Abstract
By spectroscopic and chemical tests (conversion of heme to a porphyrin), we have identified the presence of blood in the alleged blood areas of the Shroud of Turin.

Introduction
The Shroud of Turin is a linen cloth that has been dated to at least the middle of the 14th century. It has been traditionally considered the burial cloth in which the body of Jesus Christ was wrapped and placed in the tomb. As recently reported,1 there is now a widespread interest in this artifact, particularly in its image phenomena.

In October 1978 an interdisciplinary American scientific team was granted permission to carry out a series of non-destructive spectroscopic and photographic tests directly on the artifact. Also, by impressing strips of a special sticky tape (supplied by 3M Corporation) on various locations of the Shroud, it was possible to bring back samples of the surface material for further investigation, e.g., chemical. A number of investigators have subjected these samples and the spectroscopic data to various examinations to determine whether the Shroud and its image properties are a medieval forgery or the result of some older process and to try to understand the nature of this phenomenon.

Figure 1 shows that the Shroud image contains areas from the wrists, arms, and feet that correspond to the blood stigmata of a classical crucifixion. In addition to these, there appear to be head and flank wounds that also bled. All
these areas appear on the cloth as brownish red stains. This paper summarizes the initial studies to confirm the presence or absence of blood in these alleged blood areas of the Shroud.

**Spectroscopic Tests**

An x-ray fluorescence study demonstrated that no significant amounts of high atomic number elements except for iron appear on the cloth, and iron appears only in high concentrations in the blood areas.²

We received a single 2.5 x 7.5-cm (1 x 3-in.) specimen of the sticky tape from one of the blood areas. Unfortunately, from the viewpoint of optical and chemical examinations, this came with the sticky side firmly pressed down on a microscope slide. A 1000x dry microscopic examination showed several hundred linen fibrils, assorted debris of the centuries (e.g., a crimson silk fiber, an azure wool fiber), less than a dozen possible bloodstained fibrils, and a single brownish red translucent crystal. The stains appeared as a surface sheath on some of the fibrils as well as seeming to penetrate them.

To develop and check appropriate techniques we prepared a simulacrum from a sample of roughly woven undyed Spanish linen —300 years old and impregnated with 12-month old blood from one of us. This was sampled with ordinary Scotch Tape, and under microscopic examination several crystals and fibrils similar in physical appearance to those from the Shroud sample were observed. However, these all appeared to be slightly more garnet colored and less brown than the Shroud fibrils.

Both the stained Shroud fibrils and the simulated fibrils were then examined by microspectrophotometry in the visible range.³ It should be noted at the outset that there is no specific spectrum for blood *per se*; what is seen depends on the chemical state of the hemoglobin (e.g., reduced, met-hemoglobin, denatured) and on the state of aggregation (e.g., film, crystal, solution). All these fibrils showed intense Soret (400-450-nm) absorption indicative of a regular porphyrinic material.⁴, ⁵

Interestingly, the Spanish linen fibrils showed a clear spectrum in the visible with a peak at 550 nm and shapes indicative of the spectrum of reduced hemoglobin,⁶ thus explaining their more garnet appearance from the browner Shroud fibrils. Thermodynamically the latter fibrils would be expected to show the spectrum of a fully oxidized denatured met-hemoglobin, i.e., a so-called perturbed acid met-hemoglobin.⁷ Although the spectra of the Shroud fibrils are, in fact, indicative of such a spectrum, the high degree of scattering from these solid samples makes the visible band shape features less distinct and does produce peak shifts from the solution spectra (see Fig. 2 for a typical spectrum of one of the Shroud-stained fibrils). Therefore, this identification is much less positive than desired.

Fortunately, reflection spectroscopy has also been carried out directly on the blood areas of the Shroud. By using Kubelka-Monk theory⁸ these data can be transformed to an absorption spectrum.⁹ Although this spectrum is also distorted, as is expected due to anomalous dispersion, it does show more clearly the visible bands and shapes indicative of high spin iron porphyrin spectra ⁴.
In our opinion the spectral data taken in aggregate are positive in confirming the presence of perturbed acid met-hemoglobin species on the Shroud.

**Chemical Tests**

The usual forensic tests for blood involve the catalytic peroxidative action of the heme group in producing either a colored or fluorescent oxidized form of some dye. However, a number of other materials can give false positive reactions under these conditions as can even simple iron salts. Furthermore, false negative conclusions can be drawn if the material cannot be adequately solubilized, as can occur with a very aged strongly denatured sample. Therefore, we chose to use the conversion of the suspected heme group to a porphyrin, detectable by its characteristic Soret excitable red fluorescence, as a more specific test.

This conversion is accomplished by treating the heme material with a strong reductant to reduce the iron to its ferrous state and then treating it with a strong acid to displace the iron. The acid porphyrin dication so formed fluoresces red strongly under longwave UV radiation. A number of tests on the Spanish linen fibrils showed that 97% hydrazine (N$_2$H$_4$) served as the best reductant with the additional convenient property of solubilizing even strongly denatured or aged samples. Since it is a strong reducing acid, 97% formic acid proved the best acid of choice. The Spanish linen fibrils when treated with these reagents readily gave a positive test easily detected in a darkened room. (It should be noted that nanomolar solutions of porphyrins can be fluorimetrically detected in a darkened room by a dark-adapted eye.) Positive tests could even be obtained by successively exposing the Spanish linen fibrils to the vapors of the reagents for an ~15-20-min exposure in closed chambers.

We therefore peeled back the sticky tape from the glass slide and exposed the Shroud fibrils to, first, hydrazine vapor and then formic acid vapor. Irradiation with longwave UV then showed several red fluorescent spots indicative of the presence of a porphyrin species on the Shroud fibrils. We employed the vapor
method as we had hoped to take a microspectrum of the converted material to establish that it was specifically protoporphyrin IX. Unfortunately, the sticky tape was severely etched by the formic acid treatment and became optically intractable. Thus we were unable to provide this absolute final confirmation of the identity of the blood area material.

Summary
The following tests were performed:

(a) visual examination;

(b) positive association with iron by x-ray fluorescence;

(c) positive Soret absorption and reasonable correspondence to expected met-hemoglobin visible spectral shapes by both transmission and reflection spectroscopy; and

(d) positive chemical conversion to a fluorimetrically characteristic porphyrin species does confirm and give positive presumptive evidence for identification of the alleged blood areas on the Shroud of Turin as, in fact, containing blood.

This does, however, still leave open further questions. Since the Shroud has undergone a fire with exposure to intense heat under oxidative conditions, what other chemical species may be found in these same areas? Hopefully a larger sample of this same material will permit examination of this problem and allow us to further confirm spectrally the porphyrin formed as specifically protoporphyrin IX.
REFERENCES


3. We wish to express our indebtedness to Joseph Gall at Yale for access to his instrumentation and his assistance in these measurements.


5. F. ADAR, in Ref. 4, pp. 167-207.


9. R. GILBERT, Jr. and M. M. GILBERT, Oriel Corp; and S. F. PELLICORI, Santa Barbara Research Institute; personal communications; to be published elsewhere. We are indebted to these authors for the data shown in spectrum B of Fig. 2.

