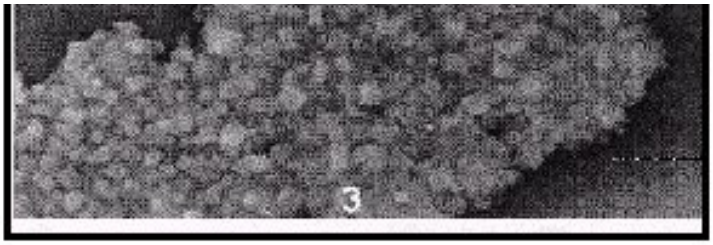


Texture fonction de la vitesse de croissance

Recent research gave us a better understanding of the link between the microscopic phenomena and the whole macroscopic form. We can observe that very different mechanisms lead to identifical forms. This is the result of *universality*, a statistic characteristis of critical phenomena. Universality stipulates that, on a large scale, certain details of the growing mechanism become unimportant : an electrochemical tree will look like a liquid tree or a colony of bacteria. In this sense growth is not sensitive to details of the process : we can say that some parameters are *unessential*. On the opposite we may have, under very similar growing conditions, very different patterns by varying an *essential* parameter. In this case the large scale pattern is changed, and there will be a macroscopic «revelation» of a microscopic part of the process.



We will show a panorama of these crystallisation phenomena very far from equilibrium, which leads to a quick generation of complex macroscopic patterns. We will insist on the connexion between the nuclear mechanism and the transport mechanism out of equilibrium, which leads to patterns dependant on certain details of the microscopic, molecular, mechanism. In principle it should be possible to use these mechanisms for revealing the presence of additives or biological interesting molecules



Agrandissements successifs

Physical mechanism of crystal growth in the presence of the impurities and crystal structure of hydrated cupric chloride (Takashi SHIBATA)

By Takashi SHIBATA[1)],

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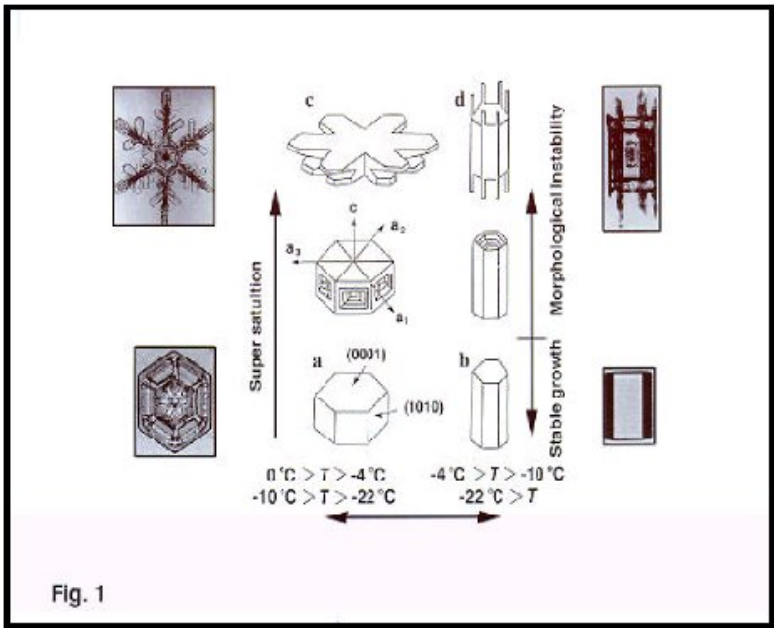


Fig. 1

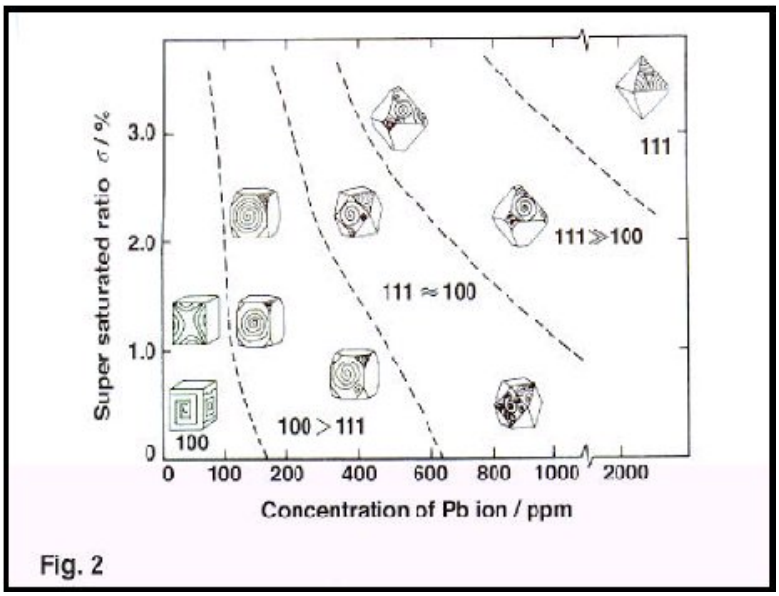


Fig. 2

Introduction

Crystal growth is quite sensitive to the surrounding conditions such as temperature, supersaturation ratio and impurities, and changing their form of crystals. The wide variety of snow crystal shapes consists of only four different kinds of crystals, platelet, prism, dendritic and needle, as a functions of temperature and supersaturated vapor pressure as shown in **Fig. 1** [1]. On the other hand, many investigations of the effect of impurities or additives have been performed. In 1783, Romé de Lisle observed a morphological change from cubic to octahedral in NaCl grown from a solution containing urea [2]. Kern has investigated habit change of KCI from aqueous solutions as a function of supersaturated ratios and concentration of Pb ion as an impurity and produced phase diagram of the morphology of KCI crystals called Morphodrom as shown in **Fig. 2** [3]. It is known that microscopic examination of slides of dried thin layers of cervical mucus

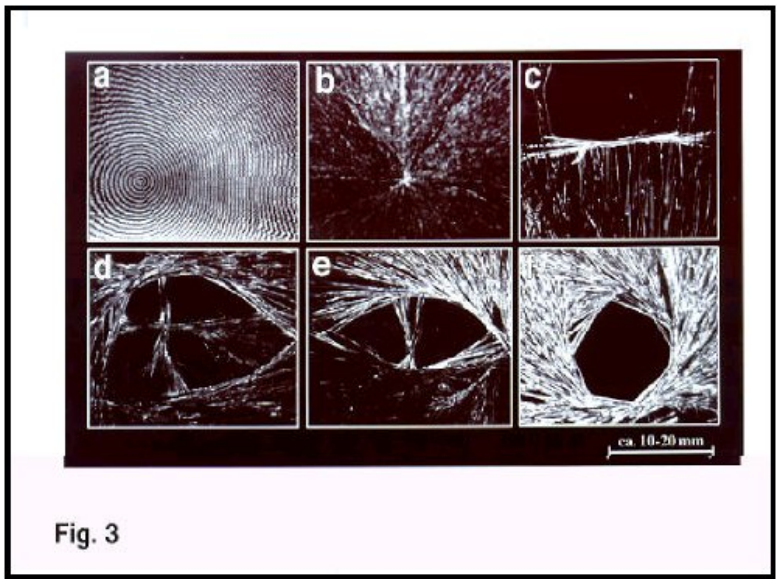


Fig. 3

secreted by the uterus has revealed crystalline morphological changes according to the menstruation cycle. The crystalline is NaCl in the cervical mucus and the dendritic growth of NaCl is caused by estrogen and inhibited by progesterone.

In addition, changes in the growth pattern of dendritic cupric chloride crystals according to the disease of the patient whose blood was added, in a small amount, to the aqueous solutions have long been recognized, i.e. since first reported by Pfeifer [4]. Detailed specific crystal growth, in terms of morphology has been studied by Selawry and Selawry as shown in **Fig. 3** [5]. Nickel also reported by produces a specific dendritic crystal growth [6] and Cocude et al. recently studied silicosis [7]. Koopmans discussed about malignant tumor [8] and Barth reported that a specific form due to tumors was detectable significantly earlier than the standard diagnosis could be made [9].

Piva et al. pointed out that some crystallization patterns correlated with variations in different serum proteins [10]. It has also been pointed out since early times that not only human blood but the plants extracts affect dendritic crystal growth by Ballivet et al.

Many studies have been performed on changes in configuration of hydrated cupric chloride crystals grown with human blood or plants extract. However, there have been few studies designed to analyze the anomalous crystal growth mechanism. We therefore have been studying it from a physicochemical viewpoint.

General theory of crystal growth

Crystal growth mechanisms are divided into three categories according to the character of the surfaces. One category is called "rough face" growing homogeneously by the solvent molecules. The second mechanism is called "two dimensional nucleation growth". In this mechanism, a molecule as a growing unit reaches the smooth face and by surface diffusion reaches the kinked face and stepped face to build in the crystals.

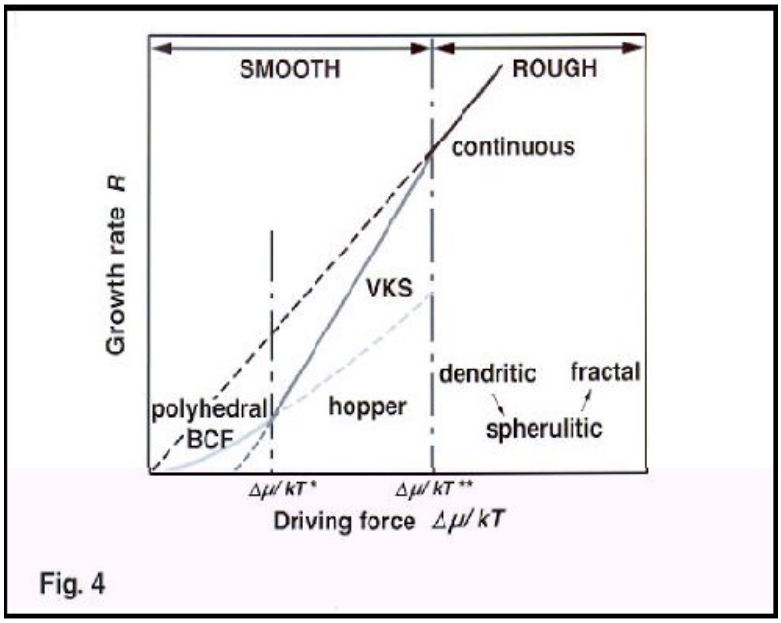


Fig. 4

The third is called the "spiral growth mechanism". A schematic diagram of crystal morphology is shown as a function of growth rate, driving force (Dm/ kT) and roughness of the crystal surfaces as shown in **Fig. 4** [11]. Curves a, b and c in this figure are the results of the relationship between R and Dm/kT, corresponding to flat growth, layer growth by the two dimensional nucleation, spiral growth respectively. The morphology such as, flat planes, hopper crystals, curved surfaces, cell structure, dendritic growth, boe-tie structures or spherulite depends on these growth rate and driving force.

Crystallization method

Hemolyzed solution was added to 30.0 wt% cupric chloride aqueous solution and solutions having a final concentration of 15wt% of cupric chloride that contained 0.1 or 0.5 vol% human blood. Hydrated cupric chloride crystals were obtained from 8 ml of the solution in 100 mm-Petri dishes under conditions of 28E C and 45 % relative humidity in our crystal growth system.

Macroscopic observation and Atomic Force Microscopy (AFM)

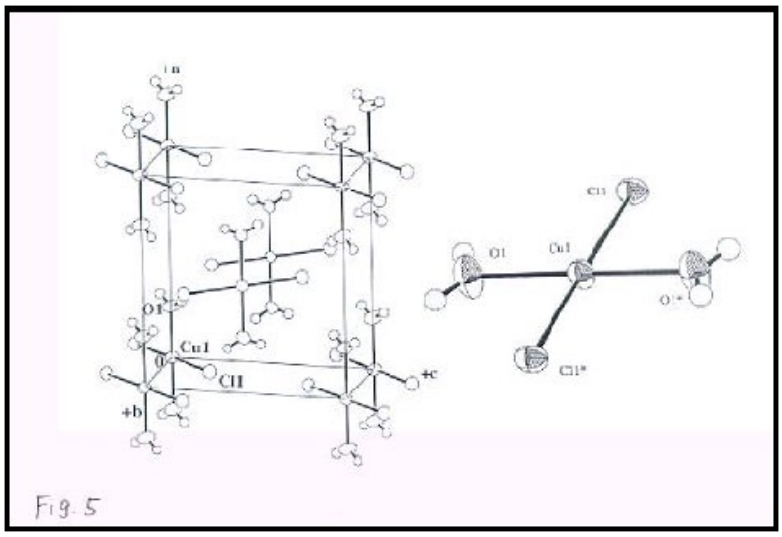
The pure cupric chloride coagulated with many very small crystal grains although dendritic growth itself was observed in only a small part. When blood was added, three concentric circles created by radial growth of the dendrites formed from the initial growth point 0 as a result of adding the blood. Blood has the effect of inhibiting nucleation generation observed by Atomic Force Microscopy (AFM) [12] and makes the crystal volume a few thousand times larger.

Hydration number by thermo-gravimetry (TG/DTA)

Green crystals first appear from the mother solution as a result of evaporation of the solvent water. After complete evaporation of the water, the green crystals turn blue. We tried to determine whether both color crystals have the same or different hydration number. TG shows that the pure blue cupric chloride has 2.16 hydration numbers, while green ones grown from solutions containing blood from healthy subjects and diabetics has 2.53 and 2.62 respectively.

Thermal behaviors are quite different between pure and blood addition as shown differential thermal analysis (DTA) [13]. Based on these findings, we conclude that the green crystals obtained from pure solutions are unstable and transform to the stable blue condition in a short time, whereas the green crystals grown after blood addition are rather stable and maintain their green color for a longer time.

Analysis of Crystal Structure



Blue and green crystals have the same crystalline structure, regardless of the presence or absence of blood. An ORTEP drawing of a unit cell and a molecule, determined by single crystal X-ray diffraction, of a blue crystal grown from pure solution are shown in **Fig.5** [14]. A pair of chlorine atoms bonds to a copper atom and a pair of oxygen atoms originating from 2 water molecules coordinates to the copper in the perpendicular direction in the same plane. The green crystal has the same structure.

The X-ray data suggest that there is no significant difference between blue and green crystals and that both crystals are dihydrated. The calculation of van der Waals diameter suggests that there is no space for residual water molecules in the lattice that has been determined. Given the discrepant results, we concluded that residual water, i.e. a hydration number exceeding 2, results in a disordered amorphous state that makes detection by the X-ray diffraction method impossible.

Disordered crystalline structure by differential scanning calorimetry (DSC)

As remarkable differences between blue and green hydrated cupric chloride dendrites are demonstrated by DSC measurement performed between - 140° C and 40° C [14], despite few structural differences between the crystals having been demonstrated by four axis goniometry using the single crystal X-ray diffraction method, it is suggested from the above observations that residual water in a green crystal, contrast to the dihydration of a blue crystal, results in a disordered crystalline structure. In spite of the green dendrites grown from pure aqueous solutions being short lived, those grown from a solution to which a small amount of human blood had been added maintained a meta-stable condition. As the thermal behavior measured by DSC of unstable green dendrites is different from that of relatively stable green dendrites, there seems to be little difference between the two in crystalline structure.

The structure of solutions

The structure of many different kinds of solutions are recently being studied by neutron diffraction, XAFS, and other methods. According to the results, Cu^{2+} and Cl^{-} is hexahydrated by water molecules in the dilute solutions [15]. The sky blue color of cupric chloride solution must be due to hydrated Cu^{2+} ions. In the concentrated solutions i.e., supersaturated solutions, two water molecules are coordinated to Cu^{2+}, from upper and lower directions of octahedron [15]. The internal plane containing Cu^{2+} of octahedron, two Cl^{-} ions and two water molecules are coordinated to Cu^{2+}. These distances are quite similar to the values we determined for the single crystal of hydrated cupric chloride. In the crystal state, two water molecules are placed by bonded Cu molecules. The reason for the green color of the concentrated solution seems to be change in electron density by the

coordination of two water molecules from axis direction of octahedron.

We suppose this sensitive excess hydration is one of the reasons that cupric chloride is sensitive to blood addition.

The distribution analysis of additives by X-ray photoelectron spectroscopy (XPS)

To understand the effect of addition of blood on the morphology of cupric chloride dendrites, the electron energy state of copper, chlorine, carbon, oxygen and nitrogen was investigated by XPS. As we reported previously, anomalous chemical shifts in the energy state were successfully observed in copper atoms and nitrogen atoms by absorption of nitrogen atoms that originated from the proteins or amino acids in blood [13].

In this study, to determine whether nitrogen from blood is contained inside the crystals or is only adsorbed on the crystal surface, we performed XPS measurements every etching the crystals by accelerated Ar ion sputtering to obtain depth concentration profiles.

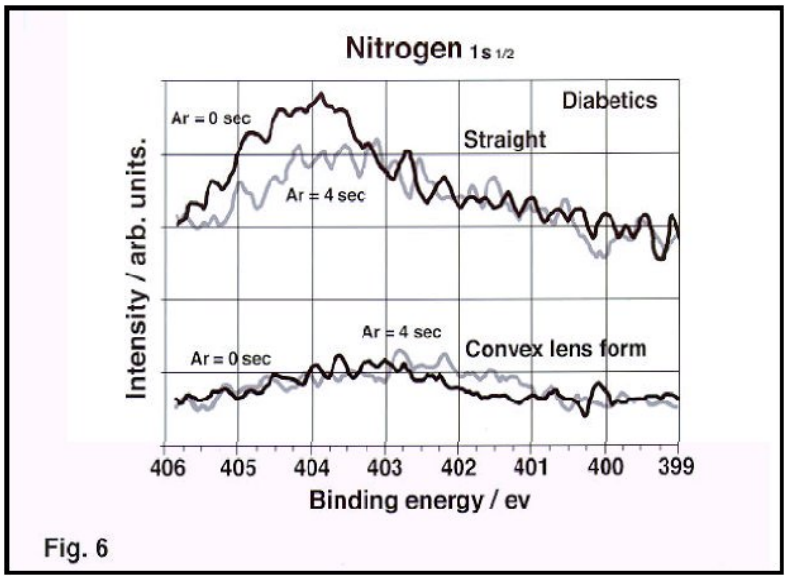


Fig. 6

No nitrogen at all was detected on either the surface or inside of the pure cupric chloride crystals. The relative concentration of nitrogen was higher on the surface, and a chemical shift between straight crystals and specific form of vending crystals grown from solution containing diabetic patient's blood was detected as shown in **Fig. 6**.

A difference in the mechanism of absorption on the surface between their crystals is respected on the basis of this observation. The nitrogen of proteins and/or amino acids from blood is absorbed to Cu on the surface of hydrated cupric chloride crystals. The blood components was under detection limits inside of the crystal. Detailed analysis is currently in progress.

The above findings show that blood has an enormous effect on the growth of hydrated cupric chloride crystals.

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Question: V. SIESO

Are the phenomena presented here reversible or irreversible? What is the influence of time? We have been speaking about mineral crystallisation but what about living systems — shells and bones for example?

Answer: V. FLEURY

Time is the only factor and the mechanism is irreversible. A mass of (n) atoms to which we add another atom becomes a mass of (n+1) atoms and so on. The nucleation mechanism is heterogeneous. The crystallisation phases are different for shellfish. The growth pattern is different because of the slime. There is a different arrangement of crystals which yield very hard shells.

Q: V. SIESO

What about other factors such as temperature, gravity? We know that there have been crystallisation experiments in outer space.

A: V. FLEURY

Gravity plays no role in it. As the density of the components is different, we can observe density gradients in the liquid. Thus, there is convection. The overall effect is that gravity increases the flow. In weightless experiments (in the space shuttle), convection was eliminated, the solutions are less disturbed and we obtain large crystals rather than dendrites. Temperature is one of the most important factors to test. Everything depends on temperature and its effect is exponential.

Q: Mrs. BALLIVET

I refer to the article by Mr. Fleury[1] and would like to go back to the photographs of trees and grains. After each nucleation we see a sort of return to the initial conditions which allow for a new nucleation.

A: V. FLEURY

The crystal may be a crystal down to the atomic scale but the fractal object is not fractal to the atomic scale. There are two scales: on one we see the trees, on the other, the little spheres. One grain represents billions of atoms, a branch is a chain of grains, one following the next. What counts is the frequency of nucleation. One grain sprouts, then another one. This can be digitally simulated. When a grain grows, it is a three-dimensional entity. The more it grows, the slower the speed. During this time the inhibitor diffuses, then another grain appears. The inhibitor affects its growth and thus the size of the grains.

Q: M. COCUDE

This question is for Mr. Shibata and concerns crystals and crystal hydration. We often speak of Copper (II) Chloride without going further, but the degree of hydration is important. In his presentation he also speaks of crystal colour, blue or green. Is there a parallel between the two? Is the blue or green colour related to a certain

degree of hydration?

With the presentation on crystallisation of diabetics' blood we arrive at the issue of medical applications which is on the programme for this afternoon. Are there significant differences between diabetics' blood and normal blood? And how do we define normal blood (reference blood samples)?

A: T. SHIBATA

For the crystallisation coloration I used a thermal method called TG-DTA[2] which shows that the blue crystallisation contains 2 H_2O ($CuCl_2$, $2H_2O$) and the green crystallisation 2.5 H_2O ($CuCl_2$, $2.5H_2O$). I hypothesised that the colour was due to the quantity of water and this was confirmed by other experiments. I used X-ray diffraction which revealed no difference, but using the DSC Technique (Differential scanning calorimetry) we found a difference and I am sure that there will be a difference at -70EC. The green crystallisation is an unstable form. When 1/2 H_2O goes out it becomes blue, but when we add blood the green colour remains.

Hydration is an unstable factor which — this is my hypothesis — allows us to distinguish reference blood from diabetic blood. In my opinion, diabetic blood should yield a 2.6 H_2O crystallisation of $CuCl_2$ while normal blood would yield 2.5 H_2O.

On the surface of the crystals we can detect nitrogen from amino acids and proteins. The use of the XPS technique (X-Ray photo-electro spectroscopy) demonstrates that these elements are absorbed on the crystal surface (exchange of peripheral electrons with the Copper (II) Chloride) but are not detected within the crystals. These elements, not just N but also C and O, influence the morphology of crystallisation.

Additional answer: V. FLEURY

I understand that when you crystallise Copper (II) Chloride in the presence of blood the blood elements are not included in the Copper (II) Chloride but only on the surface. If this is correct, it is extremely important for the crystallogenesis. If we put foreign bodies into a crystal we change its growth pattern and many other things.

Additional remark: M. COCUDE

This property might help us to see which components of the blood used for crystallisation are important for diagnostic purposes (those which can be used for identifying and classifying illnesses). It would be interesting to study this property.

Continuation of answer: T. SHIBATA

As for the definition of reference (normal) blood, this is a sensitive issue. For the experiment with the diabetics I used three criteria for defining reference blood:

Subjective: when questioned the subjects say they «feel well.»

Objective: with blood analysis: initial analysis with blood sample (no abnormal results, sugar, etc.)

Lastly: absence of diabetes.

For my research I used the blood of diabetic persons who were hospitalised but who had not yet been treated. I will discuss this further this afternoon.

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[1]Reference to M. Fleury's article in NATURE. Volume 390 11/13/1997 (p 145 - 148) : Branched fractal patterns in non-equilibrium electro-chemical deposition from oscillatory nucleation and growth.

[2] TG-DTA : Thermo-gravimetry differential thermal analysis.

II. MATERIALS AND METHODS IN USE

II ² MATERIALS AND METHODS IN USE

From left to right : J-O. ANDERSEN , J-G. BARTH, G. TEISSERON, R. NEUMANN, D. CHARPENTIER.

The technological aspects of biocrystallisation (Jens-Otto ANDERSEN)

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[1]) Department of Agricultural Sciences

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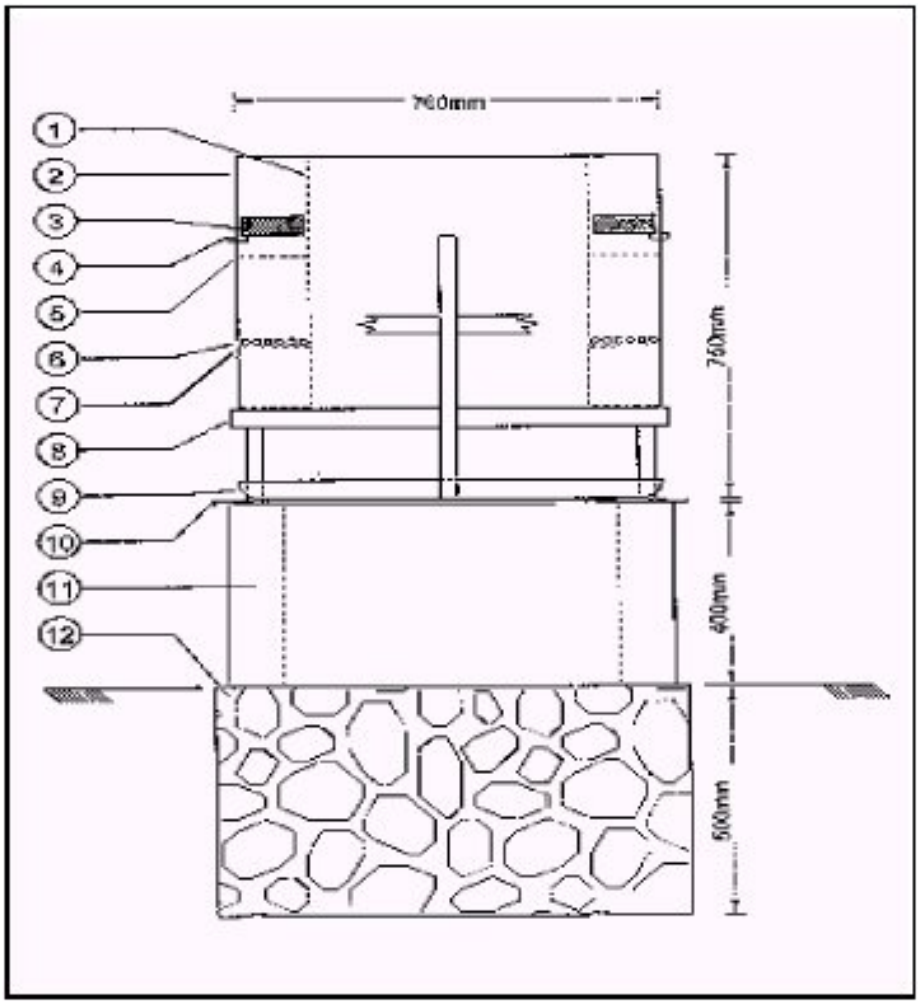
The Royal Veterinary and Agricultural University, Danemark

The bio crystallisation method, also termed «sensitive crystallisation» and «copper chloride crystallisation», was originally introduced by E. Pfeiffer in the 1930´ies. The method is today applied primarily in medical and agricultural research. The method is based on the crystallographic phenomenon that when adding specific inorganic ionic substances, and generally all organic substances, to an aqueous solution of dihydrate $CuCl_2$, crystallograms with reproducible dendritic textures are formed during crystallisation.

Reproducibility of a crystallisation process presupposes control of physical factors such as air temperature, humidity, levelling of crystallisation underlay, vibrations and particles. A broad variety of crystallisation facilities has been designed to meet these demands, from minor vessels to large crystallisation chambers that are entered when setting up an experiment. It can be argued that a major limitation for a wider application of the method is the lack of standardized and documented crystallisation techniques.



Vertical cross-section of crystallization apparatus.

1)Inner cylindric pipe. 2) Outer cylindric pipe. 3) Plane ground steel double ring with 18 interconnecting struts. 4) Exentric. 5) Upper perforated steel plate ring for air temperature homogenization purposes. 6) Constantane heating thread. 7) Lower perforated steel plate ring. 8) Steel frame construction. 9) Water bowl. 10) Foundation steel plate. 11) Concrete foundation. 12) Concrete griund block. [From Andersen et al.(1998). A refined biocrystallization method applied in a pictomorphological investigation of a polymer. Elemente der Naturwissenschaft N° 68.]

Results are reported from an ongoing study concerning the development of refined crystallisation techniques, involving control of a complex of factors influencing the course and duration of the evaporation and crystallisation phases.

A method involving a combination of an ortho-octangular inner chamber with a cylindrical crystallisation apparatus, and an outer chamber is presented. The outer chamber serves as a controlled athosphere around the inner chamber as well as a recipient of heat and humidity from the inner chamber, by way of diffusion through the wooden construction. The inner chamber has an approximate volume of 9 m^3.

Results from a reproducibility study of the method is presented, based on measurements of variations in air temperature and humidity during three similar experiments, with intended variation patterns of temperature and humidity during experimental periods of 17 hours.

It is concluded that the presented method represents a marked refinement of crystallisation techniques, relative to an earlier study performed on the basis of a cube-like chamber, with an approximate volume of 5m^3.

The refinement is attributed to a complex of factors, involving primarily the following factors:

1) Machinery generating unintended air convection (humidifier, dehumidifier, air filtrator) is placed in the outer chamber. 2) The combination of an inner ortho-octangular chamber with a cylindrical apparatus, in which the crystallisation plates are arranged in a circle, and located isotopically relative to circular heating elements below the plates, reduces markedly unintended air convection over the plates. 3) A glass ring of 35 mm surrounding the crystallisation area, which is found to reduce interference with intended gradients and microconvections in and above the evaporating crystallisation solution.

Reference:

Andersen et al. (1998): A refined bio crystallisation method applied in a picto morphological investigation of a polymer. Elemente der Naturwissenschaft, Nº 67 (in press).

Visual lecture of crystallisation patterns of cupric chloride with additives (Jean-Georges BARTH)[1]

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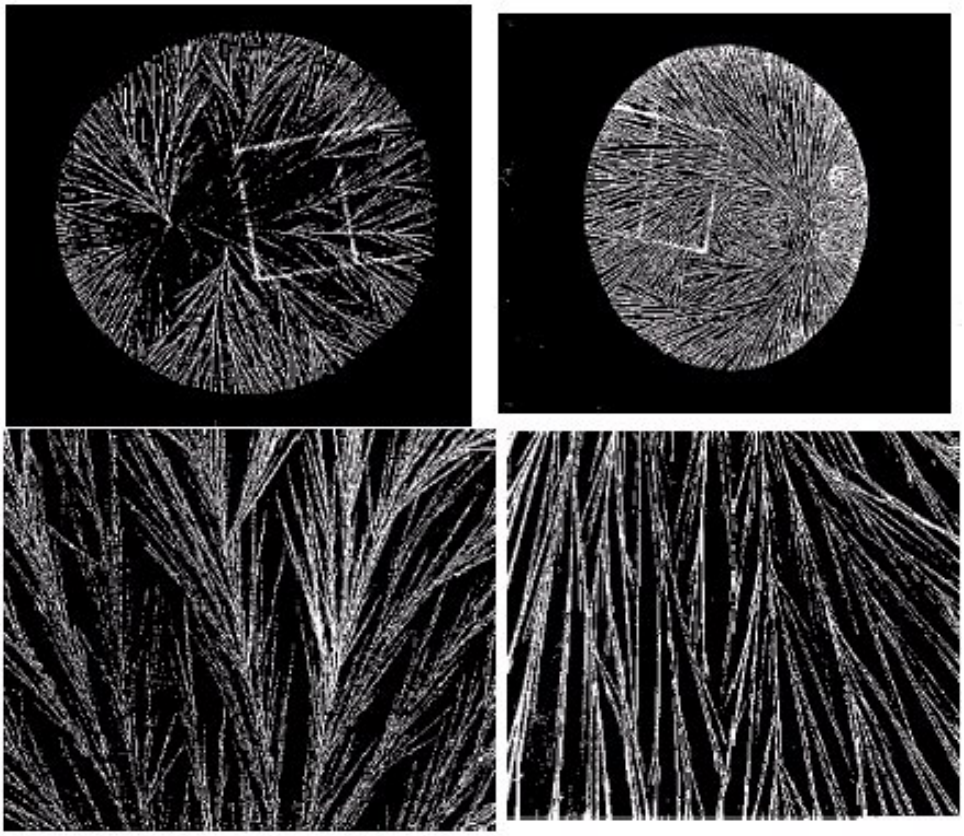


IPictures obtained with milk : crude milk on the left, pasteurized and homogenised UHT on the right.

(From KNIJPENGA H. (1980))

The matter is to study the final picture resulting from the crystallisation process (static operating). The analysing method is in search of general and particular properties, allowing comparative opinions about pictures obtained with the same or with different kinds of additives. The visual lecture demands training and the accumulation of a great number of data, allowing discriminatory comparisons or relevant orientations about diagnosis. The lecture process limits the crystallisation to a bidimensional object. With the same additive it is necessary to examine a run of pictures obtained by the same experimental conditions, especially because of the following reasons : series of pictures are not identical but homologous,because crystallisation is a growth system out-of-equillibrium ; appearing of caracteristic properties is influenced by the concentration of additive, and it is therefore necessary to analyse pictures obtained with additive in different concentrations; this way of handling is also suitable because operative

conditions are not yet well controled.

Pictures obtained with different kinds of additives are distinguishable directly, as a whole, without detailed examination. But more often it is not so, because the purpose is to distinguish pictures resulting from the same kind of additives.

General properties of the picture : relative size of central, marginal and intermediate regions are noted; picture may be orientated so lateral, lower, upper and median regions can be identified (cf. human blood). Crystal structure is described and coordination is assessed.

Particular properties : texture, vacuoles with their localisation and different little sized forms are noted and described.

The whole properties, general and particular one make it possible to describe typical pictures, and constitue the basis of a crystallographic semiology suitable for agricultural and medical purpose. These elements are exemplifyed by : a) proteine and starch pictures; b) evolution of the texture during the storage of an alimentary plant (according to ENGQUIST M.); c) texture of pictures with milk depending on processing methods for its conservation (according to KNIJPENGA H.); d) general aspect with human blood.

Caution : the operative conditions may influence the relative size of central, peripheral and marginal areas (LERAY J.) especially when initial quantity of solution is small. The distinction of structure and texture is a didactic matter, wich is eventually questionable because crystallisation can be reduced to a fractal object.

SELAWRY A. et O. (1957) : Die Kupferchlorid-Kristallisation. Gustav Fischer Verlag, Stuttgart.

LERAY J. (1973) : Profil de la surface libre d’un film liquide hétérogène. Journal de chimie physique, NE 10, p. 1428-1432.

KNIJPENGA H. (1980) : Bildschaffende Methoden .In : BOCKEMUHL J. : Lebenszusammenhänge erkennen, erleben, gestalten. Naturwissenschaftliche Sektion der freien Hochschule für Geisteswissenschaft am Goetheanum, Dornach, p. 58-60.

ENGQUIST M. (1989) : Qualitätsprüfung an Gemüse durch die Kuperchlorid-Kristallisationsmethode.

Forschungsring für biologisch-dynamische Wirtschaftsweise, Darmstadt.

Processing and analysis of crystalline growth

Case of Cupric Chloride

Static Images (Georges TEISSERON [1] & Richard NEUMANN [1])

1 Université Joseph Fourier, Laboratoire d'instrumentation micro-informatique et électronique. GRENOBLE.

G. TEISSERON

Due to the improvement of workstations, processing and analysis technics, the study of cristallization images of Cupric Chloride with biological or chemical additives can be achieved automatically.

Image processing allows the analysis of this crystalline growth whose complexity delivers a large amount of informations extracted from its texture, structure and its caracteristic pattern. Hence, this technic permits the extraction of parameters owing to the analysis of the relation between each region in time and space. We choose to do our processing on 70% of the image of crystallisation.

In order to master the crystallisation of Cupric chloride with biological or chemical additives, our laboratory acquired, at the last of 1994, an air conditionned enclosure, based on the «Pagot» model. It possesses a volume of 3.6 m3, is achieved in wood with a double box isolated on its external wall, has a weight of 500 kg and is mounted on pneumatic suspensions in order to avoid vibrations

This enclosure was modified in order to allow temperature and hygrometry regulation (température : 29EC"0.8EC and hygrometry 56% "1%). Now, the enclosure permits the simultaneous growth of 44 crystallisation rings. Such a number of synchrone crystallisations allows the

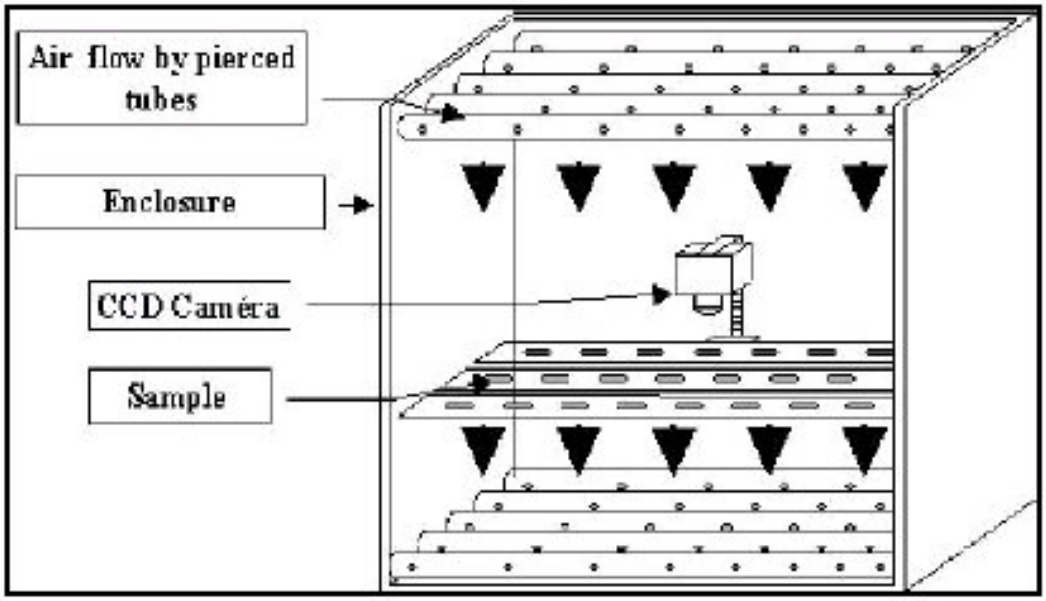
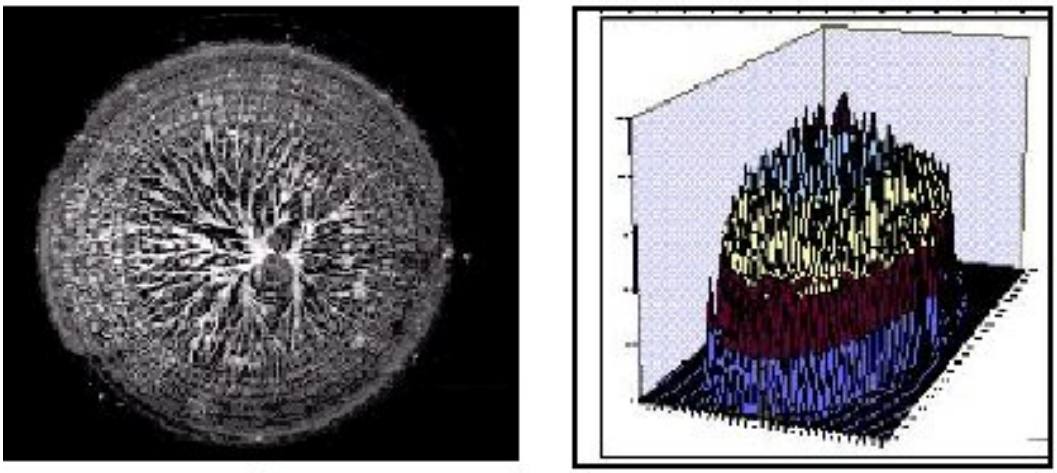


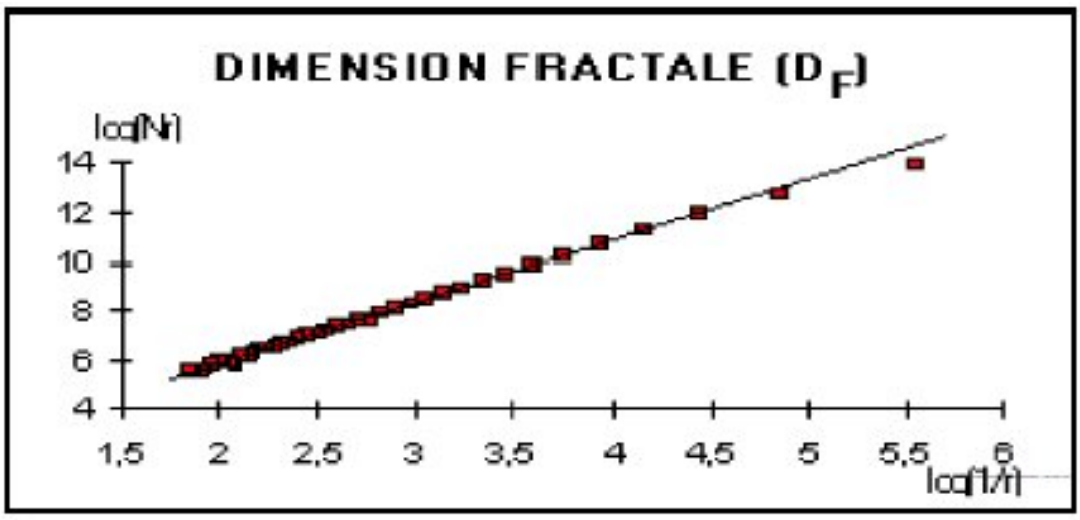
FIGURE 1 : Crystallisation enclosure

comparison of two series of crystalline growth during the same manipulation. The plate disposition in the enclosure permits globally to have the same growth conditions for the two series.



A program allowing the classification of products from different natures (by the sort, the age) was achieved which works on images representing the end of crystallisation (static images). In order to extract parameters useful for classification, crystallisation images texture was studied because its varies in regard to the additive introduced.Statistical methods, like the MPT méthod (Tonal Property Measure) and coocurence method, like SGLDM or NGLDM method, was studied ; furthermore the fractal dimension and the number of white pixels after binarization (by the method of error diffusion) was extracted.

This works allows the extraction of a maximum number of 18 parameters for each image. Data analysis was achieved owing to a classifier and a program which determines the best vectorial space use to represent the datas. Results given, indicate that texture analysis allows the discrimination between different additives with a high rate of success, whereas its delivers insufficient results during the determination of the different stages of temporal degradation of a product. Hence, it may appear that texture analysis is less sensible to state changes than to the differences of product.



c)

$$D_F = \frac{\log(N_r)}{\log(\frac{1}{r})} = 2,406485$$

FIGURE 2 : a) Initial image. b) 3D representation of the image. c) Fractal dimension of its surface.

Analysed Products (number of images)	Classification rate
UHT (22) | Pasteurized (22)	95,5 % | 100 %
UHT (22) | Pasteurized (22)	95,5 % | 90,9 %
UHT (22) | Pasteurized (22)	100 % | 86,3 %

FIGURE 3 : Classification of biological products extracted from the same species.

Cinetic Images (Richard NEUMANN)

R. NEUMANN

In order to understand how the differences between additives (differences given by species, cultivation, processing, conservation, age...) modify the developpement of crystallisations sensitives to biological or chemical additives, we have developped a tool which works on dynamics images representing the temporal evolution of the crystalline growth. Many objectives are considered during this cinetic analysis.

They must either permit the comprehension of the physical process driving to this crystallisation from and the simplication of the images in order to extract their structure.

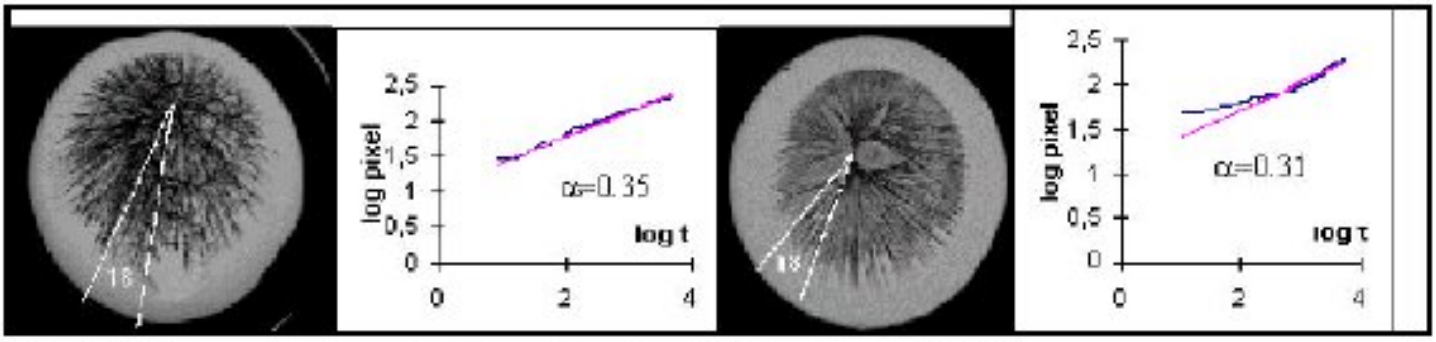


FIGURE 1 : Representation of sector 18 for pure CuCl2
Evolution of its trajectory and approximation by a ligne of a slope.
Representation of sector 18 for CuCl2 with ovalbumine.
Evolution of its trajectory and approximation by a ligne of a slope.

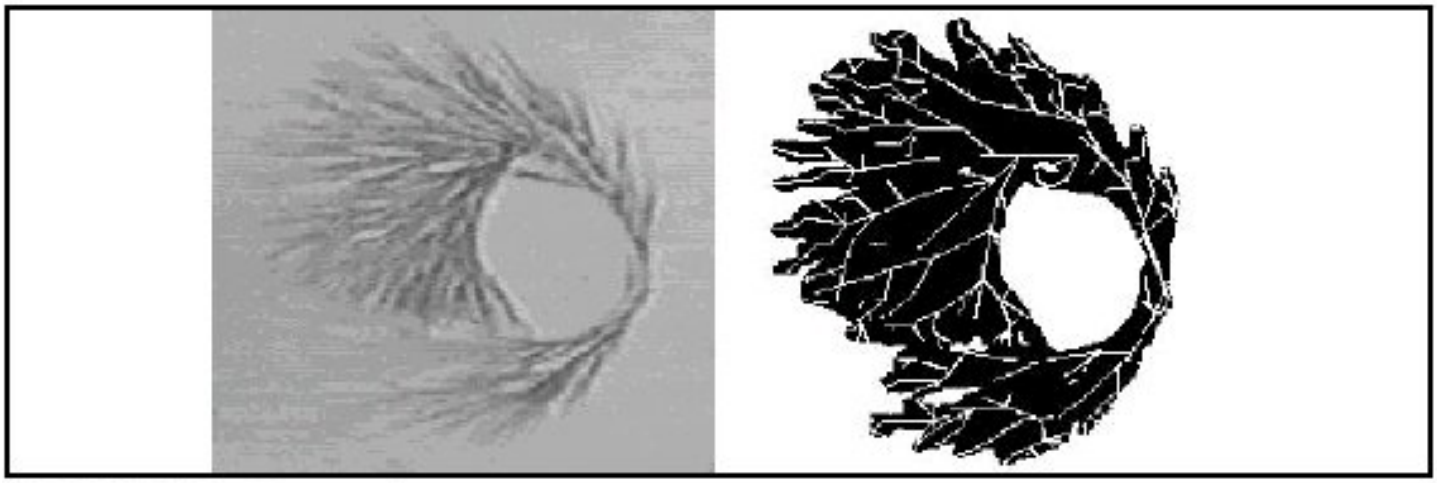


FIGURE 2 : Original image
Some image binarized with the structure extracted from dendrite tips tracking.

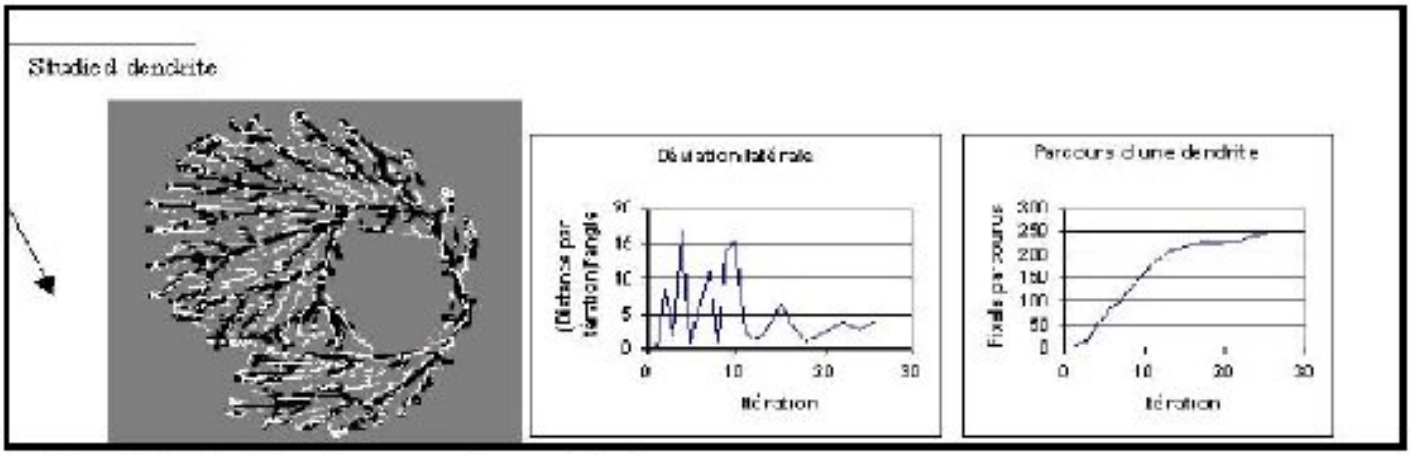


FIGURE 3 : Evolution of specified parameters
- Comparison of the structure extracted from dendrite tips tracking of the same crystallization as in figure 2 with its skeleton.
- Displacement of the dendrite.
- Lateral deviation of the dendrite per iteration.

It is the speed growth extraction of the crystalline interface which informs about the crystallisation rules of our images. An original method allowing the following of crystallisation was developped and experimentations on crystalline growth in the presence of different additives was done and permitted the extraction of a crystallisation rule (in atª).

The second objective concerning the study of crystallisation cinetic must simplify the thesigraphic images in order to isolate their structure. Previous works done on static images allowed the extraction of their skeleton but presented many defaults. Indeed, skeletized images often looked like mosaic and furthermore it always was impossible to follow the temporal path of a dentrite correctly. Our aim was to use the images growth in order to extract a staunch and interpretable structure in order to follow the evolution of various parameters such as the travelled distance and the angular deviation of specified dendrites, or the calculation of the number of sons for each branching.

This method requiered the elaboration of specific algorithms in order to extract and follow in time the dominant points present in the cinetic images. Results show a structure near from the structure of a skeleton whose all the ramifications are known and identified. The morphological validation of this arborescence was achieved by its comparison with a specific skeleton of the image.

The use of the image analysis technics and the development of original and specific methods allows us to propose few analysis tool, which either concern the study of static images for their classification, and the study of cinetic images for the analysis of the structure and the growth laws. These parameters offer various informations on the influence of the additives on the grouth mechanisms. Furthermore, these tools can also be adapted for others experimental situations. Finally an important work must be done concerning the choice and the interpretation of these parameters for an optimal use multiples.

Influence of electric and magnetic fields on sensitive crystallisation

(Dominique CHARPENTIER[1], J-G BARTH[2], M. COCUDE[3])

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3)President of the CORSS, Ministry of the Economy, Finance and Industry. 75 005 PARIS.

The crystallisation of cupric chloride solutions added with various chemical or biological additions such as juices of plants, human blood produces forms of crystallisation quite different from those of pure cuprous chloride. The aspect of these forms informs us about the nature of the impurities weather there is a systematic link between the cause and the noted effect.

The object is to study the effect of the electromagnetic radiations, considered as disturbing phenomena which can influence crystallisation and produce variations of form for the same product. The other experimental conditions by way of assumption are unchanged (temperature, hygroscopy, mode of preparation of the solutions...).

The experimentation consisted in producing electric and magnetic fields in the enclosure whose intensity between 10 and 1 000 times higher than measurements of the ambient fields (the magnetic field is lower than 100 mA/m in the frequency band between 10 kHz and 1 MHz and the electric field is lower than 3 mV/m between 10 kHz and 1 GHz) and to analyze the effect of these fields on the forms of crystallisation.

The magnetic fields were produced in the frequency band between 0,01 Hz and 200 MHz in horizontal and vertical polarization, with an amplitude varying between 10 mA/m and 200 mA/m.

No systematic modification appeared.

The impact of the electric and magnetic fields in the frequency band considered, with a recurring sweeping in frequency during all crystallisation (7 to 8 hours) does not modify to a significant degree the forms of crystallisation.

Cadre magnétique pour un champ polarisé verticalement

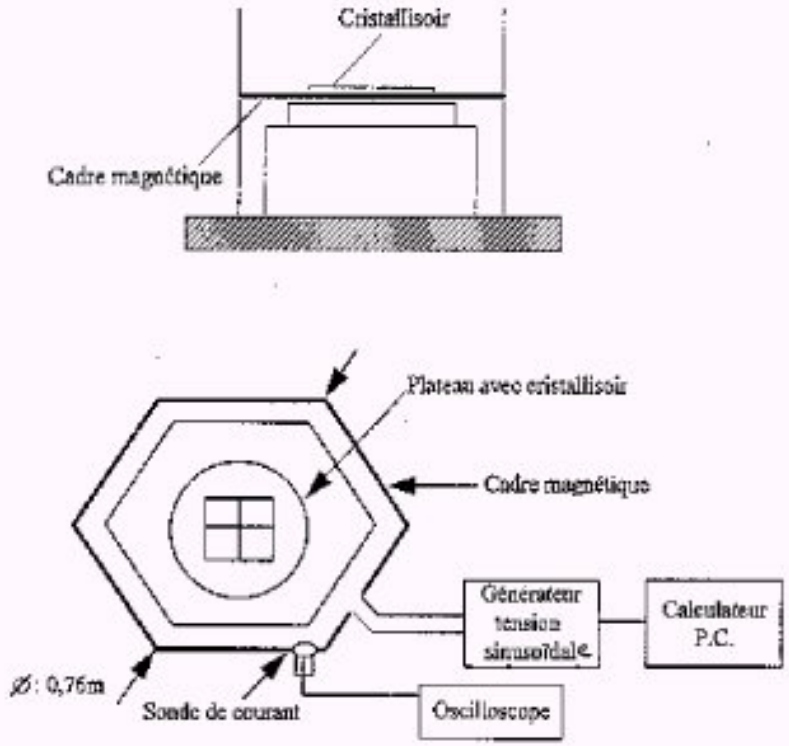
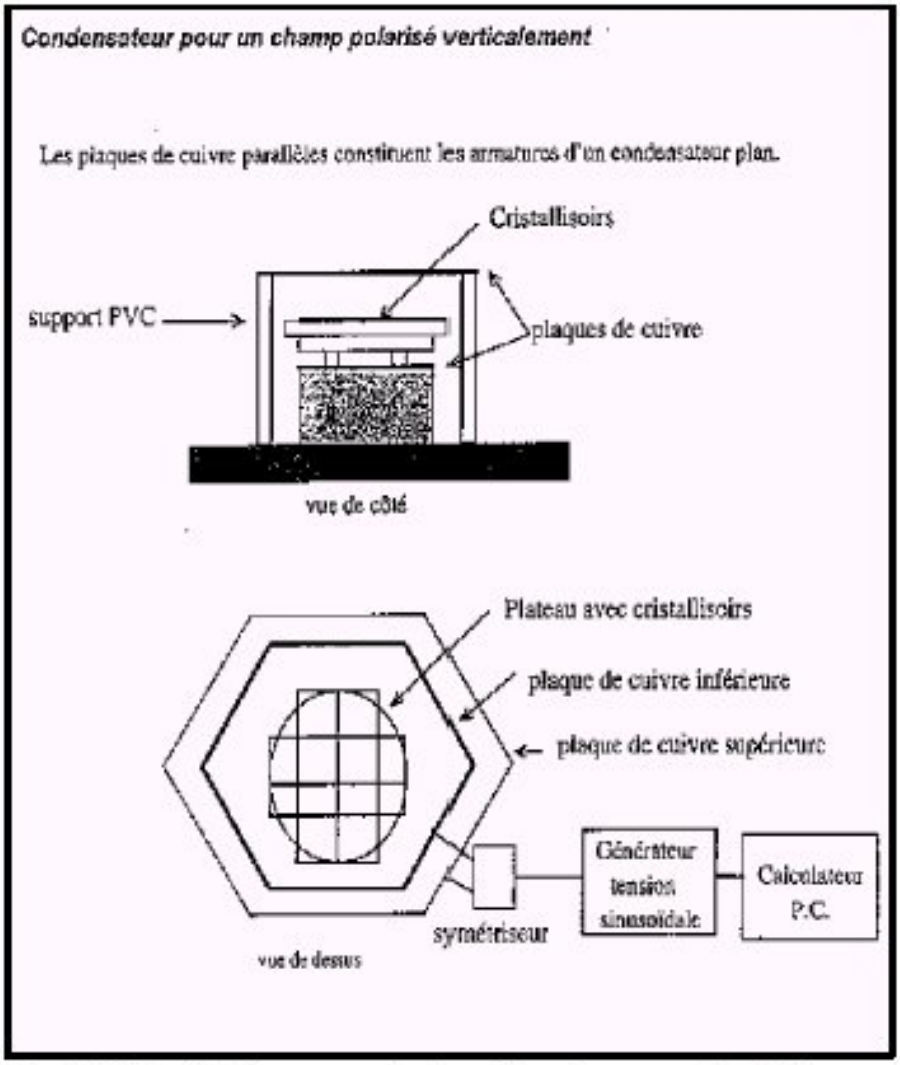


Figure 1
Caractéristiques du cadre magnétique :
hexagone, diagonale = 0,76 m
1 spire, fil de cuivre Ø : 0,5 mm

Matériel utilisé pour générer des champs magnétiques verticaux.



Matériel utilisé pour générer des champs électriques verticaux

Q: V. FLEURY

A simple comment regarding the last presentation: the images with spiral arborescence were from metal deposits which are sensitive to magnetic fields, as if each of the elementary crystals were a little magnetised needle. There is a summation of the directions of these little needles in the field which yields curved shapes on the macroscopic level.

That being said, in the images which you showed us, Mr. Barth, there are *concavities* and cavities which are formed by the intersection of the curves of small individual filament grains. This is not completely apparent when you enlarge the images. What we see is rather rectilinear rods. Do these rods appear to be straight because we see them up close although they are really curved? Or it is rather at the moment when a new rod forms on an existing one that a curve appears?

A: J-G BARTH

We would have to examine the images very carefully and seek the centres of germination which are in coordination with each other. Striations go out from these centres to the left and to the right, forming cavities.

Q: V. FLEURY

Are the individual crystals really extremely straight needles, the rods, are they really straight?

A: J-G BARTH

These are crystalline striae with a rectilinear orientation. As there are various germination centres which appear to the right and to the left, the striae which come from them form a perimeter.

Q: V. FLEURY

The question that remains unsolved is why, in general, there is gyration? In the images it is clear that the fans are curved. If an additive modifies the curve then we will have various cavities or rosettes. What is the cause of this? Mr. Neumann mentioned the measurement of this curve and you said that when we measure the growth of a dendrite over time this curve diminishes, I believe. At the beginning it is very tight and at the end very straight. Is this an effect of the thickness of the liquid in the dish?

At the beginning of the crystallisation, especially with milk, there are very large curves and at the end of the crystallisation all curve shapes disappear, the dendrites become completely straight. Based on these observations and by studying the curves we may be able to find some characteristics of the crystallisation.

In the experiment which you present, as the crystal grows, the growth slows down. The closer we get to the edge, the less liquid there is. There must be a correlation between the speed of growth and the degree of curving.

A: R. NEUMANN

Yes. There is also a correlation with the concentration of additives. There is less Copper (II) Chloride and the additives are perhaps pushed toward the edges of the crystallisation. As a result, the field of fluctuation of the

dendrites would be restricted.

At the end we had dendrites growing very slowly but in a very straight manner because they no longer had a choice of direction.

A: M. COCUDE

For the moment we are thinking in terms of planar images. But these are really three-dimensional configurations. About ten years ago, studies were undertaken at the *Ecole des Mines de Paris*. In Copper (II) Chloride crystallisation with blood additives, vacuole shapes appeared at the interlocking points, superimposed straight crystals rather than dendrites with continuous crystals. But these «vacuoles» had no continuous perimeter. The continuous appearance was the result of a perspective effect (projection of a three-dimensional reality).

Q: Ph. DESBROSSES

I have heard that there was very rigorous physical testing but I never noticed anything about tests of the air.

Could the presence of bacteria modify the results? Has the chemical composition of the water base been studied?

A: J-O. ANDERSEN

With regard to microbe infection, the concentration of Copper (II) Chloride in the solution is such that it has a lethal effect on bacteria.

Q: V. SIESO

You stressed the importance of preparing the sample before carrying out the actual crystallisation. But if we take U.H.T. milk, fresh milk and pasteurised milk, for example, these are already different samples. Before carrying out the crystallisation the sample is modified so as to combine it with Copper (II) Chloride. Is it important to make sure that the conditions are the same for samples which are different to begin with? Isn’t there a risk of introducing fluctuations in the treatment of the sample itself?

You also spoke of vegetable extracts. You do not use the raw material, you process it, you modify the sample even if, thereafter, you maintain identical conditions within the crystallisation chamber. Won’t this affect the result of the crystallisation?

A: J-O. ANDERSEN

Whatever technique is used, there will be a certain effect. For the three methods used for defrosting a frozen product we obtained three different images.

We can also work with carrot juice, with carrot extracts.

Q: V. SIESO

Is the treatment of two samples of milk absolutely identical from the beginning? Do we take this precaution which I consider essential? This point will be discussed this afternoon, there is no need to go on now.

Depending on the way we handle a given sample, the blood of one person for example, we will obtain different results. Regardless of the sample, vegetable, milk or other, we must be careful.

A: J-O. ANDERSEN

Yes, indeed.

A: M. COCUDE

That brings us to the problem of reliability of the method.

Q: V. FLEURY

When we want to make large crystals in a reproducible manner we usually put in a seed crystal to ensure that the crystallisation will begin at a certain place. Do you work the same way? With electrolysis, for example, we can be sure that it will start at the electrode. That way we are sure to have very radial images, it improves the geometry of the system. Is it compatible with your process to put a seed crystal somewhere?

A: J-G BARTH

We have never done that.

A: R. NEUMANN

We tried placing a seed crystal at a defined place by making holes in the plates in order to facilitate nucleation at that place. We didn’t obtain very encouraging results. The physicist suggested that we do this in order to have an almost certain nucleation at this point, but we were only able to get this result every other time. It’s an interesting topic and one which we would like to study further. It would be valuable to be able to choose the point of nucleation, particularly for kinetic studies.

III. CORRELATION BETWEEN THE CHEMICAL STRUCTURE OF THE ADDITIVE AND THE PICTURE

III [2]CORRELATION BETWEEN CHEMICAL STRUCTURE OF ADDITIVE AND CRYSTALLISATION PATTERNS

Jean-Georges BARTH[1]

1 Laboratoire de biochimie-coagulation. Centre hospitalier André BOULLOCHE 25209 MONTBELIARD

The crystallisation patterns obtained with biological additives, arise from complicated interactions wich are impossible to relate in detail, and to attribute to particular substance. Nevertheless it is interesting to approach the understanding of pattern forming mecanism by trying to make clear how the chemical structure of purified additives works on crystallisation patterns formation. To answer these question, additives with a well known chemical structure such as carboxylic derivates with C2, C3, C4 and C5, and natural or synthetic polymers including proteines were studied. Some connections between chemical properties of additives and crystallisation patterns are obvious, but the mecanism of their formation is not clear.

Following elements arise from these preliminary experiences :

. Strongly polar functional groups such as carboxyls and sulfonyls affect the « texture », whereas amino groups affect more the « structure ». This finding is to be qualified to the extent that the effects are

Acide-L-cystéique
3 µg/ml.CuCl2
75 µmol/ml.

Poly-L-Lysine
109 µg/ml.CuCl2
75 µmol/ml.

Poly-L-Glutamate
109 µg/ml.CuCl2
75 µmol/ml.

concentration dependent.

. Weakly polar functional groups such as alcohols and mercaptans intensify the influence of the additives on crystallisation.

Glycogène
350 µg/ml.
CuCl2
75 µmol/ml.

Amylopectine
25 µg/ml
CuCl2 -
75 µmol/ml

Inuline
350 µg/ml
CuCl2
75 µmol/ml

. The monomer wich makes up a polymer, or the primary structure of a protein appears to have a marked influence on the differentiation of crystallisation patterns.

. The three dimensional structure of a substance could play an important part in crystallisation pattern formation, as shown in comparative experiments with various glycans. Glycans with short branches at every third to fifth glucose unit of the main chain give completely different patterns compared with those obtained by addition of glycans with very short or long branches at intervals of between 20 and 33 glucose units on the chain.

These results have to be completed by other works about the influence of functional groups and to confirm the influence of primary and of tridimensional structrure of polymers and proteines. These results make possible to describe crystallographic chimiotypes typical of natural substances.

BARTH J.G.(1997): Image de cristallisation du chlorure cuivrique et Nature de l'additif. Elemente der Naturwissenschaft N° 66, p.16-42.

NB : The color of these illustrations is a result of original photo taken in polarized light.

SELAWRY A. et 0. (1957): Die Kupferchlorid-Kristallisation. Stuttgart Gustav Fischer Verlag.

Q: V. SIESO

We have studied two types of substances fundamental to living systems: proteins or amino acids and sugars. What about the third important group, lipids? Perhaps the main problem is solubility.

My second question concerns the images which you showed us. You frequently used adjectives such as «interesting», «representative», or «pertinent» to describe the images. One imagines that you made a series of images and then eliminated some which were not representative or interesting.

A: J-G. BARTH

To be fully didactic and convincing I should of course have shown you all the images obtained for a given additive. With the amino acids, for example, I studied S-methyl-cystein in concentrations of 3, 6 and 9 micromoles of additive in the mixture. We observed that when the quantity of additive was above a certain level, the crystallisation image became atypical and it was no longer possible to distinguish the features due to the additive. The same applies when the concentration of additive is too low. In that case the image of Copper (II) Chloride dominates. It is impossible to attribute specific characteristics at these two extremes. This is a general rule for all people who work on sensitive crystallisations, i.e. they have the problem of the zone of concentration in which the images reflect the additive being studied. I also spoke to you of glycogen for which the images change as a function of the additive concentration. When the concentration is low, the images look like those of pure Copper (II) Chloride. As the concentration rises, you see the images which I showed you. It would have been justifiable to show you all of these images for all the additives so you would be convinced of what I told you.

The second issue involves lipid additives. I haven't studied them but I think there would indeed be a problem of solubility to be overcome.

Comment: V. SIESO

We have conducted experiments by taking not a single, simple compound in variable concentrations but rather a mixture of two simple compounds: we observed varied images. Also, when you say that it is probably the primary structure which is involved rather than the tertiary three-dimensional structure, that is not certain at all. When we mix the protein in question with Copper (II) Chloride I wonder if this medium is not already sufficient to annihilate the tertiary structure. If not, prior manipulations could be used to denature the protein so that it would lose its tertiary structure so that we could analyse the crystallisation image after denaturing.

A: J-G BARTH

I conducted some experiments of this sort involving enzymes, for example urease or trypsin which we heated. We verified that the heat treatment removed the enzymatic activity. In this case we could conclude that the functional structure of the protein had changed. By comparing images of the treated and untreated enzyme we could see that there were differences. The idea that the tertiary structure can play a role should not be ruled out, although I should say immediately that the heat treatment certainly changed many more things than we realise. This is not easy to control. We would have to study the changes produced before we would be able to conclude that it is the tertiary structure or other modifications in the primary structure which were caused by the heat treatment.

IV. MEDICAL APPLICATIONS

IV [2]MEDICAL APPLICATIONS

General principles - Indication of risk (Marie-Thérèse PIVA)[1]

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1 - Patients from the Montpellier University Hospital Centre

We used and adapted for medical diagnostic advises the cupric chloride crystallization, first studied by E. Pfeiffer (1931) and A. Selawry (1957). Patients from the Service of Internal Medicine from the Montpellier Hospital Centre and "healthy" subjects were studied. This method is based upon the studies of different pictures that appear after evaporation of an aqueous cupric chloride solution in presence of blood in a bidimensional circular plane system.

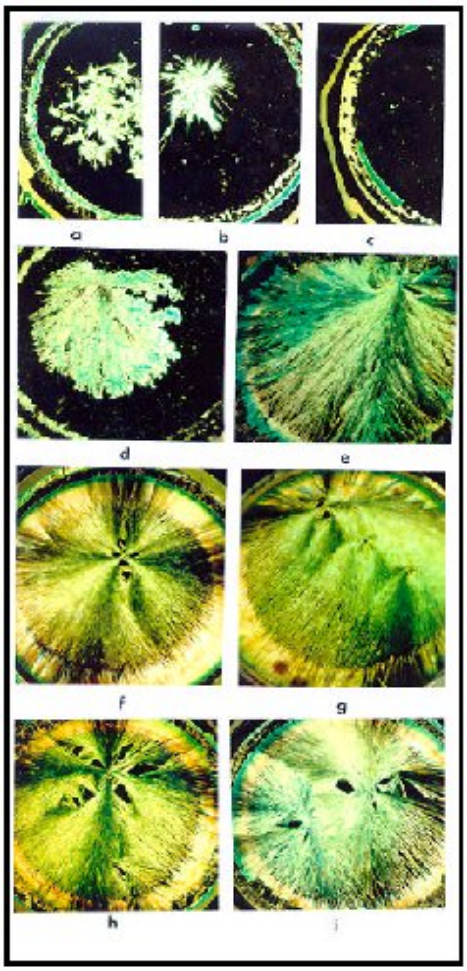


Fig 1 Formes de cristallisations observées.
a,b,c : acides aminés.
e : sérum albumine.
f, g, h, i : sang de patients

We described the methods in its various technical aspects and defined the optimal conditions to obtain these pictures. In healthy subjects (chosen as controls), the crystallization was more regular than in non-healthy subjects. In 80 patients classified into 8 pathological groups there were crystallization alterations leading to geometrical figures. The different figures obtained are listed and schematized.

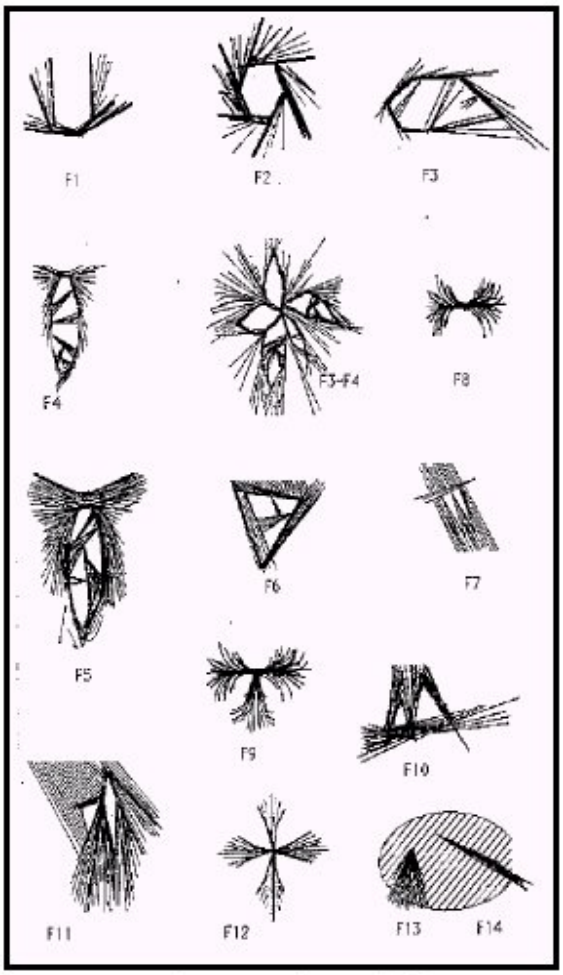


Fig 2 :Schéma des différentes "formes".

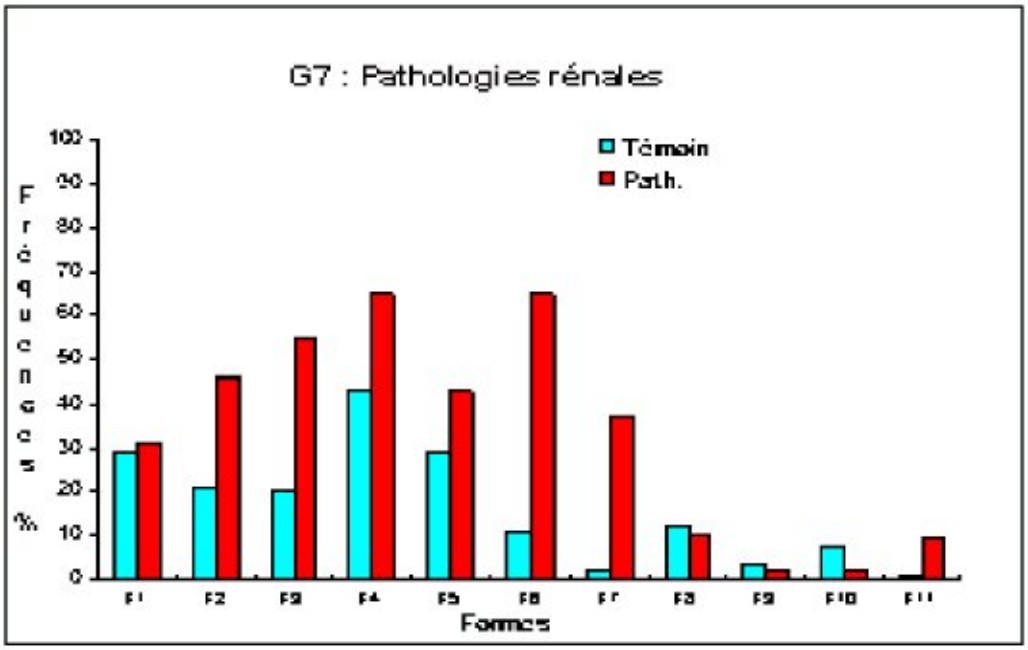


Fig 3 a Comparaison d'histogrammes montrant les profils de forme :

- **Pathologies rénales et profil témoin.**

An experimental procedure with less complex media than blood (i.e amino-acid solutions, serum albumin dilutions) demonstrates the influence of protein in eliciting forms.

The statistical study showed significant differences between most of the patient groups and a relation between a single alteration « Form » frequency and an etiological group. Some crystallization patterns were also correlated with known variations of some different seric proteins.

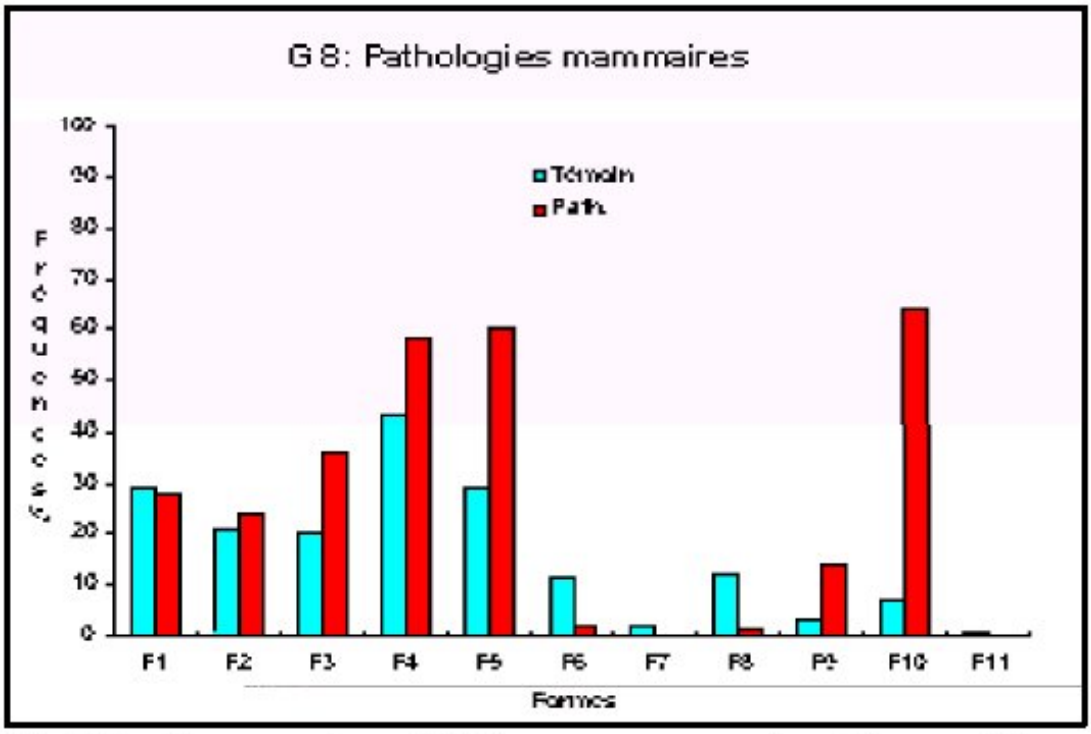


Fig 3 b : Comparaison d'histogrammes montrant les profils de forme :

- **Pathologies mammaires et profil témoin.**

This study shows a one to one relationship between pathology and form allowing one to " visualise " a pathological state on a crystallographic picture. It also allow to define for a certain number of pictures a pertinent threshold related to frequency up to which watchfulness must be set up.

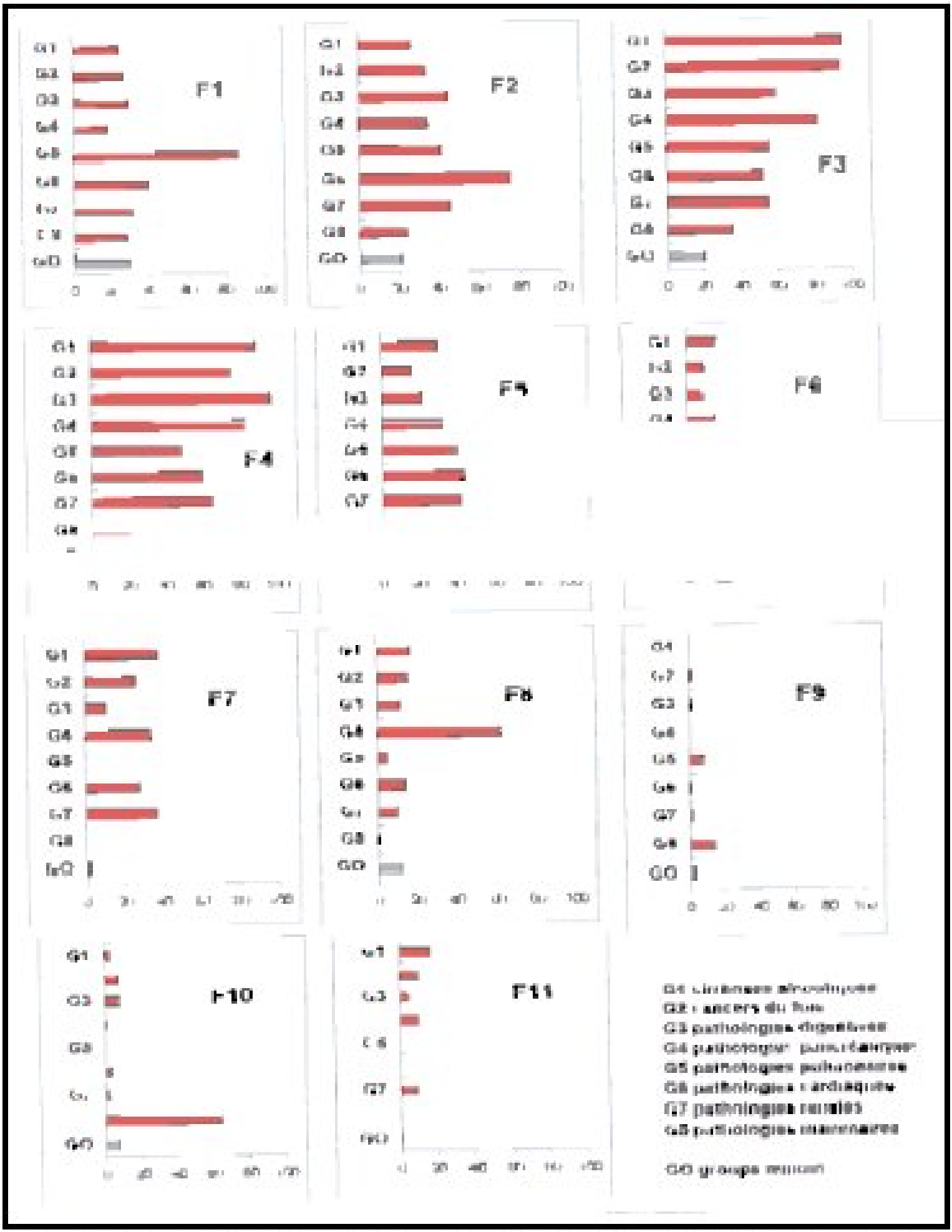


Fig 4 : Histogrammes présentant la fréquence d'apparition des 11 formes dans les différents groupes pathologiques.

2 - Patients and cancerology

German papers notably reported that the more prominent application of cupric crystallization lays in the cancer diagnosis.

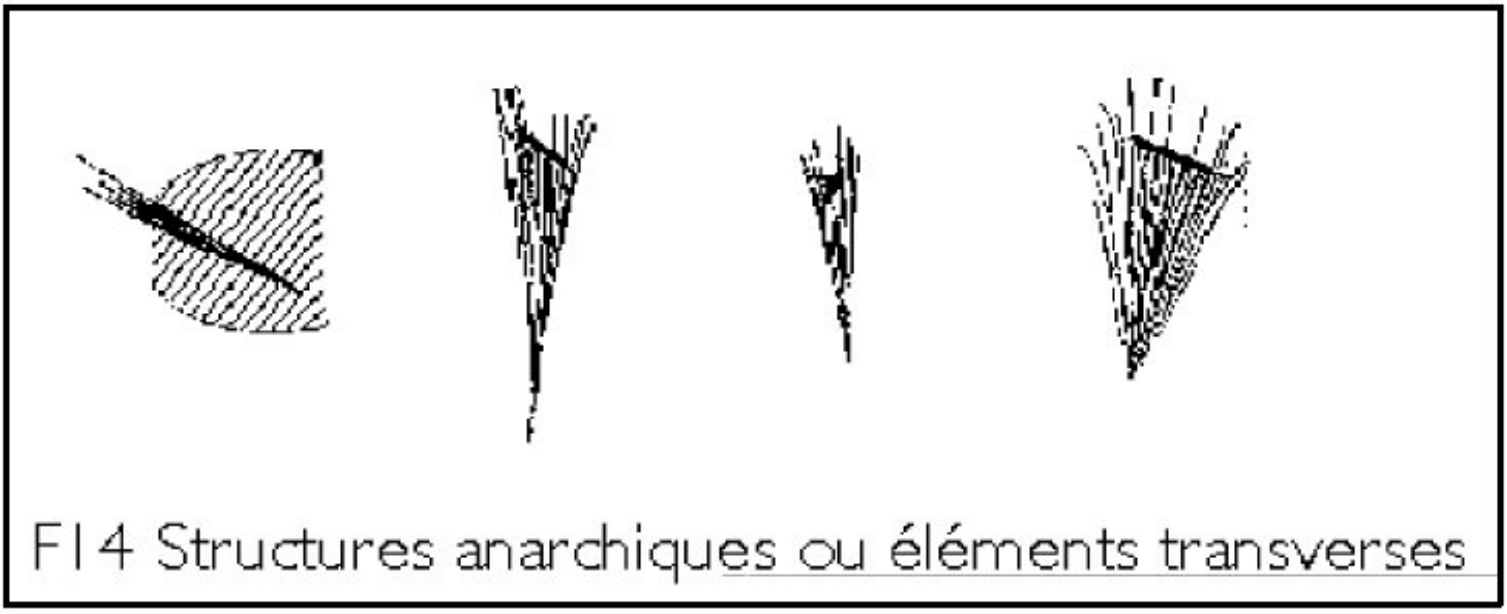
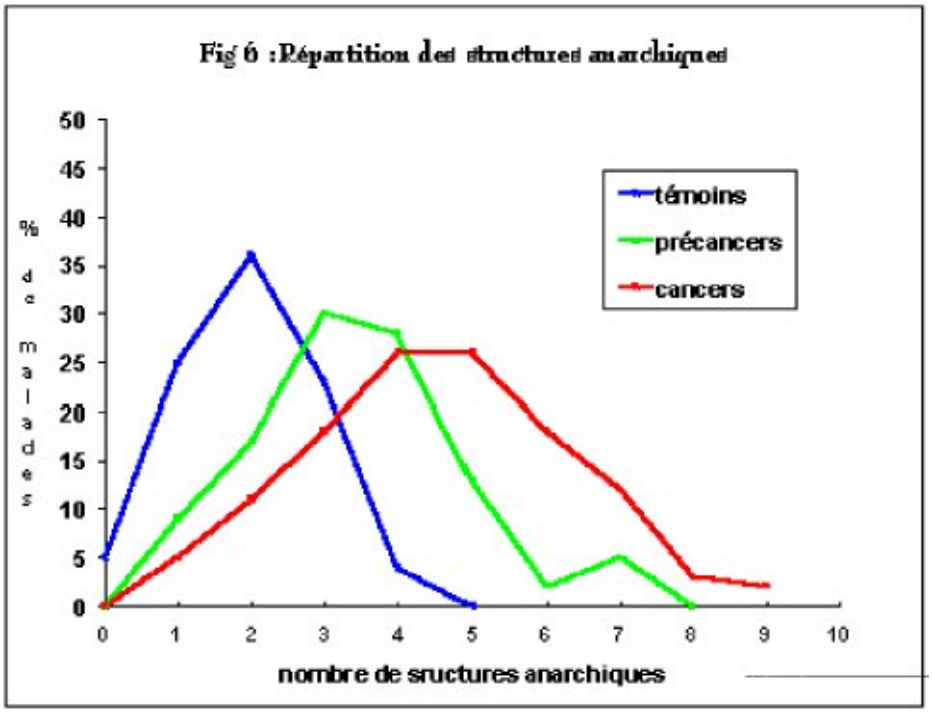


Fig 5 Structures anarchiques ou éléments transverses ou F14

These authors concluded that the sensitivity of the method is in the 56 to 97% range and the specificity in the 83 to 100% range, according to the nature and the cancer status.



The signs correlated with cancer are qualified either « anarchic structures « or « transversal elements «. These signs were also presents in some of the healthy persons. The authors considered the presence of signs for these persons as a risk indicator of cancer arising as an important number of persons of the above group developed an authentified cancer in few months or years following this first investigation.

To-day, few papers reported convenient statistical studies. Spielberger reported significant differences in the follow-up of two populations presenting or not the sign, the population with the sign developed more frequently a cancer. J-G. Barth underlined the importance of experimental conditions on the variability of expected results. Indeed, under optimal determinated conditions, he evidenced a pertinent level of n $ 4 transversal structures for 4 crystallisation sheets. (80% of 4 crystallization sheets.(80% of cancerous patients are within this level). In the group of healthy persons, 18% are above this level, and 78% of this group were found in a precancer status.

These results afforded positive insights in the hypothesis that some crystallisation signs would be considered as good indicators of cancer risk advise. This also means that a more long term prospective study will be necessary.

3 - Occupational medicine

The work of Cocude et al on silicotic patients would serve as a reference for all comparable kind of investigation. The presence of characteristic signs called « major signs « were present in 61% of patients concerned with this occupational lung pathology. This study showed also that 96% of the patients presented a clinical aggravation within three years following the first crystallisation. These signs are also present at the same time in the group of exposed patients not suffering from silicotic disease (53 %) and in a control group (22 %). Furthermore, Multifactorial Discriminant Analysis (MDA) based upon 13 selected parameters allowed a correct classification for 75 % of silicotic patients (P) and control patients (T). The healthy exposed patients are classified as 61 % P and 39 % T. This work showed a good correspondance from one part between the presence of the major sign and the inclusion in the P group (84 %), for another part between the P group of patients and their clinical aggravation (97 %). Indeed, the distribution of healthy patients in the P group according to the MDA reinforce the hypothesis that the presence of a major sign should represent a risk indicator of further development of this specific lung pathology.Nevertheless, these results needed a prospective study to be confirmed.

4 - Medical practice

The former studies set up a number of essential basis in order to use the method in medical practice either for diagnostic guidance or for the "follow up" of patients. These latter cases point out either improvements or damages even a long time before visible clinical or biological signs could be seen on a global point of view or when localized to different organs.

This method also allows a "follow up" of the therapeutic efficacy.

Crystallografic configurations of hydrated cupric chloride crystals grown from aqueous solutions with a small amount of diabetic blood added (Takashi SHIBATA)

By Takashi SHIBATA[1)], Akemi TANAKA[1)], Mitsuko KOGURE[3)]

Tomiko IGUCHI[4)] and Tomoya OGAWA[5)]

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Abstract

The crystal growth equipment controlled by computers having a window to enable observation of the growth process with various devices to regulate the factors that are supposed to affect crystal growth, such as temperature, humidity, and mechanical vibrations has been manufactured by Shibata to perform further studies to analyze the growth mechanism.

This third generation equipment is composed the following hardware: 12 sets of crystal growth units in a crystal growth chamber, units to the control temperature, humidity, air flow, and other environmental parameters, recording units including a 35-mm camera, laser-video disk-camera, and a stereoscope with a camera and video camera, pH electrode, laser sensor, Petri-dish-hand, all operated by a 6-axis robot with auto-changing hands controlling by three linked personal computers **(Fig 1)**. It also consists of a series of software that control the whole systems according to program and

outputs the controlled conditions and observation results. Pattern analysis was performed by a customized software.

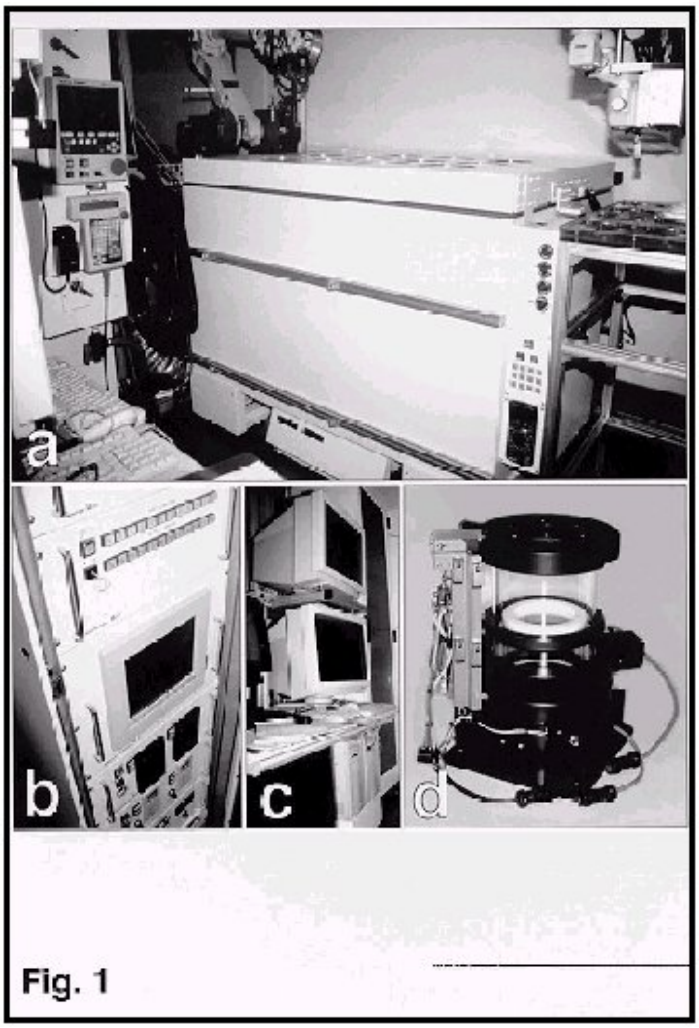

Fig. 1

Using the above equipment, we have found the specific dendritic crystal growth patterns of diabetics [1-2] and healthy subjects to differ significantly and have analyzed the correlation between pattern frequencies and nearly 90 different blood components (**Table 1**) [3]. Dendrites of hydrated cupric chloride crystallized from dihydrated cupric chloride aqueous solutions to which a slight amount of blood from 6 diabetic patients, and 6 healthy persons as controls, was added in vitro, were grown in uncovered Petri dishes under conditions of 28°C and 45% relative humidity. Multiple nucleation developed from pure cupric chloride solutions, however, addition of 0.5 %vol blood strongly inhibited nucleation. As a result, radial growth of dendrites formed from an average count of about 1.5 initial growth points (**O**) per dish was

Analyses	Items
Index of blood sugar	hemoglobin A1, hemoglobin A1c fasting blood sugar
Pancreas function	insulin , trypsin
Serum Proteins	total protein , Alb ,α1-G ,α2-G ,β-G ,γ-G, A/G ratio , fibrinogen , cerulo plasmin
Amino acids	quantitative analyses of 41 Amino acids Taurine, Thiophosphoethanolamine, Urea, Aspartic acid, Hydroxyproline, Threonine,Serine, Asparagine, Glutamic acid, Glutamine, Sarcosine, α-Aminobutyric acid, Proline, Glycine, Alanine, Citrulline,Valine, Cystine, β-Aminobutyric acid, Cystathionine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine, β-Alanine, 1-Methylhistidine, γ-Aminobutyric acid, Homocystine, α-Amino-isobutyric acid, Lysine, Monoethanolamine, Histidine, 3-Methylhistidine, 1-Methylhistidine, Carnosine, Anserine, Tryptophan, Hydroxylysine, Ornithine, Arginine, total amino acids total AA, NEAA, EAA, BCAA, EAA/NEAA, BCAA/Total AA, Fischer ratio
Lipid	total lipid , total cholesterol
Pigment	bilirubin (direct, indirect)
Nitrogen	urea nitrogen , creatinine , cretonne , uric acid
Electrolyte	Na , K , Ca , P , Mg , Cl
Metal	Fe , TIBC , UIBC , Cu
Porphyrin	protoporphyrin
Thyroid gland	total serum thyroxin
Blood counts	WBC , RBC , Hb , MCH , MCHC, Ht , MCV , blood platelet
Morphology	white blood cell , red blood cell

Table 1

achieved by adding blood.

Crystal forms in the shape of convex lenz consisting of two dendrite arches near the center of the crystal growth point were observed (**Fig 2**). Their specific crystal growth forms were divided into three categories according to the longer diameter of the convex lenz shape: small (**S**) <8 mm , median (**M**) $8 mm but < 20 mm, large (**L**) $20 mm. (**Fig 3**)

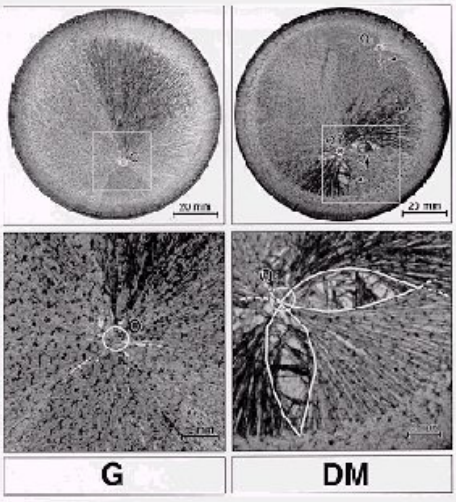

Fig. 2

S growth forms correlated with serum concentration of blood were generated by the blood of both healthy persons and diabetic patients, whereas **M** and **L** forms were specific to diabetic patients according to the results of testing by student's t test, because the number of **M** forms per twelve dishes was 14.2"1.4 when diabetic patients blood was used, versus 2.50"0.63 in the control group, and because the number of **L** forms was 6.50"0.67 and 0.33"0.13 with diabetic

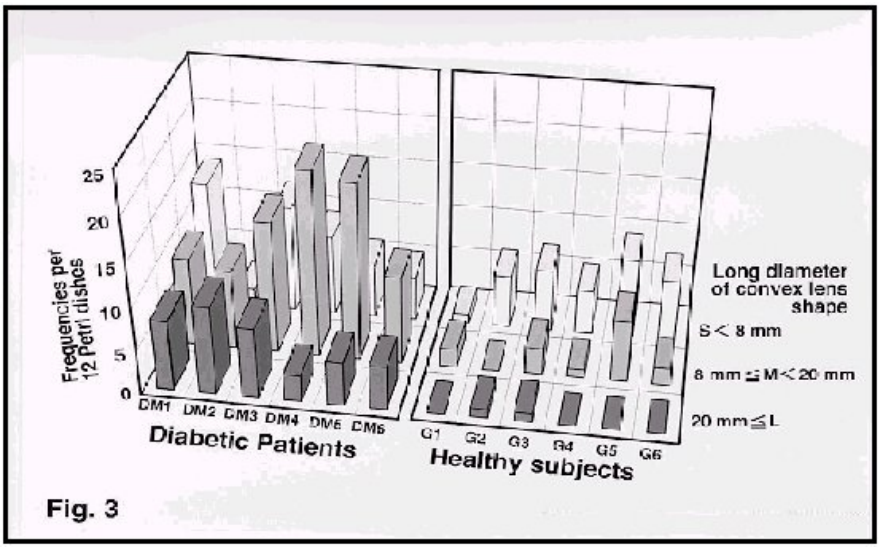

Fig. 3

patients' blood and healthy persons' blood respectively. The occurrence of these **M** and **L** forms was dependent on the concentrations of HbA1, HbA1c, glucose, creatinine, uric acid, platelets, and ceruloplasmin.

The above findings show that blood has a great effect on growth of hydrated cupric chloride crystals and that different crystal shapes form when exposed to diabetic patients' and healthy subjects' blood.

Figure 1 : Computer controlled crystal growth and analysis system designed by Shibata. a : Crystal growth chamber and 6-axis robot, b : Electric controller for crystal growth system, c : Computers, d : A crystal growth unit.

Table 1 : Ninty items of quantitative blood analysis performed [3].

Figure 2 : Typical dendritic growth pattern obtained by addition of 0.5 vol% healthy subjects' blood (G) and typical convex lens forms obtained by addition of 0.5 vol% diabetic patients' blood (DM) [3].

Figure 3 : Typical convex lens forms were divided into three categories according to the longer diameter on the convex lens shape : small (S) < 8 mm, median (M) ³ 8mm but <20 mm, large (L) ³ 20 mm. Sums of the three categories in 12 Petri dishes grown simultaneously per person are shown [3].

Références :

[1] **Selawry A und Selawry O :** Die Kupferchlorid-Krystallisation in Naturwissenschaft und Medizin. Gustav Fischer Verlag, Stuttgart (1957).

[2] **Piva MT, Lumbroso S, Sieso V et al** : Cupric chloride crystallization with human blood study of pictures obtained in different pathologies . Elemente der Naturwissenschaft 61 : 25-39, 1994.

[3] **Shibata T, Kogure M, Iguch et al** : Effects of Diabetics' Blood on the Growth of Hydrated Cupric Chloride Crystals from Aqueous Solutions. **J Tokyo Wom Med Univ** 6-7 : 346-357, 1998

Medical prospective utilisation of copper chloride crystallization in the presence of blood (Victor SIESO)

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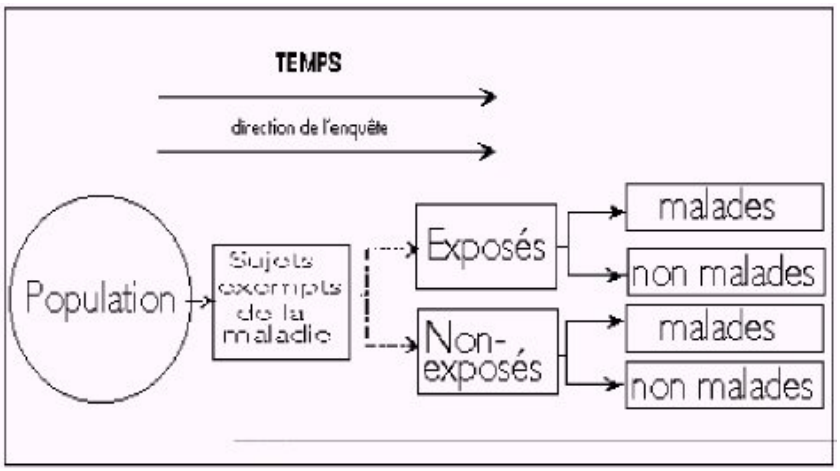
Service de Biochimie Médicale B - Hôpital Saint - Eloi, Avenue Bertin Sans - 34295 Montpellier - (France)



As some crystallization forms appear early, showing latent organ perturbations, we think that the present method would be an interesting tool needing nevertheless further evaluation in epidemiological studies.

Aim of the study

The interest of the crystallization method in the early evaluation of a pathological status concerning an healthy population, followed over a year or more by a single veinopuncture.

Fig 1 : Study of cohorts.

Method

1. Voluntary subjects recruited among

- the general population for one part :

* workers from the biochemical laboratory (occupational medicine)

* students from the medical school (preventive medicine)

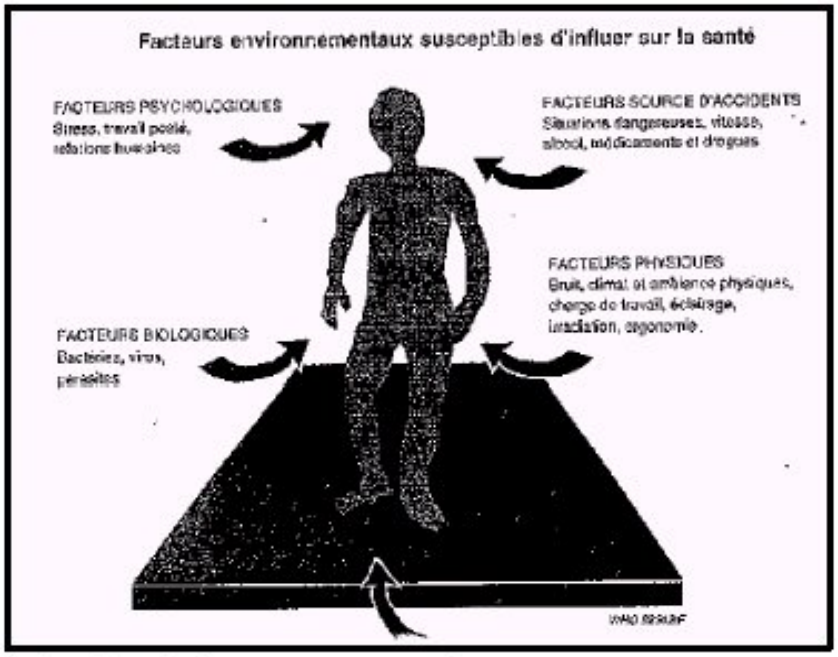


Fig 2 : Facteurs environnementaux susceptibles d'influencer la santé.

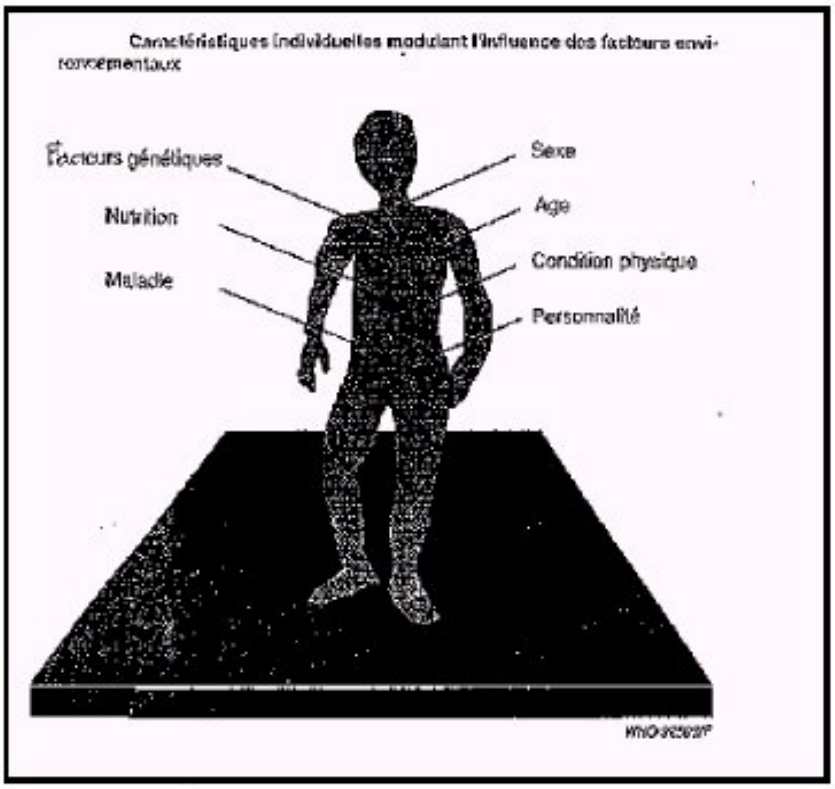


Fig 3 : Caractéristiques individuelles montrant l'influence des facteurs environnementaux

* some patients consulting in our laboratory

- a risk exposed population for another part such as workers of extractive industry.

1. The set up of a medical check-list for everybody

2. The realization of a statistical study from the different crystallization pictures obtained at different times with distinct blood samples and the comparison of them.
Conclusions

Problems

*To obtain the consent of the whole recruited population over the time of the study (i.e. to explain that the study is a preliminary scientific one and not a real "mass screening".

*To obtain the complete "follow-up" of the volunteers.

*To determine the acceptability, the feasibility and the reproducibility of the present test.

*Interpreting the cristallization pictures correlated with types of diagnosis.

*The cost of the test.

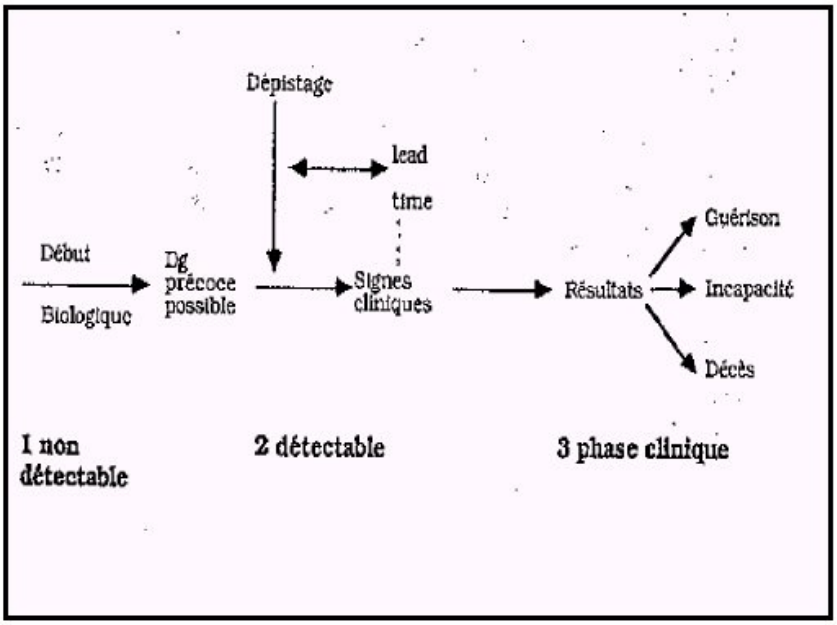


Fig 4 Place de la cristallisation dans le suivi d'une population.

Expected benefits

Not to give a diagnosis but to drive pertinently in shortened time towards more specific explorations, such as scanning, NMR imaging, echography, and so on, if necessary.

To extend this crystallization test more systematically to a clinical validation study in order to realize a suitable» mass screening « test for secondary prevention. »

Q: M. MARQUET

I would like to comment on the pneumoconiosis study: the crystallography allowed you to confirm a diagnosis which was known as well as a potential for development. This is not surprising given that this disease always has a potential for development. I wonder about the choice of the population of retired persons from whom blood samples were drawn?[1]

A: M. COCUDE

At the time the first blood samples were drawn the mines were still in operation. Dr. Amoudru who is here with us today monitored the beginning of the study. There were two types of patients namely active and retired people. They were monitored by the Coal Mines either through occupational health or post-professional services. As Mrs. Piva stated, there were three groups of subjects, those who had recognised pneumoconiosis, those who did not have recognised pneumoconiosis but who were exposed to risks, and a third group of subjects not exposed to risk.

The goal was not to give a prognosis but rather to evaluate the risk of appearance of the disease in those subjects who had no clinical or radiological evidence of pneumoconiosis. The initial objective was to find an indicator capable to anticipate the appearance of the illness in the absence of clinical signs.

Additional Comment: M. MARQUET

I saw things differently: I wondered whether crystallography might be used to confirm exposure. I was thinking particularly of another illness, asbestosis. Is there a possibility in this area?

A: V. SIESO

This is the goal of the study which was presented. For the exposed group we would choose miners and asbestos and porcelain industry workers.

A: J-G BARTH

With regard to my paper of this morning I should add that while we can see correlation between crystallisation from a patient and his illness when it is known, the opposite is more difficult. The pneumoconiosis images, which we called major signs, also appear in smokers and people with lung

cancer. Establishing a relationship between a particular crystallisation image and a medical diagnosis is possible, with a certain number of precautions, in a particular context, and taking frequencies into account. The appearance of specific crystallisation images would draw a doctor's attention to the fact that a major risk might be present.

Comment: M. MARQUET

Appearance of lung cancer, working with asbestos, smoking, it all sounds rather complicated.

Q: B. MAHIEU

The various signs found on the crystallisation plates can be considered as indicators of a change in an organ (the lungs, in the case of pneumoconiosis among coal miners). What we would like to find are early markers of biological effects, ideally specific to each pathology and detectable before the appearance of the illness itself.

The methodology proposed by Mr. Sieso seems fully suitable for finding an exposure marker. On the other hand, the number of subjects studied (50 people per group) seems too low to be able to hope for significant differences between the groups.

V. SIESO

For the planned study it would be good to have a population of one hundred people. It would also be good to have automatic image analysis.

T. SHIBATA

It is Mrs. Piva and Mr. Barth's area which interests me particularly. If I understood correctly the predictive potential of the method, there is a lapse of time between the moment when the illness is not present and when it appears. During this phase — because the method is very sensitive — we can hope to see this development on the surface of the crystal. The physical analysis methods described earlier (in the case of diabetics) are applicable.

M. COCUDE

That would make it possible to monitor a latent pathology. But I would like to ask Mrs. Piva this question: How to we use a crystallisation when there are no clinical signs?

M-Th. PIVA

That is the problem in prevention. A sign appears, a potential illness may emerge. We inform the doctor because it would be difficult to inform the patient, not knowing the psychological impact which this information could have. Sensitive crystallisation is of little value in a hospital setting because there are other investigation methods. The value of the method is in prevention, in a context of general practice, and especially in the case of occupational illnesses.

M. COCUDE

Stresses could also appear. Would crystallisation be able to reveal them?

M-Th. PIVA

We know that stress has a clinical effect on people. Since crystallisation measures the biological effects which result from them, there is a risk of crystal modification.

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[1] The blood drawing involves a simple finger prick. The few drops of blood which appear are sufficient for the crystallography study (Editor’s note).

V. THE AGRICULTURAL FOOD APPROACH

V [2]THE AGRICULTURAL FOOD APPROACH

Biocrystallization in agricultural plant quality research

(Jens-Otto ANDERSEN)

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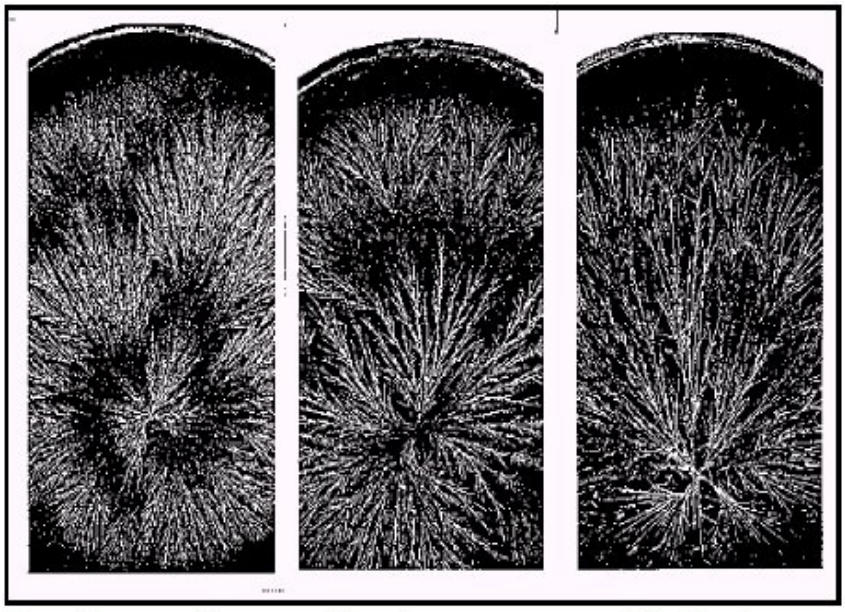
The biocrystallization method, also termed «sensitive crystallization» and «copper chloride crystallization», was originally introduced by E. Pfeiffer in the 1930´ies.The method is based on the crystallographic phenomenon that when adding specific inorganic ionic substances, and generally all organic substances, to an aqueous solution of dihydrate $CuCl_2$, crystallograms with reproducible dendritic textures are formed during crystallization.



Sections from 3 images applied in a computerized image analysis study of degradation of carrot extract over 7 days. The images represent the days 1, 4 and 7 (from left). The total number of 7x3 images were up to 100% successfully classified.

Among numerous organic compounds examined proteins and N-containing compounds exhibit unique abilities to generate complex and coordinated textures, with numerous micro- and macroscopic morphological features.

The method is today applied primarily in medical and agricultural research. A favoured field of application in the latter research area are comparative studies of the effects of different farming and fertilization systems on product quality. Examples are presented from the application of the method in agricultural plant quality research, and from correlations to traditionel chemical-analytical methods.

It can be argued that a major limitation for a wider application of the method is the lack of standardized methods for evaluating, quantifying and classifying crystallogram textures, on the basis of visual classification, or on the basis of computerized image analysis.

An ongoing study involving computerized image texture analysis is presented. The analysis is based on 256 grey-levels, five resolution scales, and 23 histogram and grey-level-cooccurrence-matrix parameters, which are combined to perform a classification by means of stepwise discriminant analysis. Results are presented from classification of a total set of 33 crystallogram images, and a subset of 21 images, originating from a carrot extract degradation experiment over seven days. Results indicate that the image analysis program can classify the images correctly according to the seven degradation days. Perspectives for applying image analysis are briefly commented.

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Balzer U. & F. (1993): Picture-developing methods. In: Mäder et al.: Effect of three farming systems (bio-dynamic, bio-organic, conventional) on yield and quality of beetroot in a seven year crop rotation. Acta Horticulturae 339, pp. 11-31.

Henriksen C.B., Andersen J-O., Laursen J. & Nielsen A.A.: Computerized texture analysis of biocrystallograms for food quality evaluation. (in prep.).

Manufacturing process with plants : contribution of the crystallization method using cuper chloride (Jean-Georges BARTH)

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C. BALLIVET : Institut Kepler, avenue Clémenceau 69230 St GENIS LAVAL.

The cuper chloride crystallization method was used to characterize the quality of many chemical and biological additives. The purpose of our study is to search operative conditions making possible to distinguish effectively two closely related additives so it would be possible to use the method as a factor for assesing a manufacturing process with plants such as medicinal plants.

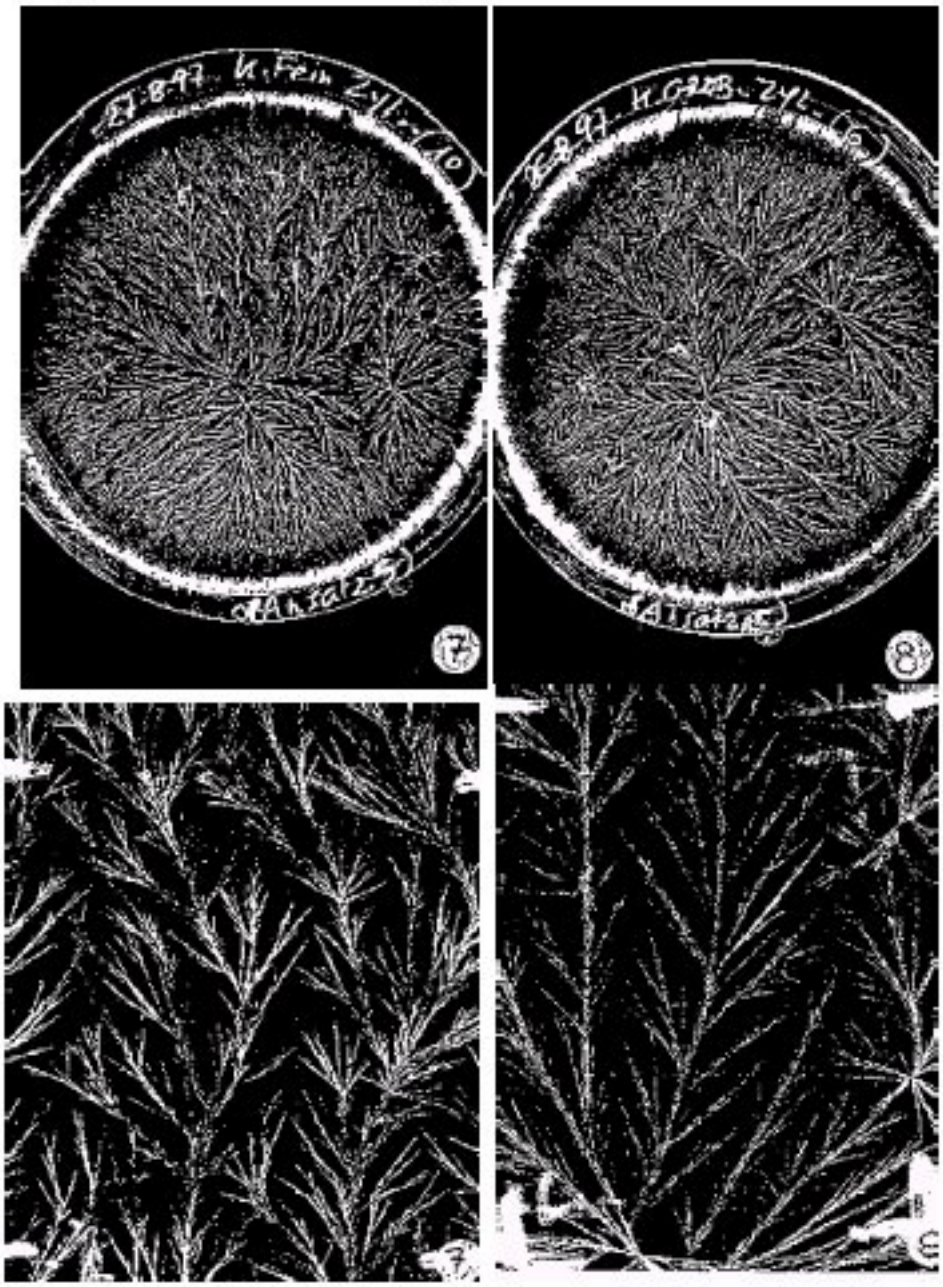
Two hydroalcoholic decoctions (DG and DF) are prepared with subterranean parts from Chamomilla recutita (L, Rauschert) got from the same harvesting. The manufacturing process was the same, except the granulometry of the cutting down of the root. Different cristallizations conditions were studied (standard, with « coat «, moist and dry conditions) and the ability of the method to discriminate was assessed with each one (succes of blind sorting 72 crystallization patterns) ; the time of the crystallization phases were noted (whole time, appearance time of the first germ and the duration of the strictly cristallization phase).

In standard conditions, moist or dry, we obtaired patterns where structure and texture were counterbalanced. It is difficult to distinguish DG patterns from DF ones, and the ability to discriminate is moderate or insignificant (74% and 0%).

With the « coat « two morphologic types are clearly characterized, where the structure is dominant in DG patterns and the texture in DF patterns. The ability to discriminate is high (94% and 100% in moist or dry conditions respectively). The «coat» leads to homogenize the evaporation time and the strictly crystallization time, in moist or in dry conditions.

Conclusion : these

Images de cristallisation obtenues en conditions sèches (HR= 70 à 73%) avec "manteau"en présence de décoction DF(7) et de décoction DG(8)



experiences show that it is necessary to search particular operative conditions for making possible to distinguish effectively related additives. The « coat « allows to give relevant patterns and satisfactory diagnoses. Other experiences are needed to explain the influence of the « coat » on the crystallization process and to consolidate the results by using other analysing methods.

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MANDERA R., BALLIVET C., KNIJPENGA H., (1990) : Untersuchugen mit der Methode der empfindlichen Kristallisation am Bilsenkraut (Hyosciamus niger). Elemente der Naturwissenschaft N° 52, p1-27.

M. LUSSEYRAN

Traditionally, when we use a physical phenomenon for metrological purposes we seek the highest degree of «determinism» possible and if possible a linear relationship which is easier to apply. In the case of crystallisation we are in a completely opposite situation. We use a physical phenomenon in what we call sensitive conditions which are clearly those of a dynamic, non-linear system in chaotic conditions. I think that this could change the point of view regarding the development of the method. This is something rather unusual, there is almost no other example.

But the conceptual framework remains to be defined. This aspect complements the research being done on structure and texture. Would it be possible to characterise the dynamic process which creates the image rather than seeking just final reproducibility? With the preceding hypothesis, image reproducibility is impossible. On the other hand, we should be able to observe the conditions which characterise the dynamic process of image formation. This would allow us to learn which still unknown control parameter is linked to evaporation speed. For example we see that the mantle and the chimney play a role. This would allow us to say whether the plate can reveal what it has to reveal. I think there is something to be found in the area of «determinist chaos» for evaluation of the proper conditions for image appearance.

M. COCUDE

You are opening up a very big issue. Perhaps the last speakers, or the first ones, could comment on this.

A: J-G. BARTH

Indeed, we are thinking about the conditions required for reproducibility of a given application, with human blood for example. The question is also valid for agro-industrial applications. I am not sure how to structure the issue practically, I am not a physicist. A physicist's contribution would be very important for work on operating conditions.

C. BALLIVET

I don't have an answer but I would like to thank Mr. Lusseyran for presenting the issue in this manner because the experiment described by Mr. Barth is quite astonishing. You saw in standard conditions the crystallisation images which we are used to seeing and which we consider successful in terms of sensitivity. The idea of testing this new system is exciting but I think that physicists will best be able to give use the concepts which we need for this work. The importance of this conference is that it has brought together several disciplines. The concepts of one of them can help the others.

M. COCUDE

You are anticipating the conclusion. That's exactly what I was planning to say. We achieved something very important today. Beyond just bringing together people who are usually very far apart geographically like Mrs. Ballivet and Mr. Shibata, we also managed to bring together people from very different fields around a common topic. The discussions have been very rich, but this is just the first step. For the next crystallisation conference (in 2000?) we should add still more fields: virtual imagery, physics, crystallography, etc. Mrs. Ballivet's comment was a very important one. At the beginning of the year there was a special issue of «La Recherche» dealing with shapes. It is amazing to see what happens in the development from the embryo to the mature organs, for example the formation of bulls' horns. With regard to crystallisation I think we must find this sort of approach. I don't know whether it is a chaotic approach or not, but we probably need a conceptual tool.

Mr. Lusseyran, could you speak on this issue? You used the word chaos. What do you mean by this exactly and how does it apply to the subject which interests us?

M. LUSSEYRAN

This is not a simple issue. I was very interested in the attempts to quantify temporal decline in crystallisation. This would mean initially using the formation phase and trying to describe it in dynamic terms, and thereafter looking to the formed image. This isn't easy because series which are long over time would normally be needed for such a study. This system is not well suited for this purpose. For the moment, I don't see a practical way to approach this. More thinking must be done to be able to understand everything from the final crystallised image.

M. COCUDE

Going back to the article from «La Recherche,» we are able to understand many things, to find out the «how», but the «why» is still very problematic. I think the same is true for our area of crystallisation.

M. CHAMBOLLE

I would like to comment on the value of such methods applied to agro-industry, agronomy, etc. It is clear that both scientists and those working in product quality control, both producers and processors, are interested in general methods which would yield discriminating results, even without knowing the exact mechanisms which govern it. However, repeatability and reproducibility are essential for these methods to become accepted. Researchers need methods which are comparable between one laboratory and another as do any other people using analyses or tests to characterise products. This is

not an area which I know well. My impression today is that people are thinking about the issues of reproducibility and repeatability and that they understand that they are crucial for these methods. They are also behind the work done in equipment design, mechanisation, robotics, etc. This aspect is nonetheless secondary with respect to our current concerns which are to link the observations of these phenomena with other phenomena, biological ones in particular. For the time being the repeatability and reproducibility for these methods is not very high. Their diffusion should therefore be handled cautiously. These methods are attractive because they appear simple — I stress the word *appear* — and because they give very discriminating results. It is important to make sure that these methods do not lead to fads with people seeking immediate applications and undertaking work which is dangerous for themselves and for the «cause» which we defend, so to speak.

M. COCUDE

You are right. The topic of our discussion today is not public, not by a long shot. There has been discussion of some alternative methods, but for sensitive crystallisation I know of no media who have taken an interest in this area.

With regard to reproducibility and repeatability, my impression is the same as yours. It is essential and, unconsciously, the people who have results to present, results which were obtained with much effort, trial and error, tend to forget all the difficulties once they achieve the result. The scientific requirements are even clearer in this area. We must be very rigorous if we want to make statements which do not require disclaimers. This is one of the major points discussed today: we can’t be too careful in establishing and following rigorous protocols.

We cannot avoid all difficulties. As Mr. Barth showed us, two different products, in two different concentrations, can yield the same crystallisations. This is a problem. Isn’t this related to the notion of universality class mentioned by Mr. Fleury this morning?

Presentation: M. ANDERSEN

In the sciences we are faced with the challenge of understanding natural phenomena both in detailed parts and in complex entities or «totalities.» For the former, we have made enormous progress in the study of minerals, chemical compounds and even atoms. In many cases computer models can provide useful information concerning the behaviour of complex systems. With regard to the complex «totality» of living organisms we are still in the same situation as the obese person who wants to play golf. His problem: when he can see the ball he can’t reach it and when he can reach it, he can’t see it. In other words, when we as scientists are confronted with living systems, we do not have standardised methods and concepts which allow us to understand the living organism as a whole. To put it another way, when we can «reach» it (using analytical methods) we are unable to «see» the living organism because the unique «totality» has disintegrated and disappeared.

We need methods and concepts which will give us the precision of analytical methods but which simultaneously allow us to see, to approach and to understand the living organism in a more comprehensive manner.

One way would be with a morphological approach: bio-crystallisation is such a method and it could provide an important contribution. This is a difficult process because complex forms are much harder to «conceptualise» and quantify than are numerical results. But these shapes offer unique information regarding various aspects of living organisms which cannot be had with an analytic approach.

CONCLUSION (Marcel COCUDE[1])

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At the end of a day which has seen many comments and questions from all of us, I would like to stress a few important points in conclusion.

*** An element of great importance is the meeting of practitioners and theoreticians**. While Copper (II) Chloride crystallisation with additives has been used for decades with certain results, there are now efforts to further validate the method.

For practitioners, the scientific method legitimises their approach and leads them to think about the theoretical bases of their work. This should lead to a better correspondence between methods and objectives.

The theoreticians have much to gain from the questions of the practitioners regarding their ways of decoding the concrete problems which arise.

A second topic for further thought is the necessary improvement of tools and techniques.

- Intellectual tools: it would be useful to apply concepts such as universality classes and determinist chaos, which have been used for some years by physicists, to sensitive crystallisations.

- Materials and methods: technology provides researchers with constantly improving means. This means observing (with more and more automated methods) the phenomenon of crystallisation itself in its different static and dynamic aspects, the nature and geometry of the deposits and their relationship with earlier deposits, the role of copper and the additives in the solutions, etc. This approach would have to acknowledge the three-dimensional nature of the phenomenon.

A third point for further thought is cause-effect relationships.

We have seen the impact of various simple additives in blood or vegetable juices on crystallisations.

Leray's work is still very significant and should be continued.

Studies to demonstrate the impact of various components, taken separately and then in combinations of two or three, could provide references for crystallisation and necessary definition of the confidence interval.

This brings us back to the issue of methods (specific climatic conditions, quality of supports, etc.) and respecting strict standardised protocols.

The last point regards applications.

Research on the crystallisation of a given additive (or group of additives) is a legitimate approach to be able, inversely, to detect the presence of certain additives by inspecting crystallisations. When we try to evaluate the «quality» of a biological material, we are faced with the enormous complexity of samples such as blood or vegetable juice, which makes it difficult, if not impossible, to completely describe them. We have great difficulty in determining which component(s) carry the pertinent information although in the case of blood we can say that proteins play an important role.

And are there really pertinent elements? Isn't it rather these elements together, taken as a whole, which carry the information through their simultaneous presence and/or their relative proportions?

This is the basis for research on using the method in a general way. Even if the human spirit is not fully satisfied until it has a rational explanation of observed phenomena, knowledge of the mechanisms does not have to be a prior condition for the use of the method.

The current state of our knowledge allows us to make certain proposals.

- In medicine, we can reasonably propose the hypothesis that crystallisation, without being a prediction, can reveal the risk of appearance of a new illness, the recurrence of a past disease, or the development of a chronic pathology. This hypothesis must be validated.

Experiments have been proposed to follow up on the studies undertaken throughout the 1990's. At the time, the authors had mentioned that they called for crystallographic and clinical monitoring of the subjects. The difficulties here are significant because many outside factors can have an influence throughout the duration of the experiment, particularly medication therapies which might be used.

- In the agro-industrial area the approach has been defined. This area deserves further efforts in light of the growing interest of the public in the quality of the food which they eat and its impact on health.

We all understand that this field of investigation and the related scientific validations are just at their beginnings. Substantial research is still needed before it can be widely used.