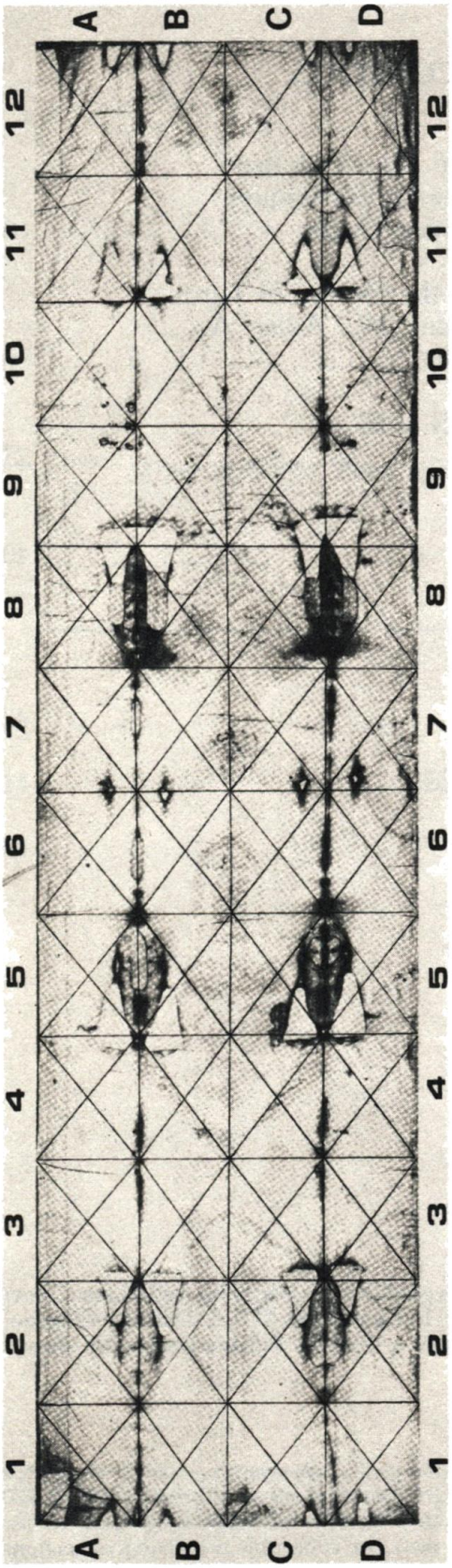

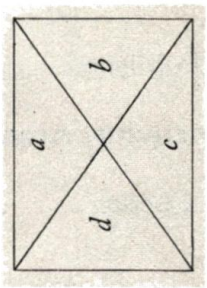

Map of the Shroud, drafted by P. Baima Bollone and Aurelio Ghio.

DEMONSTRATION OF BLOOD, ALOES AND MYRRH ON THE HOLY SHROUD WITH IMMUNOFLUORESCENCE TECHNIQUES

PIER LUIGI BAIMA BOLLONE AND AGOSTINO GAGLIO

During the tests of 1978, one of the scientists was permitted to remove fragments of threads from the Holy Shroud. This was deemed useful for biological identification purposes. Preliminary examinations, performed on samples removed from areas corresponding to stains traditionally believed to be of blood, had confirmed the actual presence of blood and other materials, among which were aloes and myrrh (Baima Bollone, 1981). Subsequent studies with fluorescent antibodies allowed us to establish the blood as human blood (Baima Bollone, Iorio and Massaro, 1981). This identification has been confirmed (Heller and Adler, 1981).* In 1982 it was further possible to ascertain some characteristics of the blood group (Baima Bollone, Iorio and Massaro).† Recent research with immunofluorescence methods has also demonstrated the presence of traces of aloes and myrrh. These traces occur both in areas corresponding to bloodstains and in other areas as well. (Baima Bollone and Gaglio, 1984).

The Aim of the Research

The purpose of these experiments is to confirm the previous findings and further assess the distribution of the different traces of biological materials with respect to the topography of the Holy Shroud. For this purpose, we have examined the trace-materials present in the concave stoppers of the test tubes in which the threads taken in 1978 were placed and preserved until the present testing. In some cases the amounts were considerable; so much so that the deposit of dust was visible to the naked eye. The test tube samples used were from C12a, C8a and D2c (see Map on page 2). The material was collected on the double-adhesive tapes designed to stick on ordinary "stubs" for electronic microscopy and on object-holding slides for optical microscopy.

Examination by SEM

A preliminary examination of the materials carried out by SEM has shown the presence of a large quantity of long fibres with an average diameter of 10-20 microns, characteristic of typical linen fibres. Cotton fibres are also found here and there. One notices also the presence of lumps of amorphous materials, with a maximum diameter of 100

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* HELLER and ADLER: *Can. Soc. Forens. Sci. J.*, 14 (3) 81-103, 1981.
† See *Sindon* #31 and *Spectrum* #6 [Ed.].

microns, some free and some attached to the pollens, spores and linen fibrils. The percentage of such plant fibres with respect to the remaining material is higher in C8a and lower in D2c.

Examination with the SEM for the elements present has shown the presence of: Na, Mg, Al, Si, P, S, Cl, K, Fe, Ca, Cu, Zn and Sb.

Identification of the Human Immunoglobulins

This research was performed with both direct and indirect methods. In the first case, total anti-serum anti-immunoglobulin and human anti-serum anti-IgG were used. In the second case, with prior incubation with Coombs serum (human anti-globulins from rabbit), antigammaglobulins of fluorescent rabbit serum were used. We used serums from the firms of Bening, Hoechst and USBC, and the method of La Cavera and Bandini (1967).

The results appear in the table below:

	Anti-immunoglobulin totals		Human Anti-IgG		Indirect
	Direct	Indirect	Direct	Indirect	method
Shroud Cl2a	+ + +	-	+ + +	-	+ + +
Shroud C8a	+ +		+ +		+ +
Shroud D2c	+		+		+ +
Timossi linen	-	-	-	-	-
Bloodstains	+ + +	-	+ + +	-	+ + +

Immunologic Identification of Aloes and Myrrh

We used an indirect immunofluorescence method. Rabbits were immunized with myrrh and aloes: Respectively, 2 grams of aloes powder and 2 grams of commercially available myrrh powder were dispersed in 20 cc. of physiological solution and left at a temperature of +4 ° C for 72 hours. Each rabbit received first an intramuscular injection of 1 cc. of the filtered aloes solution, or 1 cc. of the centrifugated myrrh intimately mixed with Freund's entire adjuvant. The injection was repeated after 20 days; after another 7 days the blood was withdrawn and the serum allowed to separate. After electrophoresis, all the injected animals showed a clear increase in their globulin fractions. At the site of the injections, granulomas were evident. From these, the microscopic samples were prepared. Upon observation of the slides, the presence of lymphocytes, plasmacytes, granulocytes (in particular eosinophils), epithelium cells and giant cells were conspicuous, along with "corpuscles" of the injected material. Other thin slices were treated with an immunohistochemical technique utilizing immunoperoxidase according to Nakane and Pierce (1967), and Sternberger (1975 & 1979), which allowed us to ascertain the presence of rabbit immunoglobulins in the plasma cells and lymphocytes as well

as at the surface of the antigenic material. The same procedure was performed for comparison on the dust samples from test tubes C12a, C8a, D2c; with linen fibres taken from a sample of Timossi's reconstruction; and with linen fibres smeared with aloes, myrrh and desiccated juice of *Allium sativum*, *Allium cepa* and *Aesculum hippocastanum*.

In this case also, the indirect immunofluorescence method was used (La Cavera and Bandini, 1967). Anti-globulin fluorescent rabbit serum from the Behring firm was employed. Examining the preparations by fluorescent microscopy (with Dialux Leitz), we observed:

— totally negative response for the experimental linen fibres of the Timossi sample and of the Shroud fibres, as compared with the controls.

— positive response for the experimental stains of aloes and myrrh, which confirms the capability of these materials to be identified with the serum obtained;

— negative response for the experimental *Allium sativum*, *Allium cepa* and *Aesculum hippocastanum* stains, which confirms the specificity of the results;

— positive response for the dust samples from the Shroud. In particular, the preparations from C12a and C8a showed the presence of fluorescent lumps either free or adherent to the fibres, while on the preparations from D2c only free lumps were observed (see cover photo, Figs. 1 and 2).

The results obtained are shown in these tables:

	Rabbit serum immunized with myrrh	Natural rabbit serum
Shroud C12a	+ + +	-
Shroud C8a	+ +	-
Shroud D2c	+	-
Timossi linen	-	-
Experimental stain with myrrh	+ + +	-
Aesculus hippocastanum stain	-	-

	Rabbit serum immunized with aloes	Natural rabbit serum
Shroud C12a	+ + +	-
Shroud C8a	+ +	-
Shroud D2c	+	-
Timossi linen	-	-
Experimental stain with aloes	+ + +	-
Allium sativa stain	-	-
Allium Cepa stain	-	-

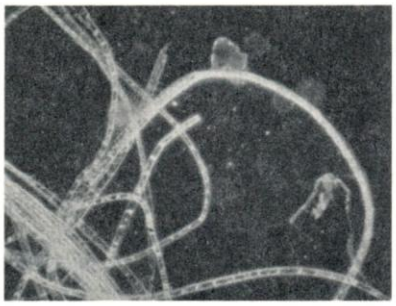

Fig. 1

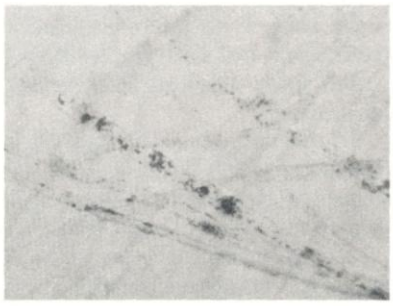

Fig. 2

Fig. 3

Fig. 4

1. Lumps on fibrils from C12a, showing positive response in tests of indirect immunofluorescence method using rabbit serum immunized with aloes. Enlarged 400X.

2. Grain on fibrils from C8a showing positive response in tests of indirect immunofluorescence method using rabbit serum immunized with myrrh. Enlarged 400X.

3. Grains of material which make up the stains in B12c; microphotograph in transmitted light. Enlarged 800X.

4. Microphotograph in contrast-phase of fibres from B12c; visible are lumps of material morphologically identical to myrrh. Enlarged 600X.

For locations indicated, see Map on p. 2.

Conclusions

Our research has confirmed the existence of human immunoglobulins on the Shroud. The preservation of such proteins over a long period of time poses the problem of the causes which have made that phenomenon possible. The present immunological investigations have also confirmed the presence of aloes and myrrh in Shroud samples. The fact that the density of the blood traces, aloes and myrrh is highest where there are bloodstains and decreases in the other areas, lends support to a connection between the blood's preservation and the presence of such plant preservatives. What is more, the demonstration of traces of aloes and myrrh adherent to fibrils removed from areas corresponding to the body imprints leads us to suppose the existence of a connection between these substances and the formation of the images.

Summary

The dust obtained from the stoppers of the test tubes (which contained some threads removed from the Shroud in 1978) was examined. The use of commercially available fluorescent anti-serums has allowed us to ascertain the presence of human globulins. Moreover, through serums of rabbits immunized with aloes and myrrh, traces of both substances have been identified.

This article was submitted in the English language.

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