JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Hematology

Research Article

Red Blood Cells on the Turin Shroud

Gérard Lucotte*[1]

[1]Institute of Molecular Anthropology, Paris, France

*Corresponding author: Dr. Gérard Lucotte; Institute of Molecular Anthropology, 44 Monge Street, 75 005 Paris, France, Tel: + 33 6 98 82 92 61; Email: lucotte@hotmail.com

Received: 11-30-2017

Accepted: 12-14-2017

Published: 12-17-2017

Abstract

We have studied by SEM-EDX analysis the red blood cells (hematies) at the surface of the Turin shroud. Sample is a sticky-tape, triangular in form, that was applied on the Face area of the Turin shroud in 1978. We found on it twenty-five corpuscules that are red-blood cells, according to morphological and chemical criteria. Concerning morphology studied by SEM, one of them is a spherocyte, four are discocytes and three are ovalocytes, five are crenocytes and one is a hematy portion ; a total number of twenty seven hematies are piled up "en rouleaux". All the twenty-five hematies found have typical elemental compositions when studied by EDX analysis ; the corresponding spectrums show that all these hematies are partially calcified, this indicates their oldness.

Keywords: Turin Shroud; Face Area; Sticky-Tape Sample; Red Blood Cells; SEM-EDX Analysis

Abbreviations

EDX: Energy Dispersive X-ray;

RBC: Red Blood Cell;

SEM: Scanning Electron Microscopy;

TS: Turin Shroud

Introduction

The turin shroud (TS) is a well known object in which a body image is imprinted [1]. Until now there are only indirect chemical evidence [2] of blood on the TS; but the visual demonstration of blood on the TS requires red blood cells (erythrocytes) observations. We have recently shown [3] by electron microscopy that at least nine typical erythrocytes are visible on the surface of the TS linen fibers.

Morphological preserved erythrocytes have been previously revealed in studies of prehistoric implements [4,5,6]). Hortolà (2002) [7] reported morphological SEM (scanning electron microscopy) analysis of erythrocytes in very ancient human bloodstrains. The main features of a SEM are their high resolution for bulk objects, and their large depth of field and electron contrast (the shadow-relief effect) ; this results in a three-dimensional appearance of the specimen image. That is the reason why this technique was applied to the detection of erythrocytes in aged bloodstains [8].

Examining blood of mummies as old as 2000 years, based on optical or electron microscopy data, postulated that blood

could be conserved. But, in fact, there are only few reports on the recovery of blood remains preserved in these mummies [9,10,11].

More recent studies on ancient blood concerns the (ca. 5300 BP) Tyrolean Iceman (commonly known as "Otzi"); the man corresponding to this wet-mummy was presumably killed by an arrow. This mummy was exceptionally well preserved; however, in contrast to the good overall preservations of its tissue, no blood has been found. Further studies, by X-ray and computed tomography imaging of the Iceman body, gave the first hinds of blood residues (the zone of the arrowhead was surrounded by inhomogeneous soft-tissue areas that were located between the rib cage and the left scapula). These areas were interpreted as being dehydrated haematomas [12] and associated with a lesion in the left subclavian artery that has led to haemorrhagic shock and the Iceman death [13]; a microscopic analysis of immunochemically stained histological tissue samples indicated the possible presence of blood residue [14]. Finally Janko et al. (2012) [15] reported the direct detection of red blood cells in tissue samples with an atomic force microscope (AFM) and Raman spectroscopy.

We present here a detailed SEM-coupled with EDX (energy dispersive X-ray) study of TS erythrocytes located in a sticky-tape sampled in the region of the body image concerning the Face [16].

Materials and Methods

The material [16] is a small (1.36 mm high, 614 mm wide) sticky tape triangle (Figure 1) at the surface of which portions of fibers, pollen grains and spores and some organic matter were deposited. As declared by Riggi di Numana [16], this sticky tape triangle is one part of a larger piece he applied directly (during the 1978 official sampling) to the TS surface, near one "blood area" of the Face.

More than 2 500 particles (greater than 1 μm) can be observed at the surface of the triangle; all of them were studied by optical microscopy, SEM and EDX analysis. For practical reasons, the surface of the triangle was subdivided into 19 sub-samples areas (areas A to S) containing almost all the particles.

Particles of the samples, without any preparation, were observed on the adherent part of the triangle surface. The observations were conducted by SEM, using a Philips XL30 instrument (environmental version) : GSE and BSE procedures were used, the last one to detect heavy elements.

Bessis (1974)[17] described the different forms of erythrocytes observed by scanning electron microscopy of blood samples. An international terminology (using uniform Greek words) has been introduced to describe erythrocytes, based on their three-dimensional morphology (and including pathologic forms). Table 1 characterizes the five abnormal forms of erythrocytes, observed by SEM, that we found in the present study on the surface of the triangle.

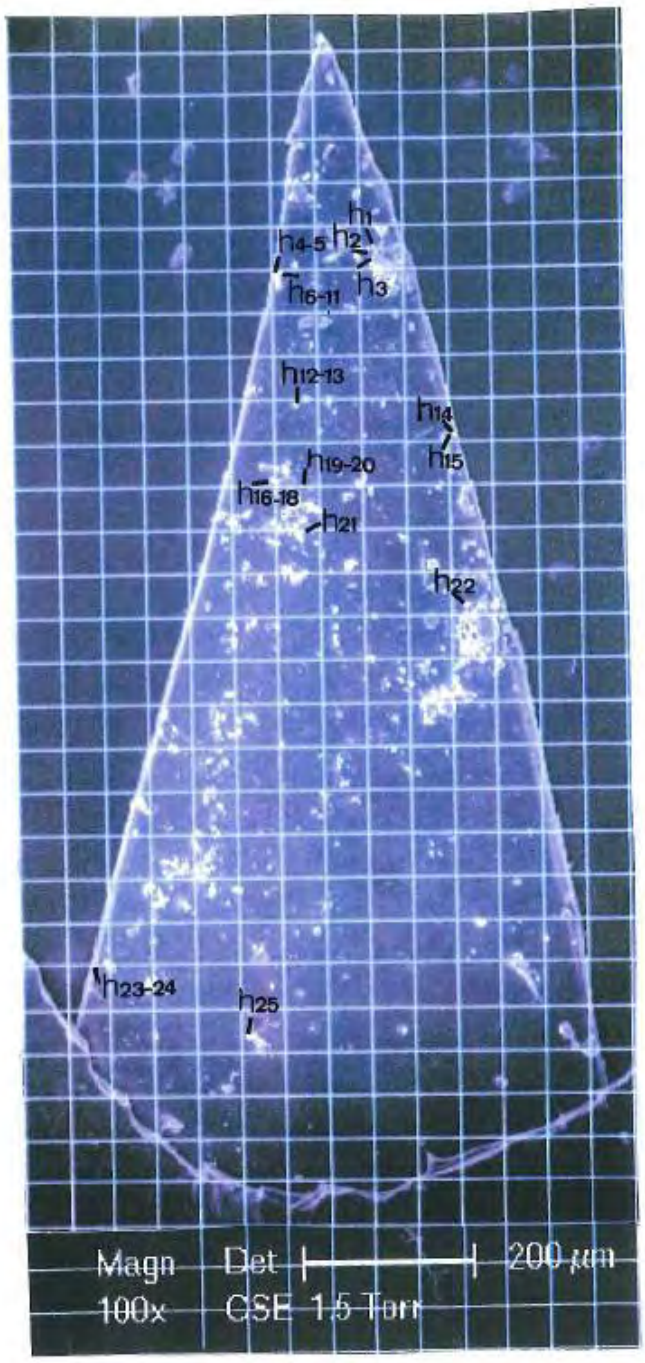

Figure 1. A SEM (x100) Photography of the Triangle. The Locations of the 25 Hematies on it are Indicated.

Elemental analysis of each particle corresponding to erythrocytes were realised by X-ray microfluorescence (XRMF), the SEM microscope being equipped with a Bruker AXS energy dispersive X-ray (EDX); the system analysis is PGT (Spirit Model, of Princeton Gamma Technology).

Table 1. The Five Abnormal Forms of Erythrocytes Found on the Triangle.

	Terminology	Description
Normal form :	*discocyte*	biconcave disc, of the normal size
Abnormal forms :	1. *spherocyte*	inflated, or spherical
	2. *ovalocyte*	oval, to elongated
	3. *crenocyte*	crenated disc
	4. *microcyte*	diameter lower than 6 µm
	5. *schizocyte*	cell fragments, arising by erythrocyte damage

The criteria adopted in the present study to establish that some particles are erythrocytes is as follows : 1. Cells with round or oval contours (in the best kept form) ; 2. Cells diameter comprised between 6 to 9 µ, approximately; 3. An adequate chemical composition, when studied by EDX, with a spectrum showing elevated peaks of carbon and oxygen (the organic matter corresponding to the proteins of the cell membrane) ; 4. An inside cell composition revealed by EDX, with adequate low concentrations of chlorine, potassium, sodium and magnesium (corresponding to intracellular electrolytes); 5. Presence of iron (the iron of haemoglobin), when the structure of the cell is altered.

Results

A Preliminary SEM-EDX Study of a Current Erythrocyte

The human erythrocyte (hematy) morphology is well known (a little biconcave disc with diameter ranging from 7.5-8.5 µ, a thickness of 1-2 mu and a smooth surface) ; but, to our knowledge, there is not any published SEM-EDX analysis of current hematies.

Figure 2 shows an example of a SEM photography of one of my own hematies. It is a hematy of one drop of my blood, that was deposited on a filter paper. The observed hematy is of little relative size (diameter of about 5µ), because of loss of the inside water at the occasion of the transfer from blood to the substratum; this hematy is coated by fibrin macrofibrils [18], sulphur-rich.

Elementary analysis of this hematy shows that it is mainly constituted by carbon and oxygen (the organic matter, corresponding to proteins of the cell membrane). Little peaks on the spectrum concern nitrogen (another component of protein membranes), chlorine, sodium and potassium (the electrolytes) ; there is also a little peak of sulphur (from fibrin), and traces of phosphorus. This hematy was submitted to an intense bombing by X-rays (that mimics aging effects) at its periphery (Figure 3). On the corresponding spectrum, calcium and iron appear (but under the form of some traces only). On the same enlarged spectrum represented on Figure 4, minor elements appear as distinctive peaks: chlorine (two peaks) and sulphur; sodium and potassium ; phosphorus ; silicium, aluminium and magnesium ; calcium, and iron (two peaks) traces.

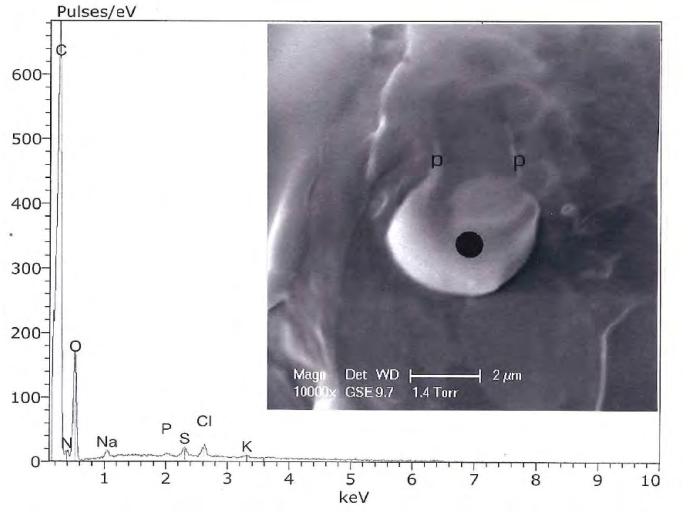

Figure 2. Above : SEM Photography (x10 000) of the Erythrocyte. The black point in the hematy center indicates the place where elementary analysis was realized. P : pedunculus of fibrin macrofibrils.Below : Spectrum at the black point. For each spectrum horizontal axis is graduated in kilo-electrons / Volts (keV), and heights of the peaks are proportional to quantities of elements found in the sample. Elements detected here are C (carbon), N (nitrogen), O (oxygen), Na (sodium), P (phosphorus), S (sulphur), Cl (chlorine) and K (potassium).

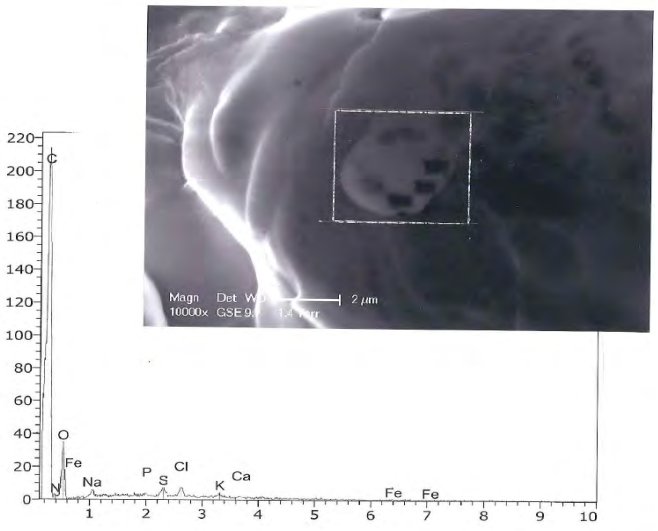

Figure 3. Above : SEM Photography (x10 000) of the Erythrocyte,

whose Border is Bombed by X-rays. Below : Global Spectrum (in the square) of the Bombed Hematy ; Trace Elements of Calcium (Ca) and Iron (Fe) Appear.

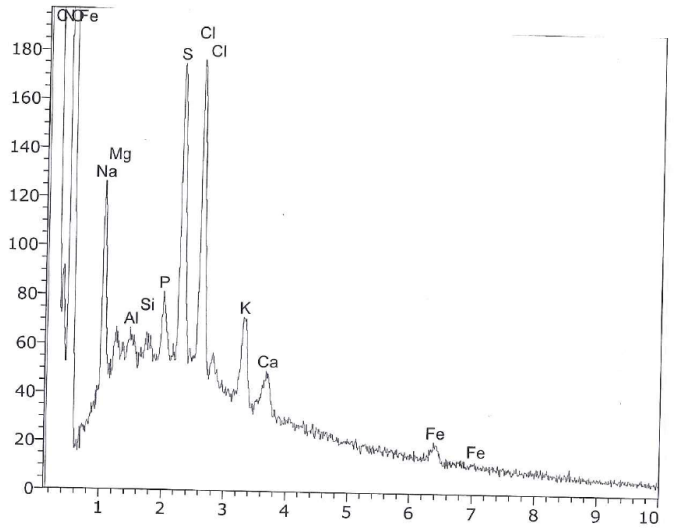

Figure 4. High-Resolution Spectrum of the Previous One.

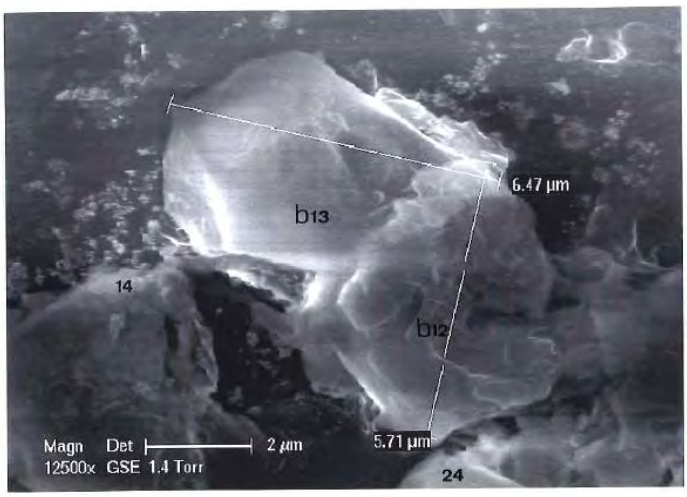

Figure 5. SEM Photography (x12500) of the b13 Particle. Adjacent Particles Erythrocyte? b12 ; b14 : lapis-lazuli ; b24 : clay, iron-rich.

A SEM-EDX Analysis of Preserved Hematies on the Triangle

There is a total number of 25 hematies, observed and analysed, on the surface of the triangle.

Particles b13, b30, b32, b64 and b65 in the B area.

Particle b13 is a typical crenocyte (Figure 5), of a size of about 6.5 µ. The corresponding spectrum (Figure 6) is that of a red blood cell (RBC), with some excess of calcium (that indicates a calcification process) and of silicium-aluminium-magnesium (that indicates a silicification process). Particle b12, adjacent to b13 and similar to it in aspect, is completely calcified.

Particle b30 is also a typical crenocyte (Figure 7), of about 5.1 µ of size, similar to b13. The spectrum concerning b30 (Figure 8) is that of a RBC, with some excess of calcium (calcification) and of silicium-aluminium (silicification).

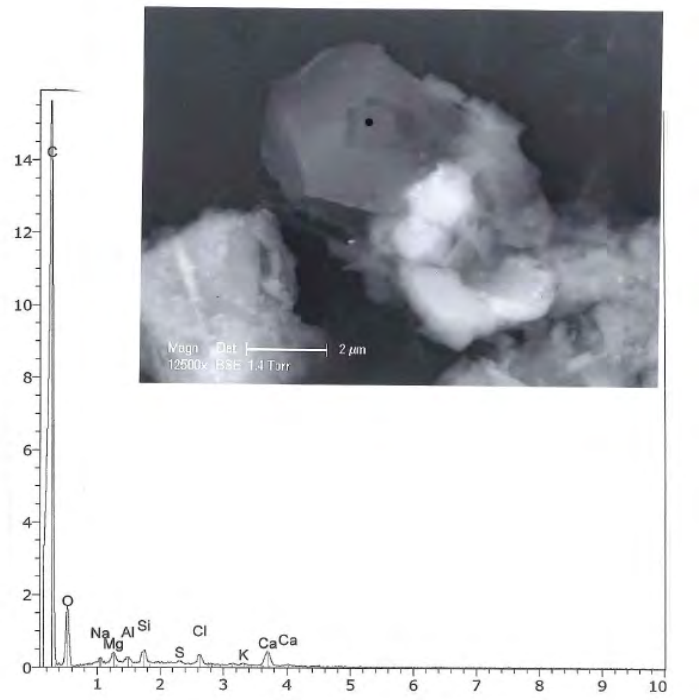

Figure 6. Above : SEM Photography (x 12 500, in BSE) of the b13 Particle. Below : Spectrum at the Black Point Indicated.

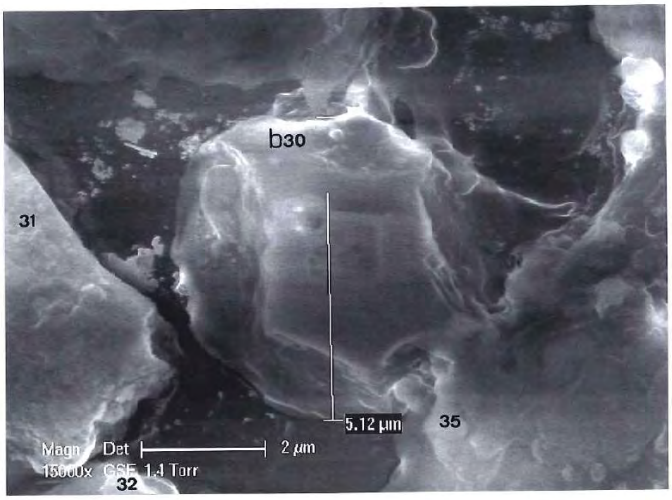

Figure 7. SEM Photography (x 15 000) of the b30 Particle. Adjacent Particles : b31 (kaolinite) ; b35 (cyanophyceae).

Particle b32 is more rounded in form than the two precedent RBCs (Figure 9); its size is of 4µ only. The corresponding spectrum (Figure 10) is that of a RBC, with evidence of silicification and calcification (and with some trace of sulphur).

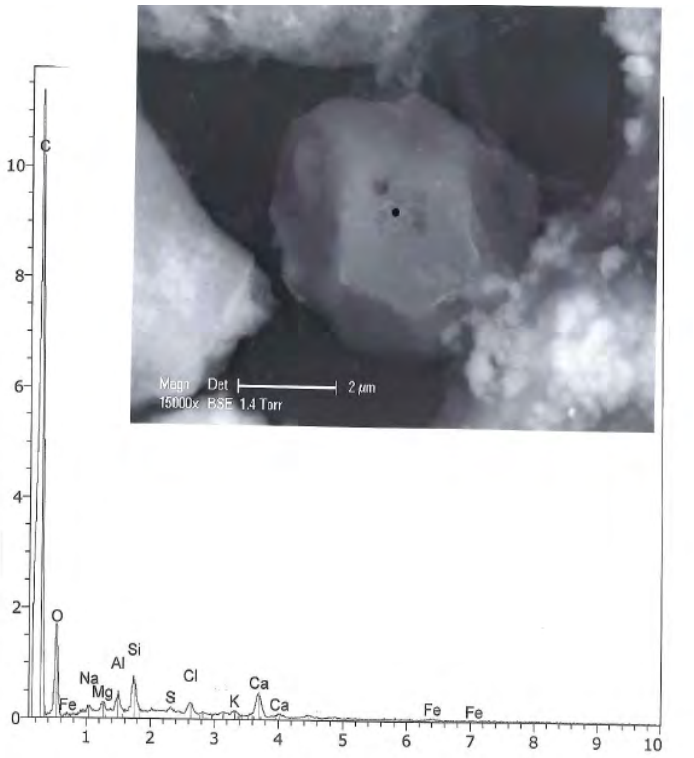

Figure 8. Above : SEM Photography (x 15 000, in BSE) of the b30 Particle.

Below : Spectrum at the Black Point Indicated.

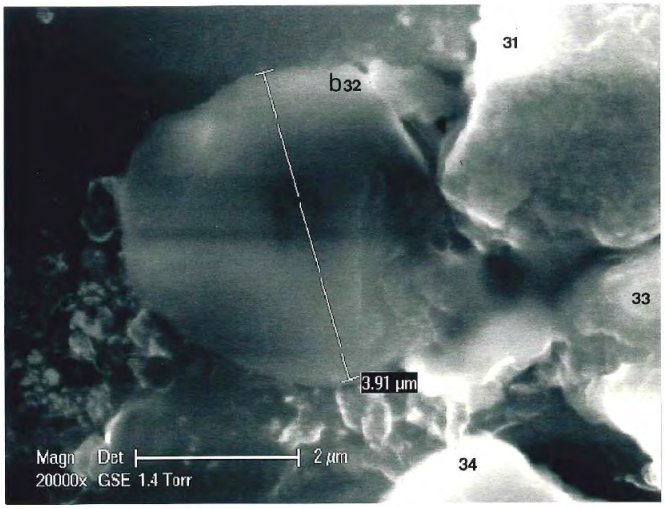

Figure 9. SEM Photography (x 20 000) of the b32 particle. Adjacent Particles : b31 (kaolinite) ; b33 (smectite) ; b34 (silice + calcium carbonate).

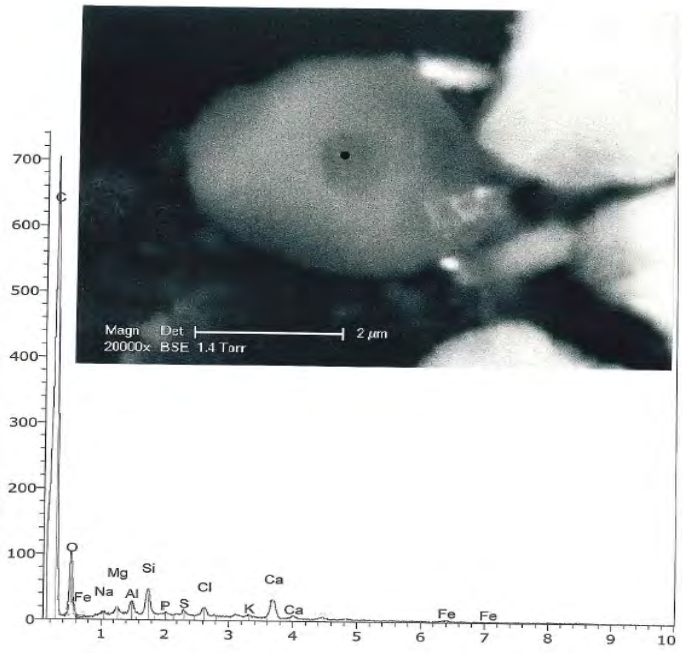

Figure 10. Above : SEM Photography (x 20 000, in BSE) of the b32 particle.

Below : Spectrum at the Black Point Indicated.

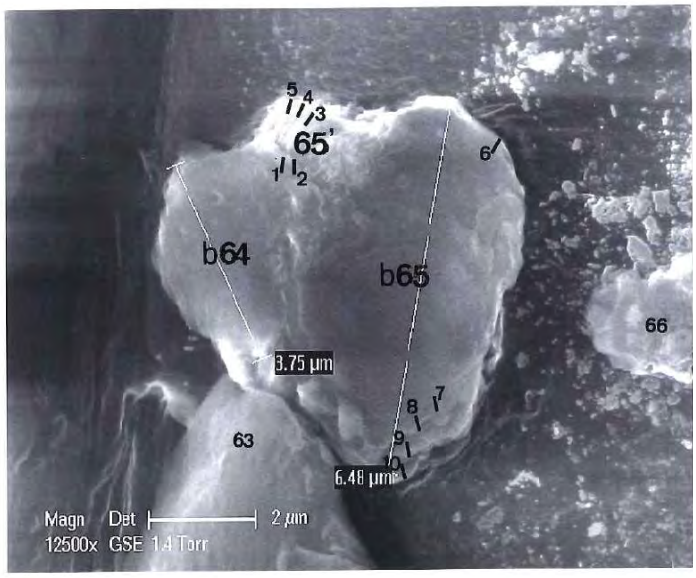

Figure 11. SEM Photography (x 12 500) of the b64 and b65 particles (b65' indicates the intermediate top region between the two) ; 1 and 2 indicate the two hematy borders of b64 ; 3, 4 and 5 indicate the three hematy borders of b65 (and 7-8, 9 and 10, the three b65 hematy borders on the other side ; 6 indicates the second b65 hematy border). Adjacent particles : b63 (clay, iron-titanium-rich) ; b66 (ostracod).

Particles b64 and b65 are ovale (ovalocytes) in forms (Figure 11), b65 covering partially b64; b65 dimension is about 6.5µ, the partial b64 dimension being of 3.5µ only. Examination of Figure 11 photography shows (regarding for upper particle borders) that b65 consists of at least six piled up hematies, and that b64 of at least two.

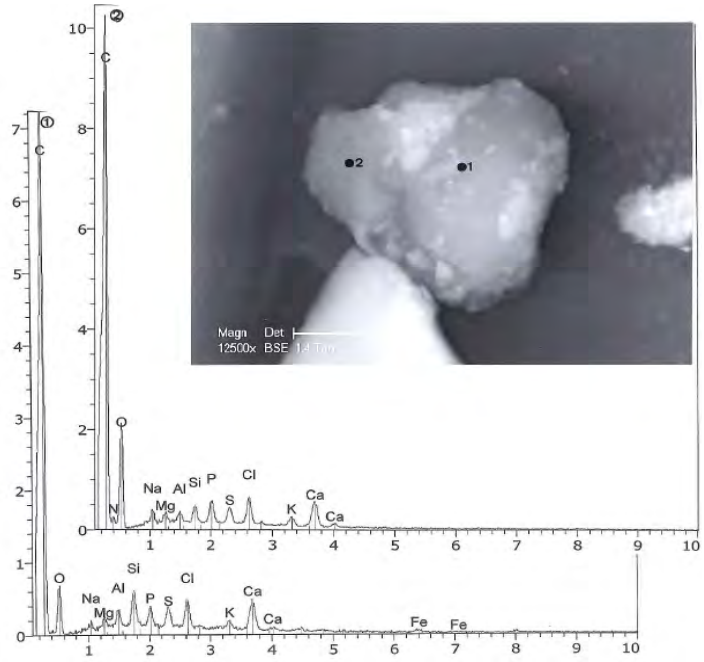

Figure 12. Above: SEM Photography (x 12 500, in BSE) of the b65 (black point 1) and the b64 (black point 2) particles. Below: Spectrums at black points 1 and 2.

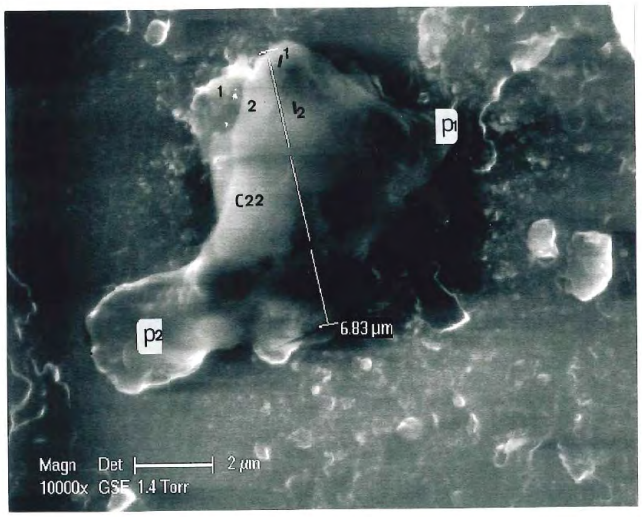

Figure 13. SEM Photography (x 10 000) of particle c22. Numbers 1 and 2 show the free parts of the two crenocytes (1 and 2 indicating the top points of the two hematy borders). The two areas indicated as p (p1 and p2) are extansion zones from the main hematy corpus.

Particles c22 in the C area.

Particle c22 (Figure 13) is angulated in contours (crenocyte) ; its size is about 6.8 µ. Examination of the photography of Figure 11 shows that c22 corresponds to two superposed crenocytes. There are two expansion pedunculus zones from the main crenocyte corpus.

Spectrum of particle c22 (Figure 14) is that of a RBC, with some evidence of calcification and silicification. Spectrum of the pedunculus p2 shows also an excess of sulphur (fibrin).

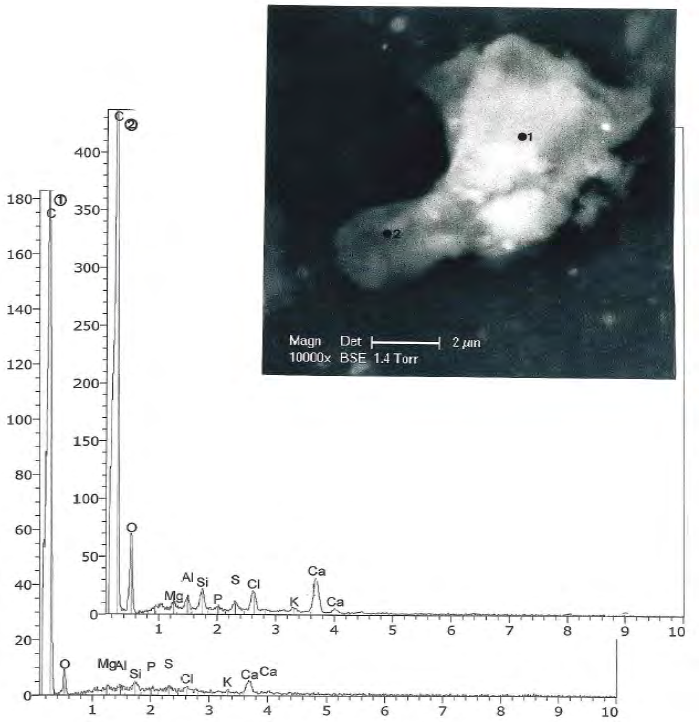

Figure 14. Above : SEM Photography (x 10 000, in BSE) of particle e22 (black points 1 and 2 concern the e22 main corpse and the left-back top P extansion). Below: Spectrums at black points 1 and 2.

Particles d3 and d4 in the D area.

Figure 15 shows particle d3, that is a quasi-perfect rounded discocyte with a smooth surface ; its diameter is about 7.9 µ. Particle d4 is a broken hematy, located under d3. Spectrums of d3 and d4 (Figure 16) show that they are two RBCs, with little evidence of calcification and silicification.

Particles e5, e22 and e58 in the E area.

Figure 17 shows particle e5, which is a perfect (round in contours) discocyte with a smooth surface. Examination of the Figure 17 photography (lower limit contours) indicates that at least two other hematies are piled up under e5. The e5 diameter is about 6µ. Spectrum corresponding to e5 (Figure 18) is typical of a RBC, with some calcification.

Particle e22 (Figure 19) is a crenocyte (length of about 7mu). Examination of its contours shows that there are at least two

superposed crenocytes. The e22 spectrum (Figure 20) is that of a RBC, with calcification and silicification.

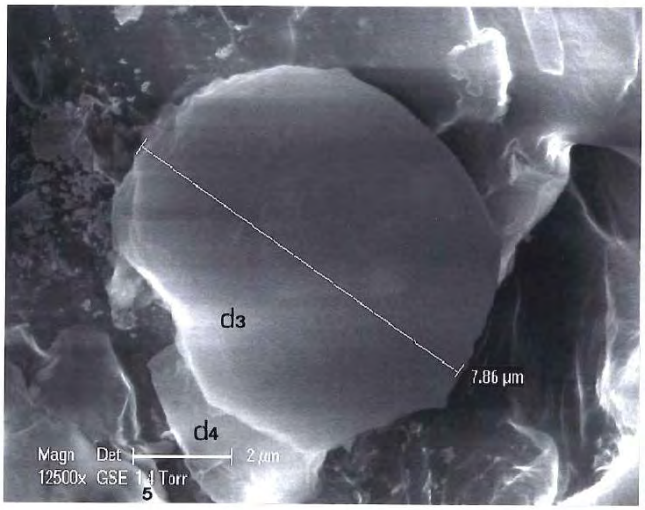

Figure 15. SEM Photography (x 12 500) of the d3 and d4 (broken) particles. Adjacent particle : d5 (dense calcium carbonate).

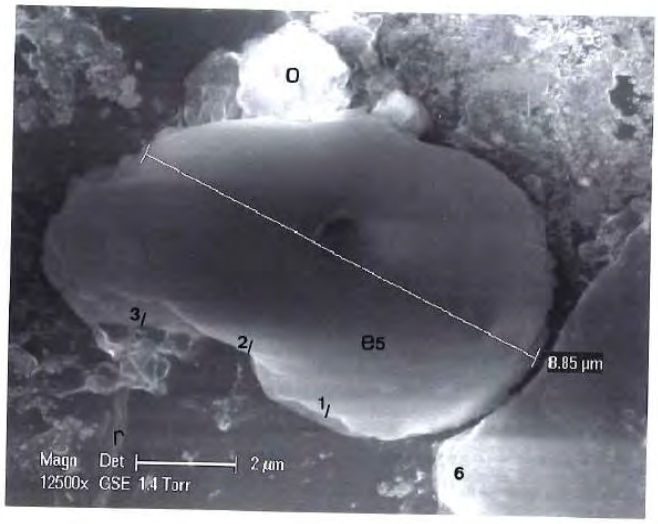

Figure 17. SEM Photography (x 12 500) of the e5 particle (1, 2 and 3 indicate the inferior limit border of e5, and of the second and the third hematies. Adjacent particles : e6 (skin cell) ; o (calcite) ; r is a hole.

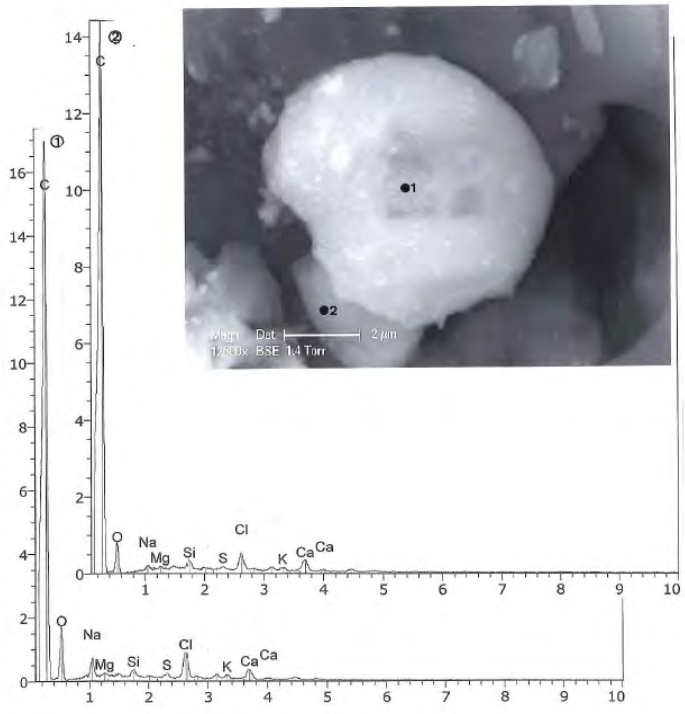

Figure 16. Above : SEM Photography (x 12 500, in BSE) of d3 and d4 particles (black points 1 and 2 concern d3 and d4 particles, respectively). Below : Spectrums at black points 1 and 2.

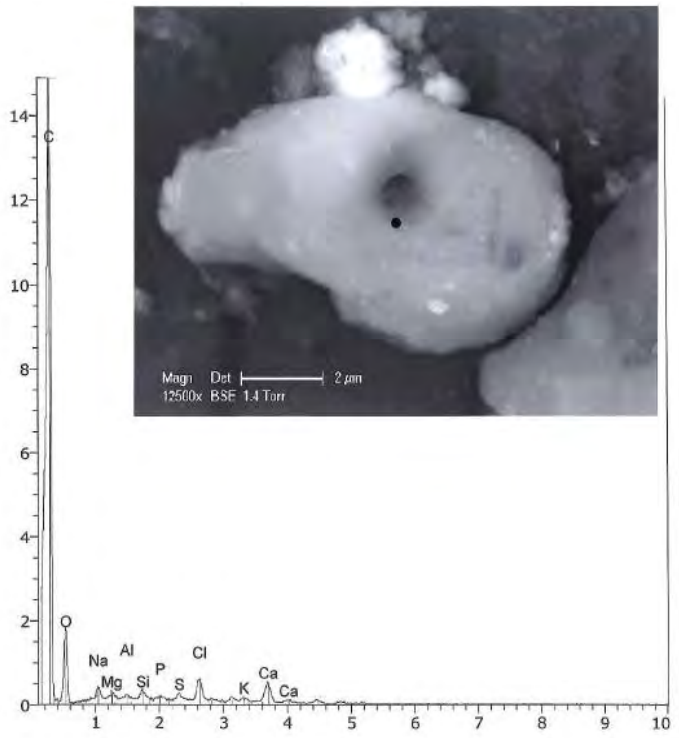

Figure 18. Above : SEM Photography (x 12 500, in BSE) of the e5 particle. Below : Spectrum at the black point.

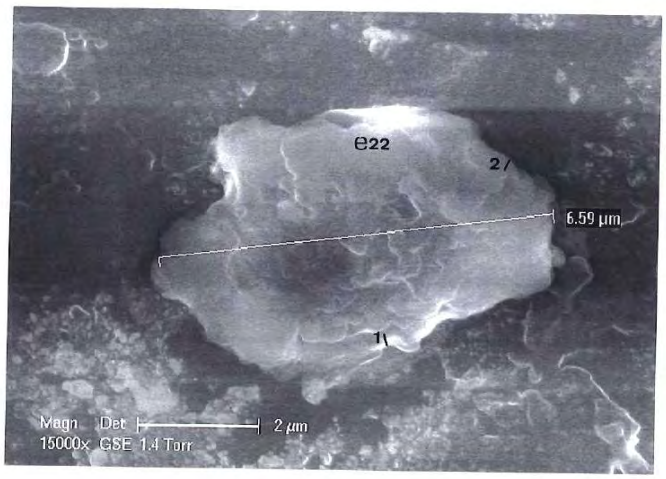

Figure 19. SEM Photography (x 15 000) of the e22 particle (1 and 2 indicate the inferior and superior borders of the e22 hematy and of the adjacent hematy, respectively.

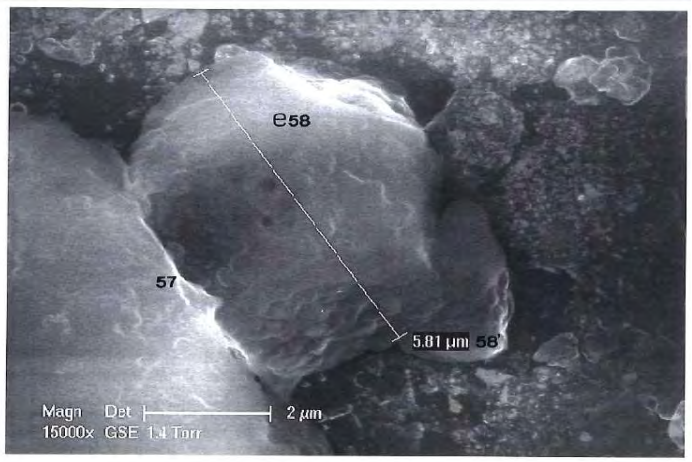

Figure 21. SEM Photography (x 15 000) of the e58 particle (e58' is a small detached part of e58). Adjacent particle : e57 (a chlorite, covered by PVC plastic).

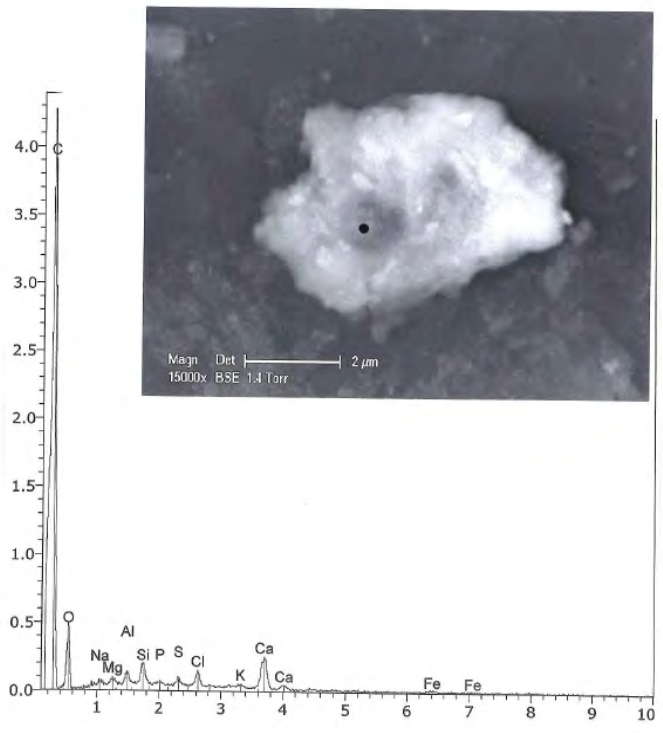

Figure 20. Above : SEM Photography (x 15 000, in BSE) of the e22 particle. Below : Spectrum at the black point.

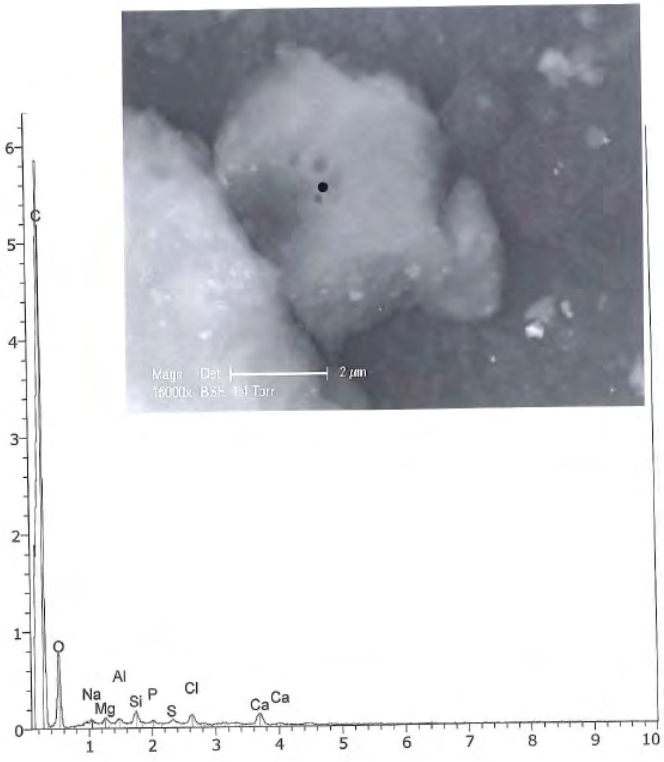

Figure 22. Above : SEM Photography (x 15 000, in BSE) of the e58 particle. Below : Spectrum at the black point.

Particle e58 (Figure 21) is a crenocyte, broken in two unequal parts (e58 and 58'). Length of e58 is about 5.8µ. The e58 spectrum (Figure 22) is that of a typical RBC, with a minimal amount of calcification.

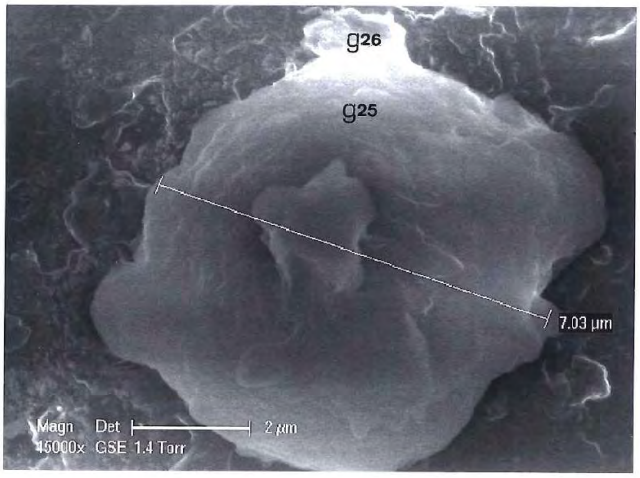

Figure 23. SEM Photography (x 15 000) of the g25 particle (g26 is a little montmorillonite particle, adjacent to g25).

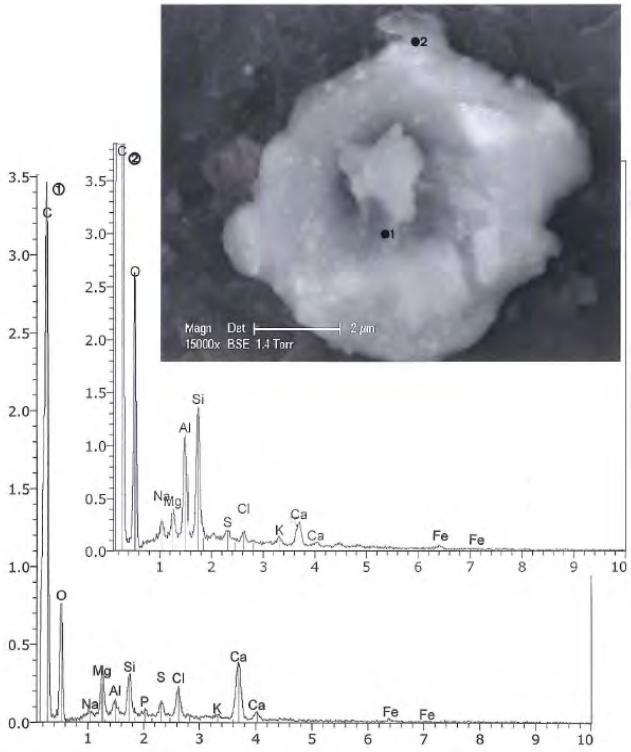

Figure 24. Above : SEM Photography (x 15 000, in BSE) of the g25 (black point 1) and g26 (black point 2) particles. Below : Spectrums 1 and 2 at the black points 1 and 2, respectively. Particle g26 is a clay mineral, of illite-smectite type.

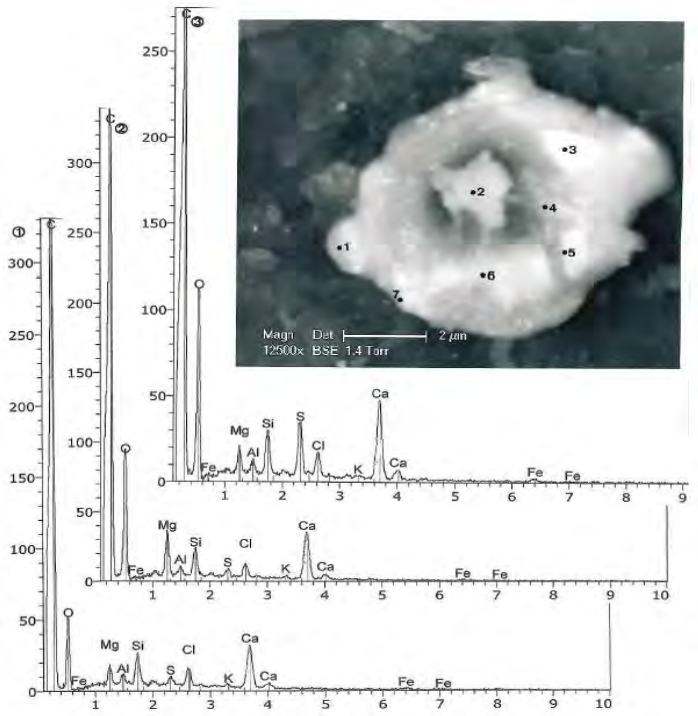

Figure 25. Above : SEM Photography (x 12 500, in BSE) of the g25 particle. Black points numbered 1 to 7 are indicated. Below : Spectrums at black points 1, 2 and 3.

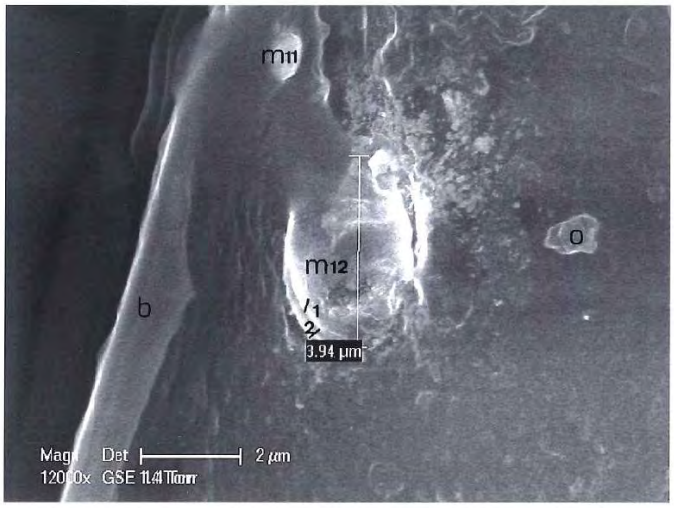

Figure 26. SEM Photography (x 12 000) of the m12 particle (numbers 1 and 2 correspond the inferior borders of the two hematies). Adjacent particles : m11 (calcite) ; o (calcite). The letter b indicates the sticky-tape border (in area M).

Particle g 25 in the G area.

Particle g25 (Figure 23) is rounded, and of 7-10µ diameter. Swollen in form, it is a spherocyte. The g25 spectrum (Figure 24) is that of a RBC, with notable amounts of calcification and silicification (there is also a relatively elevated level of magnesium).

To understand better the nature of the sub-particles deposited in the g25 surface, we have analysed separately (Figure 25) the g25 sub-areas, dense in BSE : the sub –particles 2, 4, 6 and 7 correspond to magnesium carbonate (dolomite), and the sub-particles 3 and 5 to calcium sulphate (gypsum).

Particle m12 in the M area.

Particle m12 (Figure 26) is a little ovalocyte, of about 4.5µ diameter. Examination of the Figure 26 photography shows that the two discocytes are superposed. The m12 spectrum (Figure 27) corresponds to a typical RBC.

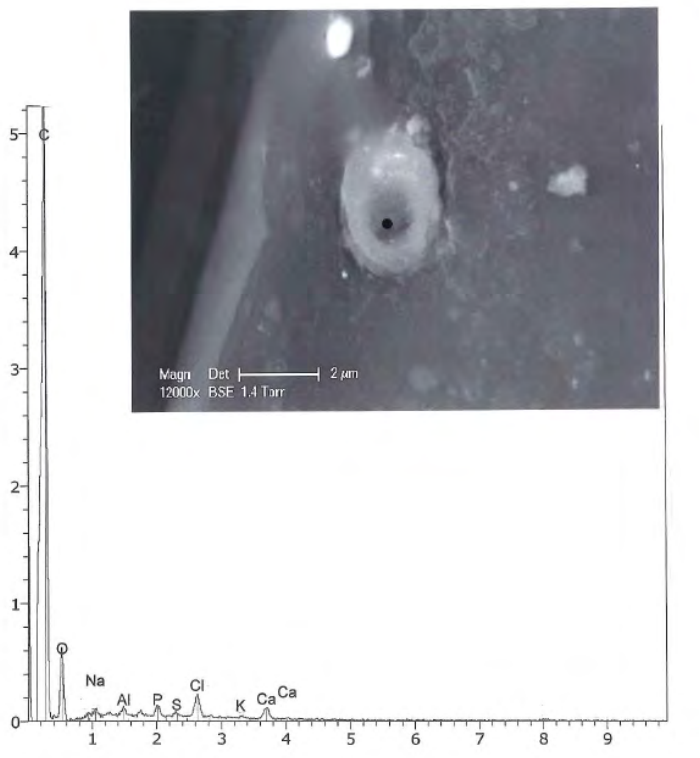

Figure 27. Above : SEM Photography (x 12 000, in BSE) of the m12 particle. Below : Spectrum at the black point indicated.

Particle o10 in the O area.

Particle o10 is a perfect rounded discocyte (Figure 28), with a smooth surface ; its diameter is about 7µ. The o10 spectrum (Figure 29) corresponds to a typical RBC, with a minimal amount of silicification. Sub-particles 1 and 2, located at the o10 periphery (Figure 30), are composed of calcium carbonate and of dense calcium carbonate, respectively.

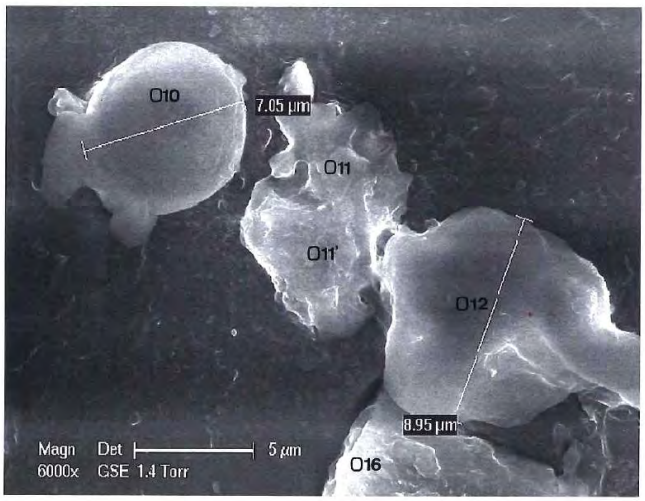

Figure 28. SEM Photography (x 6 000) of the o10 particle. Adjacent particles : o11 and o11' (lapis-lazuli) ; o12 (alumino-silicate clay, covered by PVC plastic) ; o16 (silice).

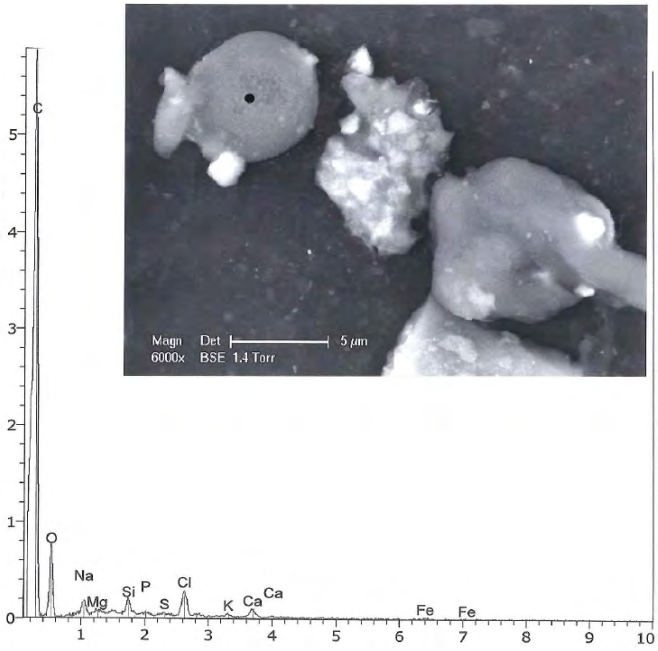

Figure 29. Above : SEM Photography (x 6 000, in BSE) of the o10 particle. Below : Spectrum at the black point indicated.

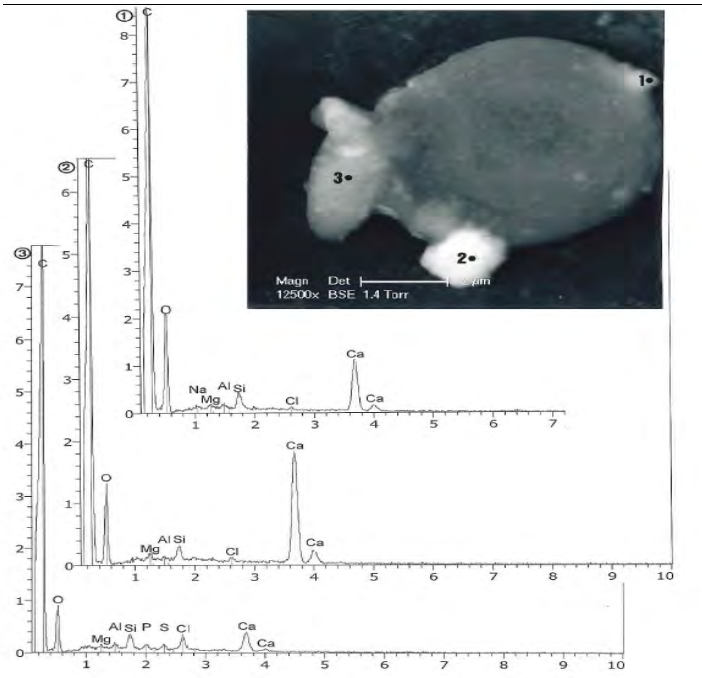

Figure 30. Above : SEM Photography (x 12 500, in BSE) of the o10 sub-particles 1, 2 and 3. Below : Spectrums of sub-particles 1, 2 and 3 (possibly, 3 is a contaminating cyanophyceae).

Discussion and Conclusions

The locations of each of the twenty-five hematies found on the surface of the triangle are indicated on the SEM photography of Figure 1. Table 2 summarizes the characterizations of these hematies.

Hematy h22 is a spherotype. All the other hematies found are flattened. Hematies h14, h16 and h25 are perfect round discocytes; hematy h3 is a rounded discocyte and hematies h4, h6 and h23 are ovalocytes. Hematies h1, h2, h12, h13, h19 and h21 are crenocytes.

So, discocytes (in the large sense, including the rounded hematy h3 and the ovalocytes) and crenocytes (they are at the first state of the crenation process) are the two main forms of hematies found on the surface of the triangle. Black and Jones (1976)[19] have described in details the progressive discocyte to crenocyte transformation (discocyte ⇒crenocyte⇒echinocyteI⇒echinocyteII⇒echinocyteIII.) during and after cardiopulmonary bypass in cardiovascular surgery. According to these authors, the initial causes of this transformation (that is partially reversible) result from physical and chemical damages of hematies, at the occasion when they are sorted-out to the body.

While most of the hematies appears as individual cells, formations "en rouleaux" occur from the ovalocytes h4-5 and h23-24 and for the crenocytes h12-13 and h23-24 (two superposed cells), for the discocytes h16-18 (three superposed cells) and for the ovalocytes h6-11 (six superposed cells).

Because of loss of the inside water when hematies are sorted-out, their volumes (and diameters) decrease; an example of this diameter reduction is shown on the photography of Figure 2. All of the hematies found on the surface of the triangle have diameters in the normal range; but we have evidence of a microcyte pattern-in diameter- for the crenocytes h2 and h21, for the ovalocyte h23, and for the rounded discocyte h3.

Hematy h15 is a schizocyte. It is also the case for the e58' fragment of hematy h21.

Table 3 gives the usually adopted concentration values of electrolytes in the human erythrocyte. One notable difference can be observed between the most elevated potassium value reported in this table and that shown in the spectrum of the erythrocyte studied (Figure 2), where the potassium level is at relatively modest value. That can be easily explained by the potassium escape during the water loss-inside : potassium ion level is at 130mM in the erythrocyte, while it is at only 4mM in the serum. Inverse is true for the sodium ion, which pass from 8mM in the erythrocyte to 140 mM in the serum.

It results from these transfers that potassium and sodium must correspond to low value electrolyte peaks in the spectrum of the isolated erythrocyte (Figure 2), that is effectively the case. Because chlorine is at a relatively elevated value – the second important one after that of the potassium in the erythrocyte (Table 3) – and because there is not significant difference values between the elevated chlorine values in erythrocyte and in serum, it results that in the isolated erythrocyte (Figure 2) chlorine corresponds to the most important electrolyte peak. Magnesium and calcium concentrations are relatively low, both in erythrocyte and in serum; it results that their corresponding peaks in the isolated erythrocyte spectrum are absent, or must be very modest in height (that is the case, respectively).

In table 3 phosphorus value – the third in importance after those of potassium and of chlorine – corresponds to a total value (in organic phosphorus, plus lipidic phosphorus originating mainly from the membrane). So, it is normal that there is a little peak of phosphorus in the spectrum of the isolated erythrocyte (Figure 2).

Calcium, silicium (and aluminium and magnesium) and sulphur levels are very low in the erythrocyte (Table 3). When their relative values in the spectrums are more elevated in hematies found on the triangle, they indicate calcification, silicification (mineralisation of hematies is a proof for their oldness) and fibrin deposits, respectively.

All the twenty-five hematies found on the surface of the triangle have a typical RBC spectrum, with some modifications

(Table 2). All these hematies are more or less calcified. A notable calcification occurs for hematies h4, h6, h16 and h 19 ; the mostly calcified hematy is the spherocyte h22. All these hematies, except for hematy 23, are more or less silicificated (with aluminium and magnesium); discocytes h14, h15 and h16 , crenocyte h21have silicium only. Hematies h2, h3 and h25 are more silicificated than calcified. The most calcified spherocyte h22 is also notably silicificated. Hematies h4, h6, h12 and the spherocyte h22 are sulphured; a fibrin pedunculus is visible in SEM for hematies h12-h13 only.

We know that erythrocytes contain about 1 pg of iron per cell. We have detected here iron traces, only for hematies h2, h3, h6, h19, h21 and h25. It remains to demonstrate that this iron is linked with haemoglobin [20], and not with the mineral part of the corresponding hematies.

We have completed our SEM observations of hematies by studies in optical microscopy (using a photomiscroscope Zeiss, model III, 1972).

Table 2. Some Characteristics of the 25 Hematies Found on the Surface of the Triangle.

Particles	Areas on the triangle	Morphologies	Numbers of erythrocytes	Elements in excess			Hematy numbers
				Calcium	Silicium	sulphur	
b13	B	crenocyte	1	+	+		**h1**
b30	B	crenocyte	1	+	++		**h2**
b32	B	rounded discocyte	1	+	++		**h3**
b64	B	ovalocyte	2	++	+	+	**h4, h5**
b65	B	ovalocyte	6	++	++	+	**h6, h7, h8, h9, h10, h11**
c22	C	crenocyte	2	+	+	+	**h12, h13**
d3	D	round discocyte	1	+	+		**h14**
d4	D	broken	1	+	+		**h15**
e5	E	round discocyte	3	++	+		**h16, h17, h18**
e22	E	crenocyte	2	++	+		**h19, h20**
e58	E	crenocyte	1	+	+		**h21**
g25	G	spherocyte	1	+++	++	+	**h22**
m12	M	ovalocyte	2	+			**h23, h24**
o10	O	round discocyte	1	+	++		**h25**

Table 3. Electrolytes (: µmol/ml) in the Erythrocyte.

Elements	**Concentrations**
Potassium	102.4 ± 3.9
Chlorine	78
Phosphorus (total)	13.2
Sodium	6.2 ± 0.8
Magnesium	3.06
Calcium	0.009 ± 0.003
Sulphur	0.004
Aluminium	0.003
Silicium	traces

Table 4 summarizes our optical studies (hematies h4, h6, h14, h15 and h23 cannot be seen under the optical microscope because they are located at the limit areas of the triangle, near the lateral folds); hematy h19 only is red in colour. Hematies h1, h2, h3, h12 and h25 appears with a clear (depending on the light incidence and of the substratum colour) corpus , with a dark piping ; hematies h16 and h21 appears also with a clear corpus, but with a sharp dark border. In hematy h22 the corpus is clear, but with a border red in colour.

In conclusion we have detected by SEM-EDX analysis a total number of twenty-five hematies on the surface of the triangle (hematy h15 is a portion of hematy) : one of them (hematy h22) is a spherocyte ; four of them (hematies h3, h14) are discocytes and three (hematies h4, h6 and h23) are ovalocytes.

Table 4. Summary of the Optical Observations Concerning Hematies.

Hematy numbers	Visible or not	Observations	Forms	Colours
h1	+	x 1 200	hexagonal	clear, with a dark piping
h2	+	x 1 200	hexagonal	clear, with a dark piping
h3	+	x 1 200	rounded	clear, with a dark piping
h4	-			
h6	-			
h12	+	x 1 200	angulous	clear, with a dark piping
h14	-			
h15	-			
h16	+	x 1 000	round	clear, with dark border
h19	+	x 1 000	hexagonal	red
h21	+	x 1 200	pentagonal	clear, with dark border
h22	+	x 1 200	round	clear, with red border
h23	-			
h25	+	x 1 200	round	Clear, with a light dark piping

The five other remaining (hematies h1, h2, h12, h19 and h21) are crenocytes. Some hematies appear as isolated, but others are piled up (hematies h4-5, h12-13, h19-20 and h23-24), or "en rouleaux" (hematies h16-18 and h6-11). All the detected hematies have an elementary composition compatible to that of typical red blood cells: elevated peaks on the spectrum of carbon and oxygen (corresponding to membrane proteins), and little peaks corresponding to electrolytes. All the hematies are partially calcified, and (but h23) silicificated ; significant values of sulphur (due to fibrin fibers) occur for hematies h4, h6, h12 and h22. There are iron traces on the spectrums, only for hematies h2, h3, h6, h19, h21 and h25.

References

1. Marion A, Lucotte G. Le linceul de Turin et la Tunique d'Argenteuil. Presses de la Renaissance : Paris. 2006.
2. Heller JH, Adler AD. A chemical investigation on the shroud of Turin. Canad. Soc. Forens. Sci. 1981,14(3): 81-103.
3. Lucotte G. Vérités sur le Saint Suaire. Atelier Fol'fer Ed. 2010.
4. Loy TH. Prehistoric Blood Residues: Detection on Tool Surfaces and Identification of Species of Origin. Science. 1983, 220(4630) : 1269-1271.
5. Loy TH, Hardy BG. Blood residue analysis of 90 000 years old stone tools from Tabun Cave, Israel. Antiquity. 1992, 66(250): 24-35.
6. Loy TH, Dixon EJ. Blood residues on fluted points from eastern Beringia. Am. Antiquity.1998, 63: 21-46.
7. Hortolà P. Red blood cell haemotaphonomy of experimental human bloodstrains on techno-prehistoric lithic raw materials. J. Arch. Sci. 2002, *29*: 733-739.
8. Hortolà P. SEM analysis of red blood cells in aged human bloodstrains. Forens. Sci. Int. 1992, *55(2)*: 139-159.
9. Lewin PK. Palaeo-Electron Microscopy of Mummified Tissue. Nature. 1967, *213* : 416-417.
10. Zimmerman MR. Blood cells preserved in a mummy 2000 years old. Science. 1973, *180(4083)*: 303-304.
11. Riddle JM, Ho KL, Chason JL, Schwyn RC. Peripheral blood elements found in an Egyptian mummy : a three-dimensional view. Science. 1978, *192(4237)*: 374-375.
12. Murphy WA, Nedden D, Gostner P, Knapp R, Recheis W et al. The Iceman : discovery and imaging. Radiology. 2003, *226*: 614-629.
13. Pernter P, Gostner P, Vigl EE, Rühli FJ. Radiologic proof of

the Iceman's cause of death (ca. 5300 BP). J. Archaeol. Sci. 2007, 34(11*)*: 1784-1786.

14. Nerlich AG, Peschel O, Egarter-Vigl E. New Evidence for Otzi's final trauma. Intensive Care Med. 2009, 35(6) : 1138-1139.

15. Janko M, Stark RW, Zink A. J. R. Soc. Interface. 2012, *9*: 2581-2590.

16. Lucotte G. Optical and chemical characteristics of the mineral particles found on the Face of the Turin shroud. Sci. Res. Essays. 2012, 7(29): 2545-2553.

17. Bessis M. Corpuscules : atlas of red blood cell shapes. Springer-Verlag Ed., Berlin. 1974.

18. Wolberg AS. Thrombin generation and fibrin clot structure. Blood Rev. 2007, 21(3): 131-142.

19. Black KL, Jones RD. The Discocyte-Echinocyte Transformation as an Index of Human Red Cell Trauma. Ohio J. Sci. 1976, 76(5): 225-230.

20. Mazel V, Richardin P, Debois D, Touboul D, Cotte M et al. Identification of ritual blood in African artefacts using TOF-SIMS and synchrotron radiation microscopies. Annal. Chem. 2007, *79*: 9253-9260.