RESEARCH ON EXTREMELY MINUTE AND ANCIENT TRACES OF BLOOD*

PIERLUIGI BAIMA BOLLONE ANNA LUCIA MASSARO

The identification of blood traces is carried out for the most part on dried stains; the fundamental characteristic of blood—which is made up of liquid plasma and cellular elements—obviously disappears in the dehydrated state. Desiccation and dehydration inhibit the action of the proteolytic cellular and bacterial activity, preserving the structure and the hematic properties and rendering their identification possible even after a long period of time (*Fallani*, 1976).

Already in 1827, *Orfila* demonstrated the possibility of recognizing the blood elements by "regenerating" the stains. A whole series of renovation liquids used for this purpose is known in the medico-legal laboratory.

Exactly one century later, *Williams* (1927) made the morphological identification of red cells in mummified tissues. The observation was confirmed by *Busse-Grawitz* (1942), *Sandison* (1955), *Rabino-Massa*, *Chiarelli*, *Sacerdote* and *Foscale* (1967) and *Rabino-Massa* and *Chiarelli* (1976). The criticisms of *Born* (1959), which attributes such findings to fungus or insect eggs, does not seem to be supported today.

The scanning electron microscope permits a refinement in the recognition of blood elements, especially in scabs. With this instrument it is possible to identify very small dried red corpuscles, thanks to their characteristic aspect of a biconcave lens. *Dixon, Samudra, Stewart* and *Johari* (1976) remark that it is possible, even in old scabs, to observe elements with this structure, especially in the area of recently broken margins. They add that in similar material the morphology of the red corpuscles corresponds to that described by *Bessis* and *Weed* (1972) in fixed preparations. *Morano* (1978), in the course of experimental research in relation to sindonology, demonstrates the difference in the aspects of fixed red corpuscles from the aspect of corpuscles in a dried stain. *Hart, Kvas, Soots* and *Badaway* (1980) were able to identify groups of red corpuscles in the desiccation dust of the tissues of a natural mummy dated to twelve centuries before Christ.

The results of this research demonstrate rather substantially that it is possible to morphologically identify single red corpuscles after a lapse of thousands of years.

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At the same time, chemical methods have also been refined. *Farago* (1965) proposed the use of benzidine to intensify bloodstains made to migrate on chromatograms in a thin strata. With this method, capable of individuating traces in the dilution of one part per million, *Bernardini* and *Masotti* (1976) identified traces of blood up to 20 years old. The method was unsuccessful, however, on samples extracted from bands of Egyptian mummies of the predynastic and dynastic periods (*Baima Bollone*, 1981).

Dixon, Samudra, Stewart and *Johari* (1976) propose the use of the X-ray dispersion microspectrometer (microwave) coupled with the scanning electron microscope. With this equipment, they resolved the characteristic spectrum of blood, constituted of known elements (sodium, magnesium, aluminum, silicon, phosphorous, etc.) in their exact relative proportions.

By the use of this technic, it is possible to determine the presence of the most minute traces of blood (*Baima Bollone*, 1981).

The problems of chronological diagnosis have not been settled so satisfactorily. One could even say that a method for the dating of bloodstains, although the problem has engaged the interest of researchers, has not yet been found (*Merli*, *Umani-Ronchi* and *Colesanti*, 1979). The reason, according to these Authors, lies in the impossibility to ascertain the influence of exogenous factors in the transformation of hematic pigment.

We omit to mention here methods of purely historical interest (as well as others not yet sanctioned by usage). For example, *Schwarzen-Baker* (1930) suggested taking advantage of the color modifications intervening in the bloodstain after parts of it were exposed to light for a half-hour and for an hour.

On the other hand, even sensitive methods such as the racemization of amino acids cannot be applied to bloodstains. As we know, living matter is composed of about twenty amino acids which have the property to deviate, toward the left, a ray of light traversing a solution; they are therefore levorotatory. After death they produce hydrolytic reactions which provoke the phenomenon of racemization, that is to say, the deviation of a ray of light to the right (therefore, dextrorotatory). Since the process occurs in function of time, and each amino acid has its own velocity of racemization, the method is used in archeology and paleontology, in particular utilizing the aspartic acid (*Bada* and *Masters Helfman*, 1976). The fact however that racemization is not verified in conditions of desiccation and in the presence of living organisms, such as the hyphomycetes present on the Shroud (*Baima Bollone, Coero Borga* and *Morano*, 1977) represents, in our case, an almost absolute preclusion of the suitability of this method.

Kind, *Patterson* and *Owen* (1972) and *Kind* and *Watson* (1973) estimate the age of bloodstains using the absorption spectrum. The second work (*Kind* and *Watson*) offers a method based on the calculation of what the Authors define as "alfa-s relation", which they use on bloodstains of less than 15 years of age. This "relation" is in function

of the variations of the absorption spectrum between 500 and 650 millimicrons of ammoniacal extracts of the bloodstain.

According to *Martone*, *De Buono* and *Della Casa* (1977), the values of the "alfa-s relation" allow precise diagnosis within 2000 hours—a little less than three months—and can therefore be used within that time period. *Kohlen* and *Oepen* (1977) confirm that the aging of the bloodstain is a function in the modifications of the "alfa-s relation", but they point out the evidence of even greater variations in bloodstains of the same age and in different sectors of the same stain; a result which would derive from variations of the hematic concentration in the sample. To overcome the difficulty, it is therefore necessary to obtain identical concentrations.

If the use of the absorption spectrum seems to hold more promise toward achieving a chronological diagnosis reaching back through centuries and millenniums, it is clear that this poses the necessity to study the behavior of a great number of cases: the Egyptian material seems to be the most favorable.

Only after having obtained data and precise operational methods will it be possible to approach the problem of the dating of blood present on the Shroud.

BIBLIOGRAPHY

- BADA L. & MASTERS HELFMAN P: "Application of amino acid racemization dating in paleontology and archaeology" in: LABEYRE J. & LAOU C., "Datations absolues et analyses historiques en préhistoire", *Méthodes et limites*, Nice 1976.
- 2. BAIMA BOLLONE P.L., COERO BORGA P. & MORANO E.: "Prime osservazioni sulla fine struttura della Sindone at microscopio elettronico a scansione", *Sindon*, anno XIX #26, Oct. 1977.
- 3. BAIMA BOLLONE P.L.: "Aspetti pratici ed iconografici dell'attività del laboratorio medico-forense", Atti Convegno di Balistica forense, *L'Aquila*, Mar. 1981.
- 4. BAIMA BOLLONE P.L.: "Indagini identificative su fili della Sindone", *Giornale dell'Accademia di Medicina*, anno CXLIV, 1981.
- 5. BESSIS M. & WEED R.I.: "Preparation of Red Blood Cells for SEM; A Survey of Various Artifacts", *Illinois Institute of Technology*, Chicago 1972.
- 6. BORN E.: "Uber Natürliche Mumifizierungen", Zool. Anat. 99, 490, 1959.
- 7. BUSSE-GRAWITZ: "Reaktionen in Mumiengeweben", Arch. Exp. Zell. Forsche., 24, 320, 1942.
- 8. DIXON T.R., SAMUDRA A.V., STEWART W.D. & JOHARI O.: "A Scanning Electron Microscope Study of Dried Blood", J. For. Sci. 797, 1976.
- 9. FALLANI M.: "Ematologia forense", Manuale di Medicina Legale, Vallardi 1976 vol. II.
- 10. FARAGO A.: "Detection of minute traces of blood by thin-layer chromatography", J. Chromatog. 21, 156, 1965.

- 11. KIND S.S., PATTERSON D. & OWEN G.W.: "Estimation of the age of dried bloodstains by a spectrophotometric method", *Forens. Sci.*, 1, 27, 1972.
- 12. KIND S.S. & WATSON M.: "The estimation of bloodstain age from the spectrophotometric properties of ammoniacal bloodstain extracts", *Forens. Sci.*, 2, 325, 1973.
- 13. HART G.V., KVAS J., SOOTS M., BADAWAY G.: "Blood Group Testing of Ancient Material" *Masca J.*, I, 141, 1980.
- 14. KOHLEN U. & OEPEN I.: "On the suitability of spectrophotometric analyses for the estimation of bloodstain age", Z. *Rechtsmed*, 79, 183, 1977.
- 15. MARTONE G., DE BUONO A., DELLA CASA E.: "Variazioni net tempo dello spettro di assorbimento di estratti ammoniacali di tracce di sangue ai fini di una diagnosi cronologica", *Zacchia*, 13, 51, 1977.
- 16. MERLI S., UMANI RONCHI G., & COLESANTI C.: "Le indagini medico-legali su macchie di sangue", L'Aquila, 1979.
- 17. MORANO E.: "Aspetti ultrastrutturali al microscopio elettronico a scansione di fibre della Sindone di Torino", *La Sindone e la Scienza: Atti del II Congresso Internazionale di Sindonologia* (ed. by Coero Borga), Ed. Paoline 1978.
- 18. RABINO-MASSA E., CHIARELLI B. SACERDOTE M. & FOSCALE C.: "Presenza di globule rossi nei tessuti di mummie egiziane", *Boll. Soc. It. Biol. Sperim.*, 43, 1333, 1967.
- 19. RABINO-MASSA E. & CHIARELLI B.: "La istologia dei tessuti naturalmente disseccati o mummificati di antichi egiziani", Arch. It. Anat. Embriol., 91, 301, 1976.
- 20. SANDISON A.T.: "The histological examination of mummified material", Stain Technol., 30, 277, 1955.
- 21. WILLIAMS S.H.V.: "Gross and microscopic anatomy of two Peruvian mummies", Arch. Path., 100, 4, Chicago 1927.