Cupric chloride crystallization with human blood
Study of pictures obtained in different pathologies

Marie-Thérèse Piva, Serge Lumbroso, Victor Sieso, Eric Monnin, Henri Mion, François Blanc, Bernard Magnan de Bornier

Summary:

We have studied the different pictures that appear after cupric chloride crystallization in the presence of blood in a bidimensional circular plane system. We describe the method in its various technical aspects and define 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. 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. This report shows a one to one relationship between pathology and pattern allowing one to »visualize «a pathological state on a crystallographic picture.

Introduction

Experimented by the German biologist *E. Pfeiffer* (1931), the crystallization method was based on the study of patterns shown after water evaporation of a cupric chloride solution in the presence of complete blood, urine, or vegetal extract. In medicine, it has been deeply investigated by *A.* and *O. Selawry* (1957), and considered to be a good tool for orientation in diagnosis. This method was commented on and criticized by *E. Nickel* (1968). Since then, as far as we know, this technique, used for diagnostic purposes, has not shown any development according to the scientific literature. Nevertheless, *Tchebotareva et al.* (1990) used it in pediatry as a pyelonephritis diagnosis element. *Cocude et al.* (1992) evaluate this method for the medical follow up of pneumoconiosis of miners. *Shibata et al.* (1993) used it to probe a population suffering from senile dementia. We have assessed the validity of this technique in a hospital environment by confrontation with different organ pathologies. We studied the relationships between different pathological states and the crystallization forms, followed-up the variations of some seric proteins (pooled within a proteic profile) (*Giraudet et al.*, 1990) and we

examined their possible correlations with the modifications observed by crystallization. Finally, we tried to understand the formation of the different configurations by a rational approach including amino acid solutions or albumin dilutions. Our assays showed the importance of complex proteic mixture in the development of specific forms.

Material and methods

Patients: 35 healthy subjects, 17 men, 18 women, (average age : 48 years) were chosen as control group G0, defined below. 80 subjects in hospital were studied : 28 women and 52 men (average age : 61.5 years). They all originated from the Service de Medecine Interne E, CHU Montpellier, France. They were classified into 8 groups (G1 to G8) according to clinical, biological, anatomo-pathological and radiological data. So, we divided our sampling selection into nine groups as follows : G1, patients with alcoholic cirrhosis (n=25), G2, patients with liver carcinoma (n=12; 6 primary, 6 secondary), G3, patients with digestive tract illnesses (n=11; 3 malignant peritonitis, 3 adenocarcinomas, 5 gastric ulcers), G4, patients with pancreatic pathologies (n=11; 2 cancers, 9 non-insulino dependent diabetes mellitus), G5, patients with lung diseases (n=3; 2 cancers, 1 chronic abscess), G6, patients with cardio-vascular pathologies (n=6; 1 mitral deficiency, 3 auricular fibrillations, 2 hypertensions), G7, patients with renal diseases (n=7; 3 carcinomas, 4 renal failures), G8, breast pathologies (n=5; 2 cancers, 3 fibroadenomas), G0, 35 healthy subjects without any of the below mentioned pathologies and free from drugs for the previous four weeks before veinous puncture were referred as the control group.

Seric protein profile: It was composed of 9 proteins : immunoglobulins (IgM, IgG, IgA), fraction 3 of the complement (C3), and liver synthetized compounds : orosomucoïd (Oro), haptoglobin (Hpt), Transferrin (Trf), Albumin (Alb), and alpha-2-macroglobulin (A-2M) quantified by immunonephelometry (*Laurent et al.*, 1980) (Array-Protein-System-Beckmann). The usefulness of this protein profile relied on the relative variations of protein levels in the blood, as assessed in numerous pathologies (*Giraudet et al.*, 1990 ; *Meliconi et al.*, 1988 ; *Jayle et al.*, 1984 ; Frot et al., 1984). The results were expressed in g/l (grams per liter of serum) and in percentage of the median regarding control values for specific sex and age.

Crystallization: Standard proteic solutions : Glycine, Isoleucine, Histidine, Aspartic acid, Tyrosine, tryptophan, cystine, arginine were purchased from Merck (Germany). Human serumalbumin (HSA) was from Sigma. Solutions were performed in distilled water or in PBS buffer pH 7.4 at concentrations ranging 0.5-15 g/l. The operating procedure for amino acid solutions or HSA solutions is the same as for blood samples, i.e. 3 ml of HSA or amino acid dilution mixed with 5 ml of cupric chloride solution dropped into a glass cupel.

Blood samples were taken by venous punction. The blood samples collected in a dry glass tube (10 ml) were immediately poured and absorbed on an ash-free filter paper of middle porosity (Schleicher and Schull Germany). These blood paper spots,

after drying at room temperature in the open air, can be stored for 10 days at 4° C. The blood paper spots were eluted in 3 ml of distilled water during 20 mn. The hemoglobin level was determined by spectrophotometry according to Van Kampen (1961). The protein contents were evaluated according to Macart (1982) at 625 nm. 3ml of hemolysate were mixed with 5 ml of the cupric chloride distilled water solution 250 g/l ($CuCl_2$, $2H_2O$, Merck ref. 2773) and dropped on a glossy glass cupel (diameter 13 cm, thickness 0.3 cm) limited by a heat-stuck glass ring (diameter 9 cm, height 1.5 cm). Before use, the glass cupels were thoroughly washed in water with a non-tensioactive compound, then plunged overnight in a sulfochromic mixture ; then, they were rinsed, dried and stored in a dust-free environment in order to avoid supplementary crystallization germs. Water evaporation and cupric salt crystallization were achieved during 12 hours in an air-conditionned enclosure (Carrier and Scada, France) controlled for temperature, hygrometry and ventilation. After crystallization, the disks were observed in polarized light (filter type HN 22 ref. 230112, SAR-ELEC, France), under a binocular microscope (Zeiss technical 2, citoval 2) in order to study crystalline morphology, in a relative humidity of less than 70% so us to avoid crystal rehydration. The plates were stored in dry air, or photographed for records.

Preliminary studies allowed us to define optimal experimental parameters.
1.) *Optimal crystallization parameters:* The processing of the plates depended on several factors : 1 – Controlled physical parameters : the assays were performed between 27.5°C and 29°C, with hygrometric fluctuations between 60 and 65%, and an air speed of about 0.8 m/s. Outside these values, patterns were not easily identifiable. 2 – Protein contents per plate : different tests with blood protein contents (Pr) ranging from 0.5 mg to 20 mg for a constant 1.25 g quantity of $CuCl_2$ have demonstrated that optimal configurations in shape, size, and number of the different patterns were obtained with $Pr/CuCl_2$ ratios of about 5.10^{-3} (Pr=6.25 mg). On the contrary, addition with relatively equal amounts of amino acid solution or even serumalbumin showed incomplete pictures of crystallization (Fig. 1a,b,c). We failed to improve the crystal network by progressive addition of amino acid solution (or amino acid mixture) to a defined preparation of serumalbumin (data not shown). 3 – Number of plates : series of 3 to 50 plates from the same blood sample were indispensable in order to obtain a satisfactory pattern. They showed a great number of analogies, but repeated assays from a same patient showed differences in patterns in spite of this. At least 4 plates were generally necessary for minimal information. Sufficient statistical information was provided by 20 plates, occasionally more were needed, especially with multiple-diseases or patients submitted to radiotherapy or chemotherapy. For a given patient, results were expressed, for each form, in relative frequency.

2.) *Definition of the »Crystallization Forms«:* Cupric chloride alone cristallized in a disorderly manner, covering only part of the glass plate surface whatever its initial concentration and with the same volume conditions. It developped clusters of needle-shaped crystals around multiple centres (Fig. 1d, 2a). After addition of a small quantity of a blood sample (less than 1 mg of protein per test), the crystallization became

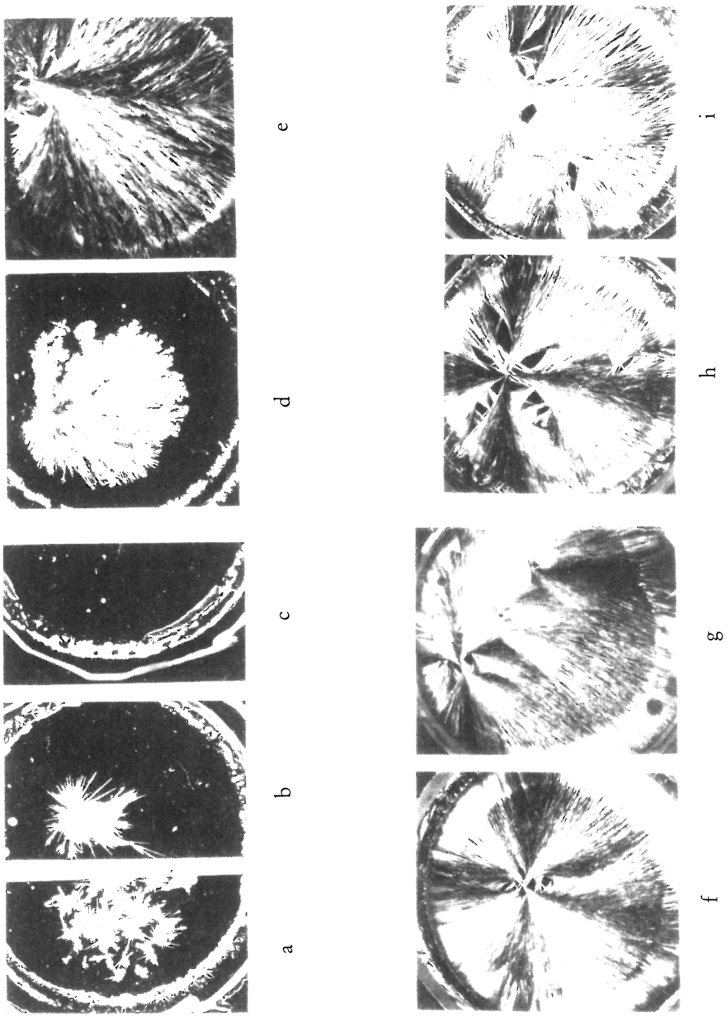


Fig. 1. Photographic patterns from plate crystallization of different samples.
a: 3 mg of histidine.
b: 3 mg of histidine with 3 mg of glycine.
c: 3 mg of glycine.
d: crystallization with cupric chloride on its own.
e: 0,.3 mg of blood.
f, g: blood samples of control subjects.
h: crystallization from subject with alcoholic cirrhosis.
i: crystallization from subject with renal adenocarcinoma, renal tuberculosis and cardiac ischemia.

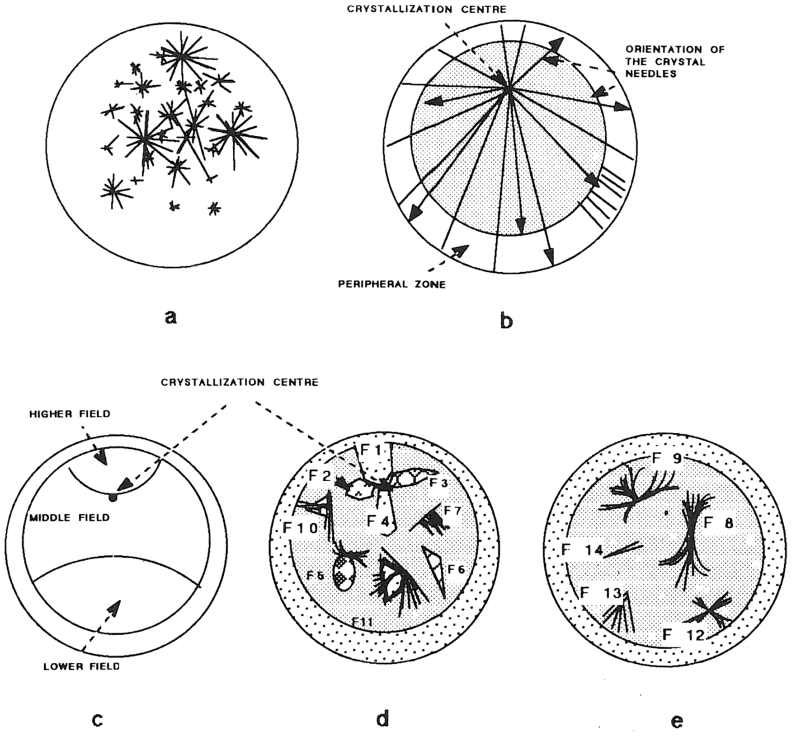


Fig. 2. Schematic patterns of crystal organization.
a: crystallisation with cupric chloride on its own.
b: general organisation of the crystallisation in the presence of blood.
c: schematic organization of the three fields.
d: preferential-fields forms, F1, 2, 3, 4, 5, 6, 7, 10, 11.
e: unsetted-fields forms F8, 9, 12, 13, 14.

organized, covering the whole plate and defining a main centre (Fig. 1e, 2b). We carried out a great number of tests with blood from control subjects. In the absence of clinically detected pathology, the plates showed a crystallization centre from which thin aggregate crystal needles radiated in a regular manner (Fig. 1f, g, 2b). Usually the crystallization centre did not correspond to the plate centre. The aggregate crystal lengths allowed us to define three fields : a higher field with short crystals, a middle field with medium size crystals, and a lower field with long crystals (Fig. 2c). In these fields, both for clinically defined pathologies, as well as in the control subjects, geometrical figures

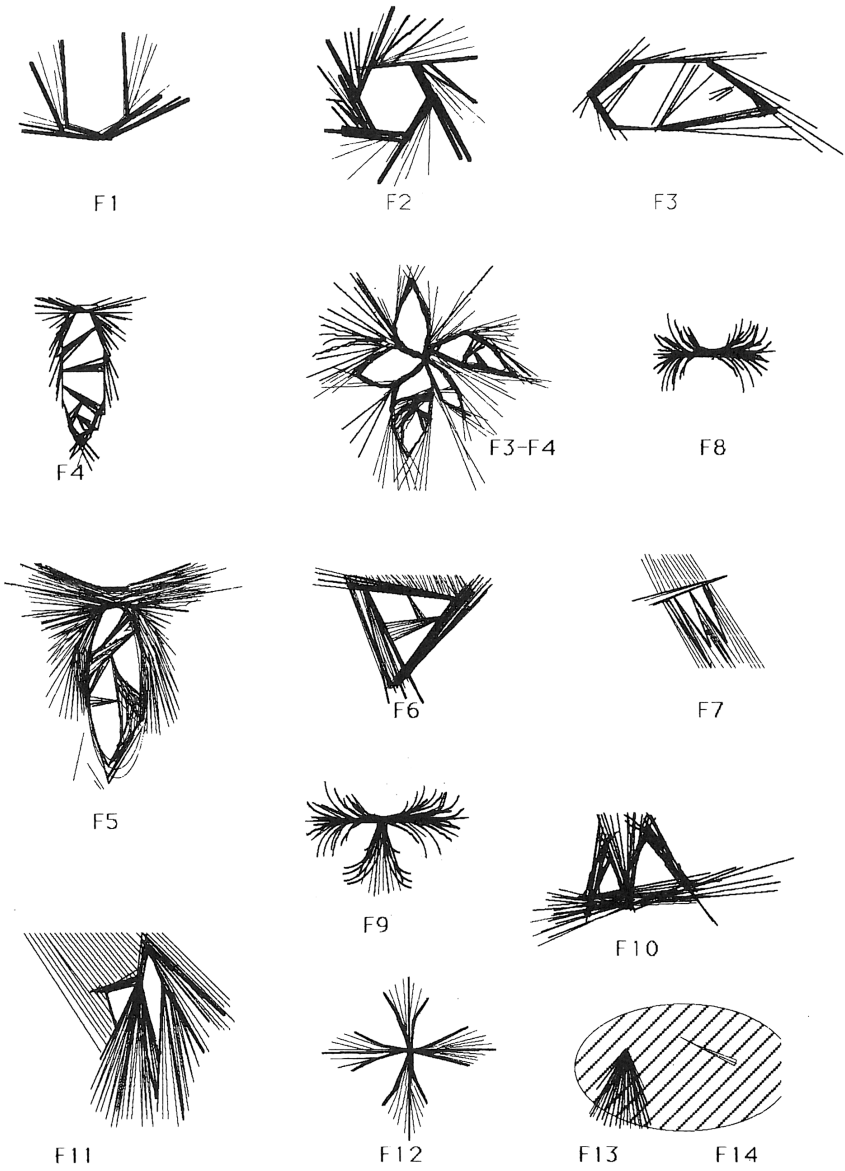


Fig 3. Schematic patterns of the different »Forms«.

appeared designated as »Forms«. We thus determined 14 forms, F1 to F14, shown schematically in Fig. 3. Some were photographed (Fig. 1f – i). Forms 12, 13 and 14 were most often associated with the others, and are not taken into account in the present study. F8 and F9 occurred indifferently in the 3 fields, while F1 exclusively occurred in the higher field. Forms F2, F3, F4, F7, F10 were generally in the middle field, but F2, F3, F4, could also be observed in the higher field. F5, F6, F11 were in the lower field or at the boundary of middle and lower fields. (Fig. 2d and 2e summarize these positions). The peripheral zone was differentiated from the rest of the plate by a thinner crystallization.

Statistical evaluation: Comparisons between the above groups for each of the 20 studied parameters (11 crystallization forms, and 9 seric proteins) were carried out by the Kruskal-Wallis test and or by one-way variance analysis, after transformation of the variables into arc sine $\sqrt{\%}$ for crystallization form frequencies, and logarithm for protein concentrations. These transformations allow the variables to be quantitative and stabilise the variance. The correlation between the different parameters were carried out using distribution free tests (Spearman's rank correlation coefficient). Comparisons between each two pathological groups were performed with Mann-Whitney test.

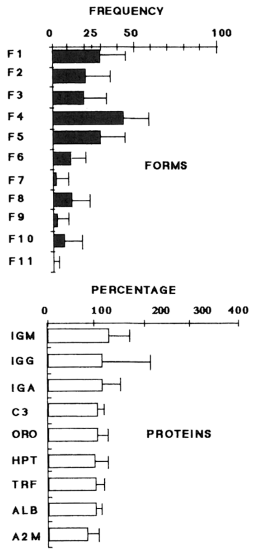


Fig 4a G0 control group

Fig 4. Bar diagrams of the averages of occurrence frequencies of the forms and the averages of the proteic parameters expressed in percentage of the normal median value (100%) for each group

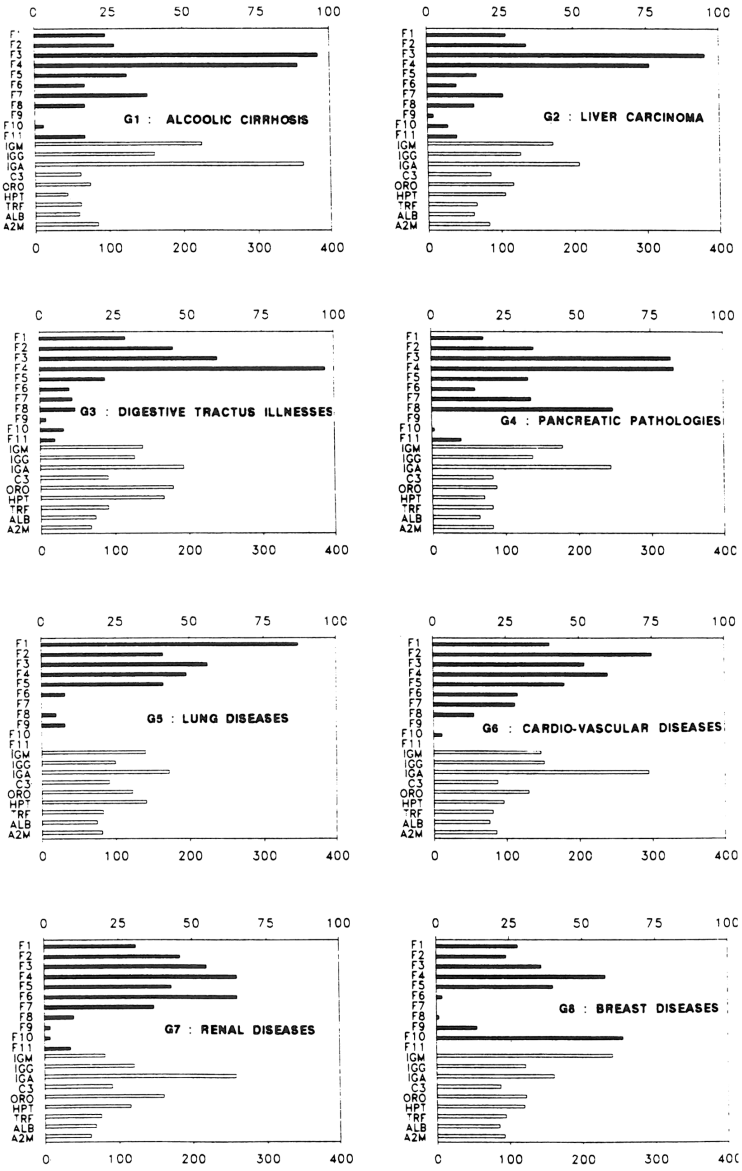


Fig. 4b G1 to G8 pathological groups.

Results

With individual amino acid solutions or their mixture, the crystallization pattern resembles cupric chloride by itself (Fig. 1a, b, c). Only human serumalbumin with an amino acid mixture gave rise to some incomplete forms, so, we did not take into account these patterns in the present study.

We evaluated the percentage variation of control patients (GO group) for both crystallization forms and proteic profile, as shown in Fig. 4a . The results of each pathological group, for both crystallization forms and proteic profiles are shown by the various bar diagrams (Fig. 4b). The following results are obtained taking account of this GO group. We have observed that:

- Form F3 and F4 frequencies were prominent with liver diseases (respectively 96 – 95% and 89 – 76%). In these groups (G1, G2), acccording to the protein spectrum, gamma-globulin levels were much higher with cirrhosis than with liver cancer, and the liver proteins levels were markedly decreased (especially in the case of cirrhosis),
- F4 frequency (97%), IgA, orosomucoïd and haptoglobin were prominent for digestive tract diseases (G3),
- F3 (82%), F4 (83%), and F8 (62%) frequencies and IgA increased in group G4 (pancreatic diseases),
- F1 (87%) frequency prevailed in G5 (lung diseases),
- F2 (75%) frequency and IgA were increased in G6 (cardio-vascular pathologies),
- F6 (65%), F4 (65%) and IgA increased less in G7 (kidney diseases),
- F4 (58%), F10 (64%), and F5 (60%) frequencies, and IgM were the noticeably increased variables in group G8 (breast diseases).

The Kruskal-Wallis test displayed significant differences in the protein contents for five proteins among the eight groups (IgM, $p=0.03$; IgA, $p=0.01$; C3, $p=0.01$; ORO, $p=0.0003$; HPT, $p=0.0003$), but there was no difference for IgG, Trf, Alb, A-2M. The variance analysis confirmed these results.

Fig. 5 shows the frequencies of the crystallization forms in the pathological groups. The Kruskal-Wallis test (global comparison) showed significant differences ($p<0.05$) for all forms except F1 and F5. The variance analysis confirmed these results; furthermore, it revealed a significance for F1 ($p<0.05$).

The comparison between each of two groups by the Mann-Whitney test showed a significantly high frequency of F1 ($p< 0.05$) in groups G5 (lung pathologies) and G6 (cardio-vascular diseases). It was noteworthy that the Kruskal-Wallis test penalized the small number of patients studied in G5.

The Mann-Whitney test applied to F5 only, showed significant differences between breast pathologies (G8) and G1, G2 and G3. The other statistical tests did not show significant differences for F5 in the groups considered.

Table I a recapitulates results of the Mann-Whitney test comparing, for each form, each pathological group (G) to the pooled 8 other groups ($\bar{G}$). The forms, F1, F2, F3, F4, F6, F8 and F10 did appear with significant frequency, respectively in lung, heart, liver, digestive-tract, kidney, pancreas and breast diseases. The Spearman rank

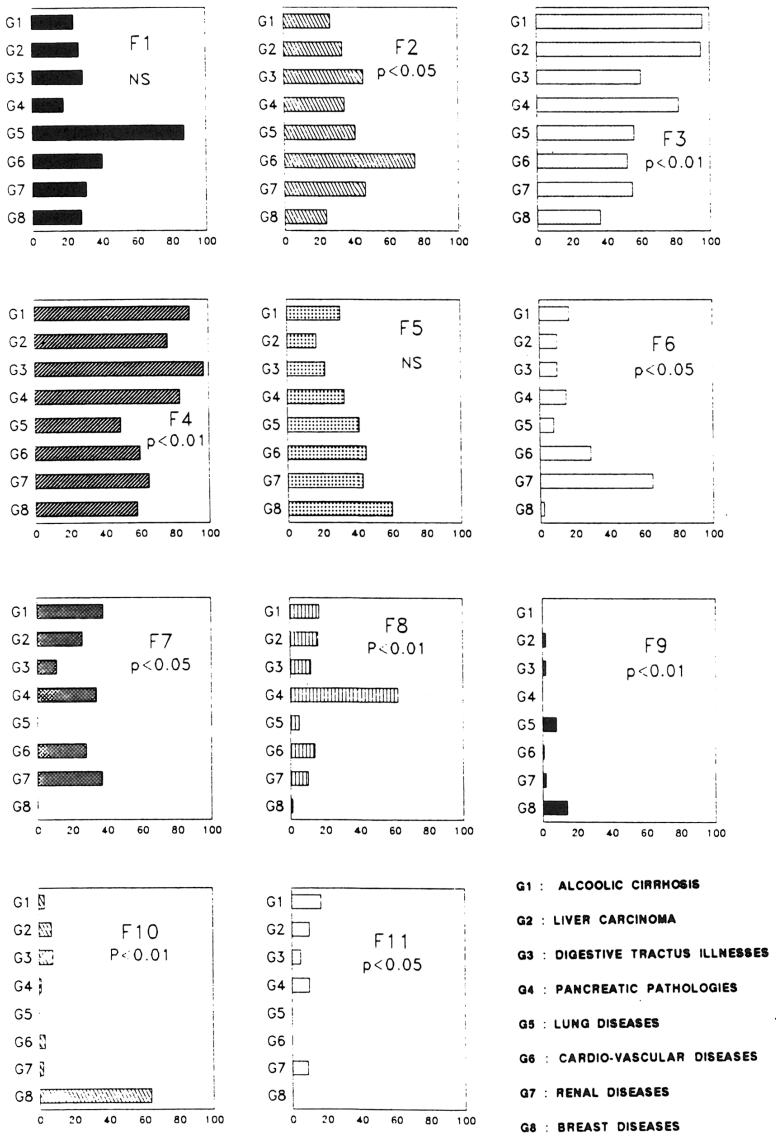


Fig. 5. Bar diagrams of the occurrence frequencies in the different pathological groups (G1 to G8) for the 11 forms.
The significant degrees of the Kruskal-Wallis test (p) are mentionned for each form; F1 and F5 only are not significant (NS).

test in the studied population (80 subjects) showed a significant correlation between some forms : F3 and F4 (r = 0.46 ; p< 0.00001), F3 and F7 (r = 0.4 ; p< 0.0005), F3 and F11 (r = 0.36 ; p< 0.OO15), F10 and F5 (r = 0.41 ; p< 0.002), F10 and F9 (r = 0.38 ; p< 0.0007).

I - a

	$G1/\bar{G1}$	$G2/\bar{G2}$	$G3/\bar{G3}$	$G4/\bar{G4}$	$G5/\bar{G5}$	$G6/\bar{G6}$	$G7/\bar{G7}$	$G8/\bar{G8}$
F1	NS	NS	NS	NS	0.0002	NS	NS	NS
F2	NS	NS	NS	NS	NS	0.003	NS	NS
F3	10-8	0,001	NS	NS	NS	NS	NS	NS
F4	0.003	NS	0.0002	NS	NS	NS	NS	NS
F5	NS	NS	NS	NS	NS	NS	NS	0.016
F6	NS	NS	NS	NS	NS	NS	0.0002	NS
F7	0.007	NS	NS	NS	NS	NS	NS	NS
F8	NS	NS	NS	2.10-8	NS	NS	0.026	NS
F9	0.002	NS	NS	NS	NS	NS	NS	0.001
F10	NS	NS	NS	NS	NS	NS	0.037	2.10-8
F11	0.002	NS	NS	NS	NS	NS	NS	NS

I - b		IgA	C3	Oro	Hapt	Trf	IgG
F3	R	0.4	-0.33	-0.51	-0.34	-0.4	
	p	0.004	0.0001	0.002	0.0003	0.0001	
F4	R	0.32			-0.43		0.45
	p	0.005			0.0001		0.0001
F7	R	0.25	-0.48	-0.43	-0.53		
	p	0.02	0.00001	0.0001	0.0001		
F11	R	0.38	-0.29	-0.23	-0.23	-0.36	
	p	0.00001	0.0002	0.03	0.004	0.001	
F9	R		0.34				
	p		0.002				

Table 1. a, Results of the Mann-Whitney test comparing, for each form, each pathological group (G) with the 8 other pooled groups ($\bar{G}$)
NS : No significant difference.
b, Correlations between proteins and forms
R : Spearman's rank correlation coefficient.

Table I b depicts the significant correlations between forms and proteins.

Discussion

In this preliminary work, statistical results indicate that some pathologies induced specific modifications of crystallization, towards certain forms which appeared with a higher frequency than in the control group or other remaining pathological groups. Indeed, statistical treatment of experimental data showed significant differences for the defined parameters, in most of the eight groups studied, i.e. the 11 crystallization forms and the 9 seric proteins. Moreover, correlations were found between the frequence of some forms and the variation of some proteins of the seric spectrum. The simultaneous presence of several forms on a plate whatever the pathology (or the clinical status generally) was not easily explicable. It might be an expression of organ interdependency (e.g. heart, lung and liver) or the occurrence of connected pathologies within the same subject. It was noteworthy that most of our patients combined hepato-digestive diseases with another illness (e.g lung or heart diseases).

A systematic study of patients with a single pathology is necessary for a proper study of the processes causing a related crystallization pattern modification. The present results obviously did not attempt an explanation for the complex molecular processes involved in the generation of crystallization patterns. It seems that greater attention must be devoted to the relative composition of the different protein species of the blood sample than to the quantitative proteic content. Large variations of one or several protein species (as assessed by seric profile) did not seem to be a sufficient criterion for clearly explaining the genesis of forms. Indeed, the observed heterogeneity should not be uncertain and the process did not seem to be as a matter of chance. Proteins present in the sample would exercise a determining influence. *Neuhaus* (1957) supposed that proteins in solution during crystallization participated in different ways in the establishment of the forms described: by their tensio-active properties, by modification of viscosity (i.e. antidiffusive properties), as impurities, with adsorption on one side of crystal, thus driving the nascent form, before they were repelled on the growth front or on other sides of the crystal. *Shibata et al.* (1993) showed, using X ray photoemission spectrometry (XPS) the presence of chemical bonds between $CuCl_2$ and proteins coming from blood. He found that the spectra obtained from blood of patients showing no pathology differed from that obtained from those showing a pathology. These among other results may contribute to understanding the frequency modifications and pattern changes.

Spherolytic crystallization generates roughly typical forms as F4, F3, F1. In this respect, the crystallographic results concerning the control group were in good agreement with the »crystallographic norm «as defined by *Nickel* (1968). In order to explain the vacuole formation (i.e. the surface empty of crystal), this author spoke of protein dependant growth inhibition. As soon the local microheterogeneity was created, crystallization started irreversibly. This peculiar desequilibrium, »primum movens «of the phenomenon, would originate other homogeneity-breaking points, leading to physical gradients (pH, temperature, concentration) (*Pfeiffer*, 1975; *Leray*, 1968, 1970, 1973). Other parameters have been included for the interpretation of the crystallization forms: presence of irregularities at the glass surface of the plate, existence of air micro-bubbles,

dust seeds, density differences, formation of Bénard cells (*Vidal*, 1989). In this respect, we observed on our plates, in superimposition with the crystal texture, an alveolar network originating from figurative elements of whole blood made insoluble by water evaporation. The theoretical approach to such a phenomenon was hard and hazardous (*Thom*, 1977).

Models have been proposed in this connection. *Turing* (1952) suggested the presence of morphogenetic fields and proposed a consistent interpretation. Briefly, the macroscopical pictures could be seen as an order emergence by fluctuation and we must place it in the frame of non-equilibrium processes. Such systems have been described as »chaotic «and they could give new insights in many physical or biological events not satisfactorily explained at the present time (*Goldbeter*, 1990).

We also think that crystal growth starting around the crystallisation centres (considered as germs, like local proteic precipitation) occured with preferential orientation. Local salt super-saturation might also contribute, more than gravity, to the different aspects macroscopically observed. Several works have demonstrated the interest of this crystallization test not only in the diagnostical orientation in general medical practice but also in the cancer screening (*Pfeiffer*, 1931 ; *Selawry*, 1957 ; *Hoffmann*, 1985 ; *Barth*, 1990), and as a possible pre-clinical disease screening method in the field of industrial toxicology (*Cocude et al.*, 1992). Our study did not demonstrate a causal relation between a defined pathology and a given »crystallization pattern«. Indeed we demonstrated the occurence of a relation at a statistically significant level between a pathological group and the prevailing frequency of a given crystallization form. In this respect we might consider the whole profile of crystallization form frequencies to be fully significant for the pathological state of a patient. These patterns could serve as guidelines for more targetted clinical investigations. In addition, in the pathologies studied here, we concluded according to the seric profile, that variations of proteins, expressed as percentages, were not very important. So, we shall direct our attention on other kinds of pathologies, where the protein spectrum is caricaturally altered, as seen in inflammatory diseases like auto-immunity ; or in immunological disorders, as hypersecretion of globulins, advanced denutrition states (neoplasias for instance, where the form F14 is often present).

Nevertheless, for the most groups, statistical evidence demonstrates an univocal relationship between »Form «and pathology. These first results prompt us to investigate more accurately this peculiar domain of protein semiology.

* * *

Acknowledgements
We thank Professor *Jean Pierre Daures* (Department of medical data processing and statistics of the Montpellier-Nîmes C.H.R.U) for contribution to the statistical analysis. We appreciate very much the technical assistance of *France Palaysi* and we express our gratitude to *Christine Ballivet* (Laboratoire de l'Institut Kepler, Avenue G. Clémenceau – 69230 – Saint-Genis-Laval – France) for her frequent, stimulating and helpful discussions.

References

Barth, J.-G. (1990): Empfindliche Kristallisation – Krebs und Präkanzerose. Elemente d. N. 52, p. 42 – 51.

Chebotareva, V.D., Maidannick, V.G., Paderno, V.N., Pokrasen, N.M. and *Martynenko, A.N.* (1990): Diagnostic potentialities of the crystallooptic method in children with pyelonephritis. Pediatria, 5, p. 46-51 (in Russian).

Cocude, M., Barth, J.-G, Bruyet, B. et François, P. (1992): La pneumoconiose des houilleurs et son suivi médical. La méthode des cristallisations sensibles au banc d'essai. Mines et Carrières. Industrie minérale, 74, p. 41-47.

Frot, J. C., Hofmann, H., Muller, F., Benazet, M.-F. et Giraudet, P. (1984): Le concept du profil protéique. Ann. Biol. Clin., 42, p. 1-8.

Giraudet, P., Coudon, B. et Postel, P. (1990): Exploitation clinique des profils protéiques. Technicon.

Goldbeter, A. (1990): Rythmes et chaos dans les systèmes biologiques et cellulaires. Ed. Masson, Collection Biologie Théorique.

Hoffmann, C. (1985): Test de cristallisation sensible avec du sang en rapport avec le cancer. Thèse médecine 303, Université Louis Pasteur, Strasbourg.

Jayle, M. F. et Engler, R. (1984): Les différents profils des variations des protéines plasmatiques dans les états inflammatoires. Path. Biol., 22, p. 645-650.

Laurent, P., Figueroa, N. et Zeller, H. (1980): Néphélométrie, principes, applications à l'immunoprécipitation en milieu liquide. Rev. inst. Pasteur Lyon, 13(2), p.153-179.

Leray, J. (1968): Growth Kinetics of hydrated cupric chloride. J. Crystal Growth, 3,4, p. 344-349.

Leray, J. (1970): Etude géométrique des configurations engendrées par la cristallisation dendritique de solutions salines concentrées. Bull. Soc. Fr. Mineral. Cristallogr., 93, p. 201-212.

Leray, J. (1973): Profil de la surface libre d'un film liquide hétérogène. J. de Chimie Physique, 10, p. 1428-1485.

Macart, M. and Gerbaut, L. (1982): An improvement of the Coomassie blue dye binding method allowing an equal sensitivity to various proteins: application to cerebrospinal fluid. Clin. Chim. Acta, 122, p. 93-103.

Meliconi, F., Parracino, O. and Facchini, A. (1988): Acute phase proteins in chronic and malignant liver diseases. Liver, 8(2), p. 65-74.

Neuhaus, A. (1957): Die kristallographischen Grundlagen der »Diagnostischen Kristallisationen«. p. XIV-XL. In *Selawry, A.* und *Selawry, O.*: Die Kupferchlorid-Kristallisation in Naturwissenschaft und Medizin. Stuttgart.

Nickel, E. (1968): Die Reproduzierbarkeit der sogenannten »empfindlichen Kupferchloridkristallisation«. Bull. Soc. Frib. Sc. Nat., 2(II), p. 65-179.

Pfeiffer, E. (1931): Studium von Formkräften an Kristallisationen. Dresden.

Pfeiffer, E. (1975): Sensitive Crystallisation Processes, second Printing, New York.

Selawry, A. und *Selawry, O.* (1957): Die Kupferchlorid-Kristallisation in Naturwissenschaft und Medizin. Stuttgart.

Shibata, T., Takakuwa, Y., Obata, H., Tanaka, A., Kogure, M. (1993): A blood crystallisation method for diagnosis, test of treatment effect, severity, and early discrimination of diseases for the aged. Annual Report of Comprehensive Research Project on Aging and Health Vol.6. Central Office for the Research Grant for Comprehensive Research Project on Aging and Health, Tokyo, in press (in Japanese).

Thom, R. (1977): Stabilité structurelle et morphogenèse. Paris.

Turing, A. M. (1952): The chemical basis of morphogenesis. Phil. Trans. R. Soc. Lond., B237, 37.

Van Kampen, E.J. and *Ziljstra, W.G.* (1961): Standardization of hemoglobinometry II. The hemoglobincyanide method. Clin. Chim. Acta, 6, p.538-544.
Vidal, C. (1989): Les réactions oscillantes en chimie. La Recherche, 20, 216. p.1478-1485.

Marie-Thérèse Piva[*,**], *Serge Lumbroso*[***,*], *Victor Sieso*[*], *Eric Monnin*[**], *Henri Mion*[***], *François Blanc*[**], *Bernard Magnan de Bornier*[*].

[*] *Laboratoire de Biochimie Médicale B – U.F.R. de médecine – Institut de Biologie – Bvd. Henri IV – F-34059 Montpellier.*
[**] *Service de Médecine Interne – Hopital Saint-Eloi – Avenue bertin Sans – F-34059 Montpellier.*
[***] *Service de Biochimie Médicale B – Hopital Saint-Eloi – Avenue Bertin Sans – F34059 Montpellier.*

Correspondence to:
Dr. Marie-Thérèse Piva
Laboratoire de Biochimie Médicale B
Institut de Biologie – Bvd. Henri IV
F-34060 Montpellier