actions catalyzed by the two enzymes, it is quite possible that botryococcene synthetase is derived from squalene synthetase, and the first step in this process was a mutation that repositioned the cofactor.

Conclusions. From a combination of model studies, stereochemical experiments, and inhibition kinetics, we have made process in elucidating the mechanisms for biosynthesis of 1′–1 and 1′–3 isoprenes. A central role for c1′–2–3 cyclopropylcarbinyl diphosphates is indicated for non-head-to-tail isoprenes with 1′–1, 1′–3, 2–1′–3, and c1′–1–2 fusions, with the regioselective formation of metabolites such as squalene, botryococcene, or phytoene by their respective synthetases primarily a consequence of electrostatic interactions in enzyme-bound intimate ion pairs. Much less is known about the cyclopropanation step. Ortiz de Montellano and co-workers[57] have evidence for distinct donor and acceptor sites for the two molecules of farnesyl diphosphate participating in the reaction. Although the regioselectivity they observed for their analogues is suggestive of an electrophilic cyclopropanation, the mechanism of the reaction remains to be established. Further work in this area will be difficult until a reliable source of soluble purified enzyme is secured.

I express my sincere gratitude to the dedicated students and postdoctorals in my group whose enthusiastic contributions made this work possible, and to Hans Rilling and Bill Epstein for sharing their interests in isoprenes with me. I also thank the National Institutes of Health (GM 21328 and GM 25521), the National Science Foundation (DCB 8803825), and the Amoco Corporation for support.

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The Shroud of Turin: Blood or Artist's Pigment?

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The scientific controversy over the Shroud of Turin is unusual in its disparity of opinions, the one-sidedness of those opinions, and its nearly dozen-year length. Although the 1988 carbon-14 date confirms a medieval origin, I reached that conclusion a decade earlier on the basis of polarized light microscopy.[1-3] I hope that a review of this evidence will not only reemphasize the importance of objectivity in scientific research but also serve as a reminder of the unique capabilities of this "mature" technique. The light microscope was invented in the 1600s, and it became a useful chemical analytical tool in the 1800s.

The Shroud of Turin (Figure 1), a linen cloth measuring 1.1 × 4.3 m, depicts two images, back and front views, of a naked crucified man. A beautiful image that fits perfectly the New Testament version of the crucifixion, it has been an object of veneration since it first appeared in history in 1356—this, in spite of the fact that Bishop Henri of Troyes, in whose diocese it was first exhibited, said it was "cunningly painted as attested by the artist who had painted it."

A group of scientists from the Shroud of Turin Research Project (STURP) spent five days and nights in Turin in October 1978 examining the Shroud. They studied it visually, photographically, and spectroscopically and took sticky-tape lift samples (18 × 37 mm); 18 from body- and blood-image areas, and 14 from nonimage areas as controls (Figure 1).

The STURP scientists identified the Turin Shroud image as blood and oxidized/dehydrated cellulose.[1] I, instead, found no blood and established the presence of $Fe_2O_3 \cdot xH_2O$ and HgS corresponding to two common artist's pigments of the 14th century, red ochre and vermilion, with a collagen (gelatin) tempera binder. My microscopical studies were made on thousands of fibers and particles on the 32 Shroud tapes.[2,3]

Characteristics of the Image

To the unaided eye, the Shroud image is yellow in most body-image areas (Figure 2), but red in the blood-image areas (Figure 3). Microscopically, the image consists of yellow fibers (Figure 4) and red particles (Figure 5); the red particles are more abundant in the red blood images, and the yellow fibers are the major colored substance in the body image.

A careful microscopical survey of the 22 image tapes and 10 nonimage tapes shows, without exception, tiny red particles in body- and blood-image areas but no red particles on the fibers in nonimage areas. They are readily apparent as easily visible red incrustations in most blood-image areas, but more widely dispersed red particles in the body-image areas. The red particles require careful high-magnification light microscopy (600–1000×) to see and identify.

A representative group of 12 of the 32 tapes were also carefully examined for the presence of yellow fibers, and

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(1) Schwalbe, L. A.; Rogers, R. N. *Anal. Chim. Acta* **1982**, *135*, 3.
(2) McCrone, W.; Skirius, C. *Microscope* **1980**, *28*, 105.
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Table I
Energy Dispersive X-ray Analysis (EDXRA) of Blood-Image-Area Sherds

particle	diameter[a]	color	peak heights[b] Na	Mg	Al	Si	P	Hg/S	Cl	K	Ca	Fe
A	12	orange	7	8	12	35	16	37	46	1	40	28
B	9	red	2	1	3	8	5	40	18	1	15	3
C	23	red-orange	1	<1	14	28	2	9	2	2	3	66
G	12	red-orange	<1	2	25	57	2	14	20	4	45	65
H	9	red-orange	1	2	8	30	4	55	22	3	36	26
I	23	orange	5	1	12	45	4	13	60	5	47	12
J	11	yellow	3	2	3	9	3	5	13	2	65	<1
K	11	red	7	5	24	53	4	9	35	9	52	22
L	5	orange	9	3	28	50	10	50	20	7	60	34
M	11	none	1	2	<1	3	1	4	5	<1	66	<1
N	9	red-orange	5	1	15	27	2	3	7	3	10	66

[a] Average of two largest diameters in micrometers of these flat particles. [b] In units above background but proportional to emitted X-ray photons.

Table II
X-ray Diffraction of a Second Blood-Area Sherd from Tape 3-CB

unknown		vermilion		calcite		hematite	
d	I/I_0	*d*	I/I_0	*d*	I/I_0	*d*	I/I_0
3.36	100	3.35	100	–	–	–	–
3.05	40	–	–	3.04	100	–	–
2.88	40	2.86	100	–	–	–	–
2.72	80	–	–	–	–	2.69	100
2.53	80	–	–	–	–	2.51	50
2.32	weak	2.38	10	–	–	–	–
2.22	weak	–	–	–	–	2.20	30
1.84	80	–	–	–	–	1.84	40
1.71	80	1.74 / 1.68	25 / 25	–	–	1.69	60
1.46	20	–	–	–	–	1.48 / 1.45	35 / 35

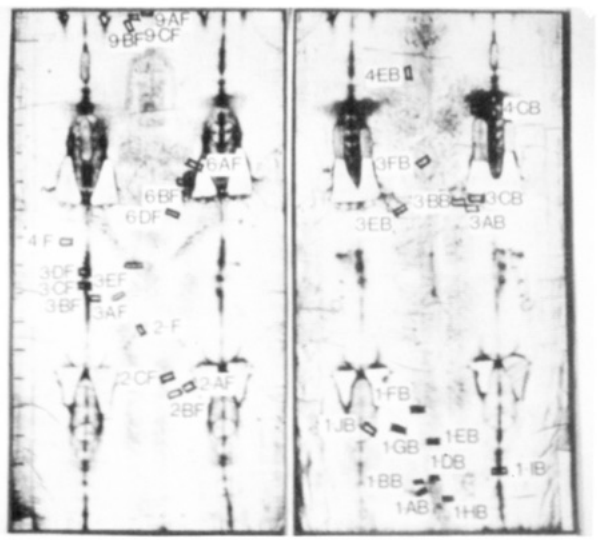

Figure 1. Photograph of the Turin Shroud with tape sample locations.

counts of yellow colored (Figure 4) versus uncolored fibers were made. The results are based on a total count of more than 8000 fibers on image and nonimage tapes. There are 10–26% (average, 19%) yellow fibers in nonimage areas and 29–72% (average, 46%) yellow fibers in image areas. Details of these studies can be found in ref 2–5 (reprints are available from the author). The significance of this discoloration of the image fibers lies in its suggestion that the image was applied as a liquid suspension of red particles, that is, a paint. Paint media and varnishes commonly yellow with time.

Microscopical Identification of Red Ochre

Microscopically, the submicron, ultrahigh refractive index red particles are opaque by transmitted light. Such particles are differentiated from truly opaque particles only by reflected light and/or high magnification (Figure 6). These red particles are found on the fibers of all image tapes and have varying degrees of hydration, color, and refractive index (from about 2.5 to 3.01); these properties are characteristic of the artist's earth pigment, red ochre. Common worldwide, this pigment has been used by artists for at least 30 000 years. The highest refractive index particles are anhydrous, crystalline, highly birefringent hematite with indices of 2.78 and 3.01. The iron earth pigments are hydrous iron oxide ranging in color and refractive index from yellow ochre to red ochre depending on their history. Long times or high temperatures cause the particles to lose water, becoming redder. This raises the refractive index and may result in crystallization of hematite, anhydrous Fe_2O_3. A significant proportion of the Shroud red ochre is hematite, thus accounting for the observed birefringence of many of the individual particles. Figure 7 (compare Figure 6) shows a modern red ochre, Morellone Buonamici (Forbes Pigment 6.02.15).

The composition of the Shroud red ochre was confirmed both by electron microprobe (Table I) and by X-ray diffraction[4] (Table II). The XRD data were obtained on single red particle aggregates (blood-image sherds) similar to that shown in Figure 8, but amounting to <1 ng of which possibly 0.2–0.3 ng is

(4) McCrone, W.; Teetsov, A.; Andersen, A.; Hinch, R.; Humecki, H.; Majewski, B.; Piper, D. *Microscope* **1981**, *29*, 19.
(5) Feigl, F.; Auger, V. Translation by Oesper, R. *Spot Tests in Organic Analysis*, 7th ed.; Elsevier: New York, 1966; p 219.


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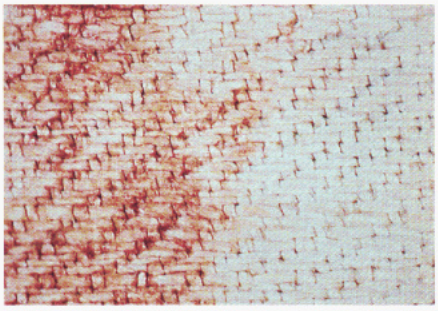
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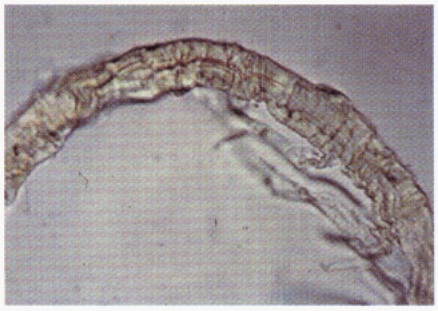
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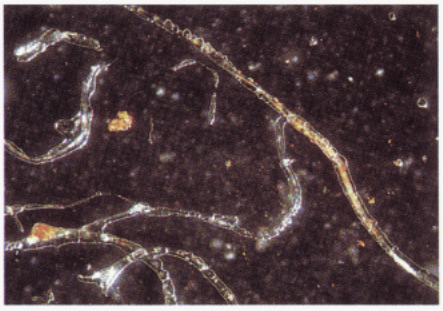
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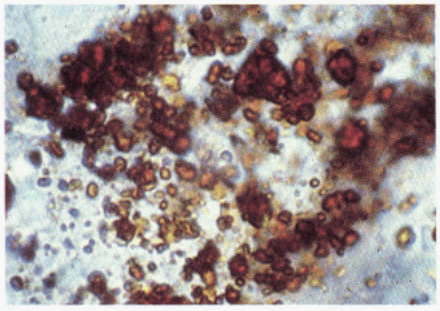
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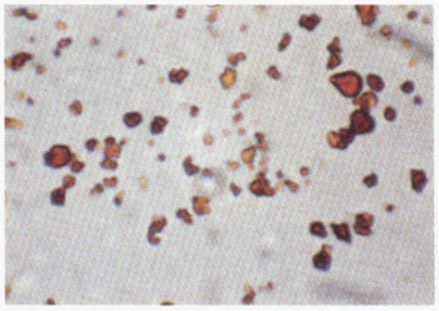
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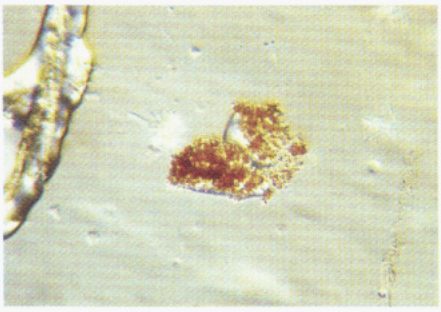
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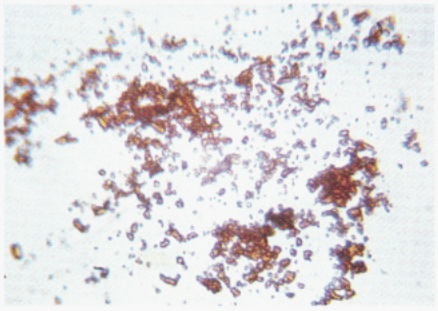
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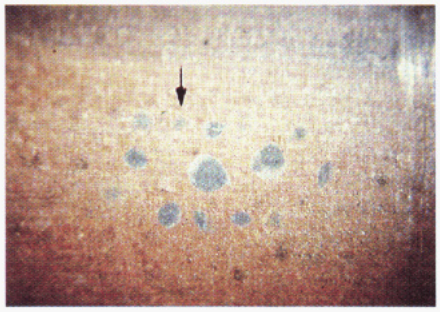
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Figure 2. Yellow body-image area on the Shroud; photomacrograph by Mark Evans, 9×.

Figure 3. Red blood-image area from small of back; photomacrograph by Mark Evans, 22×.

Figure 4. A yellow linen fiber from tape 6-AF, 220×.

Figure 5. Image particles on Shroud tape 3-CB, 55×.

Figure 6. Red particles on a Shroud linen fiber, 3-CB, 1650×.

Figure 7. Modern red ochre pigment particles, 1650×.

Figure 8. A blood-area red pigment aggregate, 220×.

Figure 9. Vermilion in a single Shroud particle aggregate from tape 3-CB, 220×.

Figure 10. A group of microchemical tests for mercury (mirror formation by precipitation from HgS solution by metallic copper). The arrow indicates the test result for a Shroud particle aggregate from tape 3-CB, 16×.

pigment. Less than one-half of the pigment is crystalline (hematite or vermilion), hence the lines in the XRD pattern are very spotty and difficult to measure. The agreement with known hematite data is, nonetheless, convincing.

Microscopical Identification of Vermilion

The blood-image areas show incrustations of a red substance with indications of "spalling" (Figures 3 and 8). Many loose particle aggregates, picked from the blood-image tapes, show red particles different in shape and color from red ochre (Figure 9), but characteristic of the artist's pigment, vermilion (HgS). Artists have used at least three microscopically different vermilion pigments, all with the same crystal structure as the mineral cinnabar. The most common vermilion pigment is the ground mineral cinnabar. The other two are synthetic mercuric sulfides, one a modern wet-process product, and the other, a dry-process form first prepared by alchemists about 800 A.D. All three are readily distinguished microscopically. Significantly, the vermilion on the Shroud is the 800 A.D. form.

The chemical composition of this second red pigment was established by polarized light microscopy (PLM), by electron microprobe,[4] by XRD (Table II), and microchemically (Figure 10). The PLM microchemical test requires wet ashing of one of the blood-image sherds to remove the organic binder, dissolution of the HgS crystals in HIO_3, and precipitation of a mercury mirror with metallic copper. All of this is done in a <100 μm diameter droplet on a copper penny (Figure 10) as a source for copper. Many tiny simulated known vermilion blood sherds were tested on the same penny to develop this procedure, which was then used to test

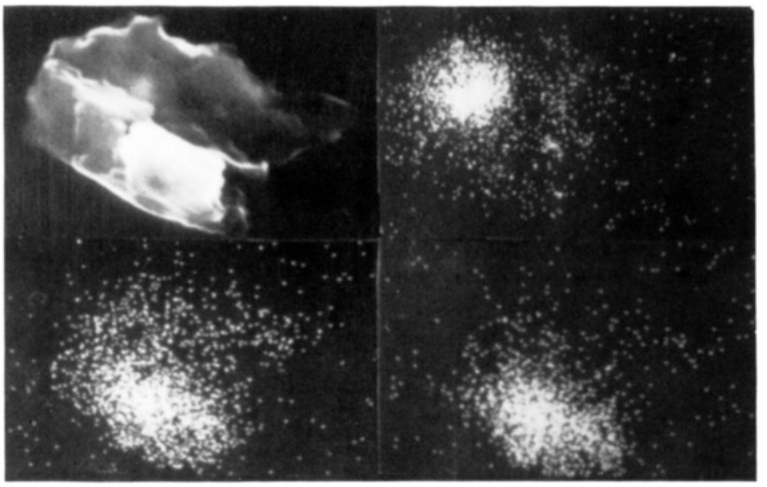


Figure 11. A 3-CB blood-image-area sherd: (a) back-scattered electron image (upper left); (b) an iron map (upper right); (c) a mercury map (lower left); (d) a sulfur map (lower right), 3000×.

one of the Turin Shroud sherds (arrow in Figure 10).

The same XRD pattern identified hematite in this blood-image sherd (Table II) and also showed the strongest lines for cinnabar, confirming vermilion. A number of these blood-image sherds were analyzed by electron microprobe[4] (Table I). Figure 11 shows a back-scattered electron image of one sherd and the corresponding Fe, Hg, and S elemental maps. The fact that these maps show discrete volumes for these elements, and Table I shows varying ratios for the two different pigments, proves there must have been two different paint applications, one a red ochre paint, and the other, a vermilion paint. The different paint sherds show varying amounts of red ochre relative to vermilion; this also supports the application of two paints. Furthermore, no vermilion pigment particles were observed on any of thousands of body-image tape fibers. It seems reasonable that the Shroud was first painted with a red ochre paint and then the blood images were enhanced with a vermilion paint.

The finding of HgS as an artist's pigment on the Shroud is highly significant. One might argue that Fe_2O_3 could be formed from blood, but it is not possible to explain HgS as vermilion except as an artist's pigment. The energy dispersive spectrum in Figure 12 and similar spectra for 10 other blood-image sherds were measured to give the quantitative elemental compositions tabulated in Table I.

Microscopical Identification of Collagen

If the image on the Shroud is a painting, we must find a paint medium. Because the image is so faint, the paint layer will be almost invisibly thin in most areas. However, I did find evidence of a medium as a dried film, or other accumulations, on the Shroud linen fibers. On one tape (1-AB) I observed two fibers "cemented" together with a yellowed residue. Further examination by PLM at 200–400× shows paint residues on a number of fibers (Figure 13) and very thin paint layers stripped from the fiber surfaces by the tape lifts (Figure 14). Therefore, I decided to test the image fibers for paint media (drying oils, gums, tempera) and blood using microhistological staining reactions. A bromcresol green test for drying oils was negative, as was an orcein test for gums. However, tests for protein, using amido black as well as fuchsin, show thin stained paint residues, and

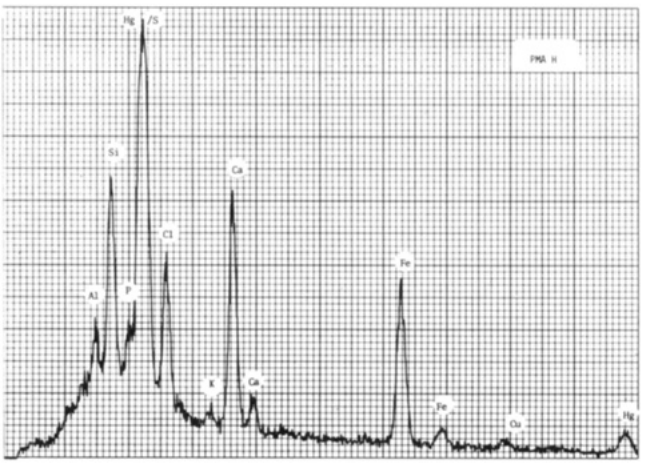
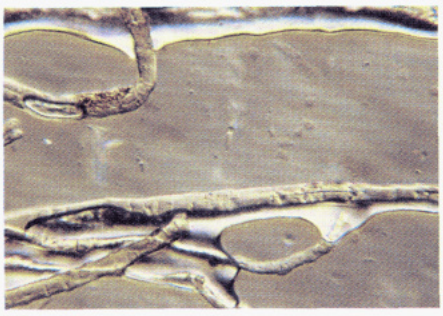


Figure 12. Energy dispersive spectrum for a single blood-image sherd showing both Hg and S characteristic of vermilion and Fe characteristic of red ochre.

occasional accumulations of paint (Figure 15) on the image-area fibers from several tapes including 1-AB, 3-CB, and 6-AF. In many of these accumulations, red pigment particles are observed. Figure 16 shows one blood-area sherd from tape 3-CB; the collagen is stained blue with amido black, and a hint of red pigment is apparent within the sherd.

Before continuing with further tests, I prepared two paints in order to produce Shroud-like images: a 3% aqueous blood solution, and a collagen tempera prepared from gelatin.[6] The tempera paint was prepared with 10 ppm red ochre in a 1% aqueous gelatin solution. A noted Chicago artist, the late Walter Sanford, painted Shroud-like images with both paints; transparent (adhesive) tapes of these images were compared with the Shroud tapes. I also applied drops of each paint, one at a time with drying between each drop, to linen strips to observe the spot color and shape (Figure 18). Each drop contains known amounts of iron in the form of red ochre or blood. The blood-image spots are made up 1, 2, 3, ..., 10 drops/spot. The red ochre/tempera spots are made with 5, 10, 15, ..., 80 drops/spot. A glance shows that it would be impossible to paint the Turin Shroud with diluted blood because of the brown color

(6) McCrone, W. *Wiener Berichte uber Naturwissenschaft in der Kunst* **1987/1988**, *4*/5, 50.



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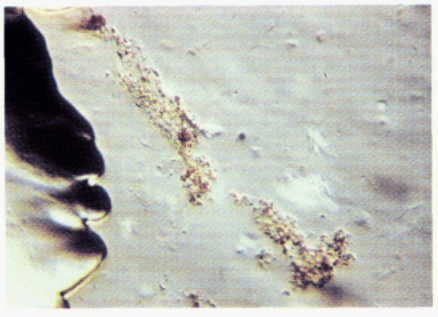

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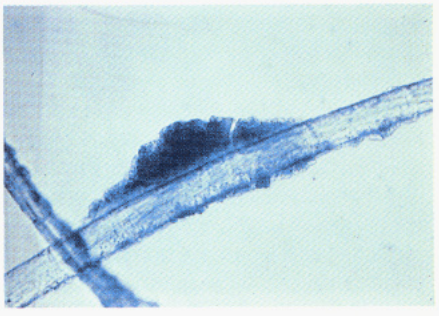

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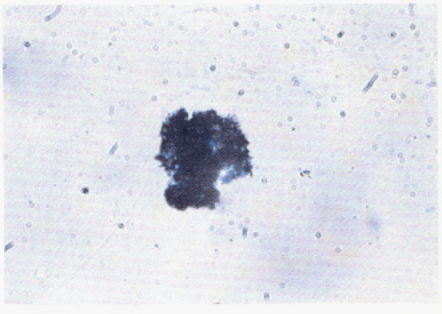

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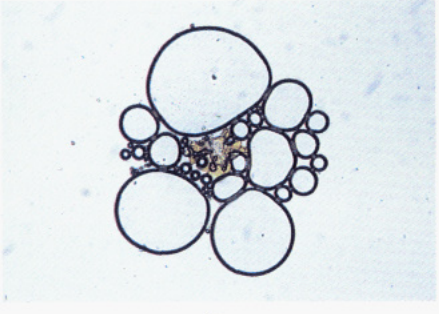

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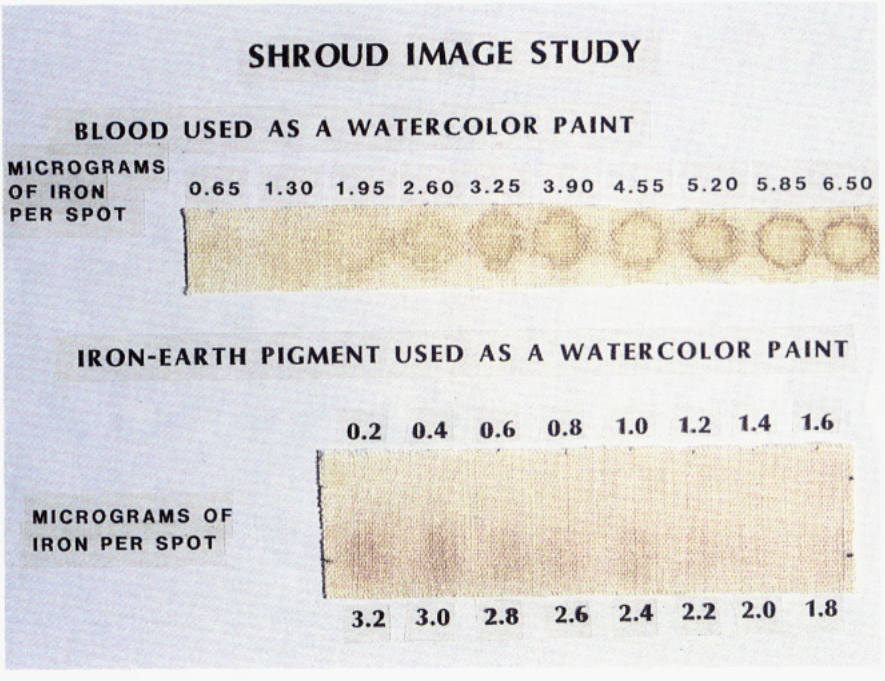

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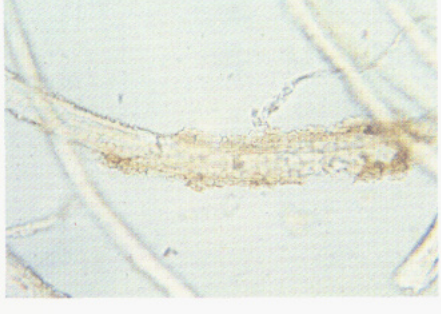

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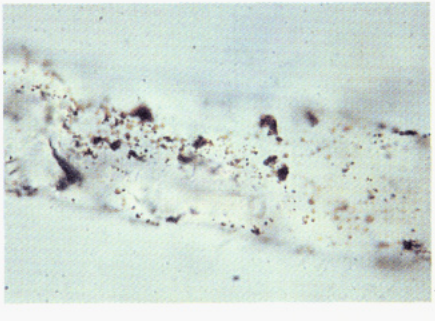

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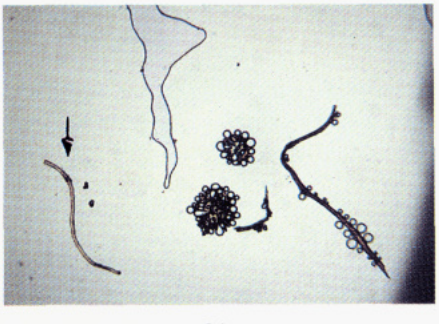

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Figure 13. A Shroud linen fiber showing a paint residue with encapsulated red ochre pigment particles, 220×.

Figure 14. A thin paint film stripped from blood-image fiber by the tape and with dispersed red ochre particles, 66×.

Figure 15. A dried paint residue on a 1-AB Shroud linen fiber stained with a protein stain, amido black, 220×.

Figure 16. A Shroud 3-CB amido black stained blood-image sherd, 220×.

Figure 17. A real-blood particle catalytically decomposing NaN_3 in solution to give N_2 bubbles, 66×.

Figure 18. Each succeeding spot has an increasing number of drops of paint.

Figure 19. Blood-coated linen fibers from a modern shroud painted with a diluted-blood "paint", 660×.

Figure 20. A red ochre paint-coated linen fiber. The red ochre was modern commercial pigment, and the medium was collagen (gelatin), 1100×.

Figure 21. A linen fiber from the Turin Shroud (arrow) and two real-blood-coated linen fibers and blood particles in NaN_3 solution showing N_2 bubbles around the blood, but none around the Turin Shroud fiber, 66×.

and halo shape of the spots. The red ochre spots, on the other hand, match both the shape and color of the Shroud image.

The tapes of Sanford's two paintings, one with blood-painted images and one with red ochre images, yielded coated fibers (Figures 19 and 20). The paint

prepared from a modern red ochre produces coated fibers indistinguishable from Turin Shroud tape fibers in color and particle dispersion. They also test positive for protein with amido black in the same way. The diluted-blood-painted images, when taped, are very different from Turin Shroud image tapes (Figure 19). The brownish gelatinous blood coating on the linen fibers of Sanford's blood-painted shroud shows no red particles and a low refractive index of only about 1.55. I also tested the Turin Shroud blood-image-area tapes from the right-side lance wound for blood. I obtained negative results with all forensic blood tests tried. These included Takiyama, Teichman, and benzidine as well as H_2SO_4 treatment followed by UV fluorescence. These tests are positive for fibers from Sanford's blood-painted shroud, but negative for Turin Shroud fibers and Shroud blood-image sherds. One might argue that first century blood, or even 14th century blood, would behave differently, yet similar tests on Persian burial silks and Egyptian mummy wrappings show no difference from my year-old blood spots.

Amido black is a stain for any proteinaceous substance; hence I could only conclude that the paint medium was blood, or a tempera produced during the Middle Ages from egg, cheese, or collagen, the latter often from parchment (collagen) scraps. However, if the Shroud paint medium is collagen, it would show one unique difference from egg or cheese tempera, *and* from blood. Collagen lacks cystine and cysteine, two sulfur-containing amino acids present in other proteins (including blood). A standard PLM test for sulfur-containing substances in particle form is the catalytic decomposition of NaN_3 in solution to yield a froth of N_2 bubbles.[5] Figure 17 shows such a reaction between NaN_3 and a particle of dried (real) blood. Collagen tempera shows no evolution of N_2 in a NaN_3 solution; blood, egg, and cheese tempera evolve N_2 from NaN_3.

The test based on catalytic decomposition of $NaN_3 \rightarrow N_2$ by sulfides and disulfides[5] was then applied to known Sanford-painting blood-image particles and to a Turin Shroud fiber. I mounted two linen fibers, and two blood sherds taped from Sanford's real-blood shroud, shown on the right side of Figure 21, and one linen blood-image fiber from the Turin Shroud, on the left. Bubbles of N_2 from the surrounding NaN_3 solution are apparent on the real-blood-image particles and fibers, but none around the long Turin Shroud fiber. The paint medium on the Shroud forms no N_2 bubbles with NaN_3, hence it does not contain sulfur-containing amino acids. Because that medium is known to be proteinaceous by histological staining, it must be collagen.

Painting of the Shroud Image

These results suggest that a talented artist carefully studied the New Testament, sources of information on the crucifixion, and other artists' paintings of Christ. He then thought about a shroud image in terms of a dark tomb. Instead of the usual portrait with normal light and shadow, he assumed that the image could only be produced by body contact with the cloth. He painted directly on the cloth to image the body-contact points (forehead, bridge of the nose, cheekbones, mustache, beard, etc., over the entire body, front, and back). This automatically creates a negative image; areas that normally catch available light and appear bright, like the bridge of the nose, would instead all be dark. However, those dark areas appear bright on a photographic negative. He decorated the body with blood stains as required by the New Testament descriptions. These he rendered dark on the Shroud, hence they form a photographic positive image superimposed on the otherwise negative Shroud body image.

The Shroud artist used a style of painting and painting materials common in Europe during the 14th century. A chapter entitled "Practice of Painting Generally During the Fourteenth Century" in an 1847 book[7] by Sir Charles Locke Eastlake entitled *Methods and Materials of Painting of the Great Schools and Masters* covers precisely the Shroud-like images. He refers to the process as the English or German mode of painting faint images ("grisaille", a light monochrome image). As Eastlake writes: "...After this linen is painted, its thinness is no more obscured than if it had not been painted at all, as the colours have no body." "The peculiarity of the English method appears to have been its absolute transparency." "A manuscript of the time contains directions for the preparation of transparent colours for painting on cloth." Eastlake continues: "The Anglo-German method appears, from the description, to have been in all respects like modern water-colour painting—except that fine cloth, duly prepared, was used instead of paper."

The Opposition

The October 1988 results of the carbon dating of the Shroud by the Universities of Arizona, Oxford, and Zurich Technological Institute[8] with a date of 1325 ± 65 years have not lessened opposition to the painting hypothesis by many Shroud believers. Some STURP authors admit that the Shroud has protein, Fe_2O_3, and HgS in image areas, but attribute the protein to blood, the iron oxide to retting of the flax to form linen fibers, and the vermilion to contact of the Shroud with painted replicas (to transfer the divine powers of the Shroud). Many members of STURP, and others convinced of the authenticity of the Shroud, explain away the carbon-dating result by invoking impure linen samples or a change in the date induced by the resurrection, or by saying that the dating was not a test of the Shroud, but the Shroud (known to be first century) was a test of carbon dating.

The STURP scientists find no pigment particles at 20–50× (I used 400–2500×). They find no cementation of the fibers nor evidence of capillary flow. I observe many visual evidences of a paint medium. They are not microscopists and, particularly, not microscopists trained in the study of pigments and paintings. They are not familiar with the microscopical appearance and behavior of tiny samples, in this case, both very thin collagen paint medium and small amounts of very tiny particles in watercolor images. There is no way, at 50×, that anyone could recognize the red particles as Fe_2O_3 and as red ochre or the HgS as a ninth century vermilion, and no way anyone could see that the pigment particles are cemented into an organic matrix (paint

(7) Eastlake, C. L. *Methods and Materials of Painting of the Great Schools and Masters*; Chapter V (1847); reprinted by Dover: New York, 1960.

(8) Damon, P. E.; Donahue, D. J.; Gore, B. H.; Hathaway, A. L.; jull, A. J. T.; Linick, T. W.; Sercel, P. J.; Toolin, L. J.; Bronk, C. R.; Hall, E. T.; Hedges, R. E. M.; Housley, R.; Law, I. A.; Perry, C.; Bonani, G.; Trumbore, S.; Woelfli, W.; Ambers, J. C.; Bowman, S. G. E.; Leese, M. N.; Tite, M. S. *Nature* **1989**, *337*, 611.

medium), and to the fibers. At 400–2500×, I readily observe these features.

The amounts of pigments and medium on the body-image areas and some of the blood-image areas, barely visible microscopically, demonstrate that absorption spectroscopy on 1-cm^2 areas by Pellicori[9] and others could not have detected them.

The results published by STURP authors in more than 30 papers are consistent with the sensitivity of the instruments and techniques they used. Rogers and Schwalbe[1] summarize the STURP findings. Their grossly disparate conclusions read in part as follows: "The primary conclusion is that the image does not reside in an applied pigment. The reflectance, fluorescence and chemical characteristics of the Shroud image indicate rather than the image-recording mechanism involved some cellulose oxidation/dehydration process."

There are very small amounts of pigment and medium on the body-image fibers, undetectable except by careful light microscopy at a minimum magnification of 400×. The blood-image areas hold more solid material as red ochre, vermilion, and collagen tempera but, still, much less than a normal painting. Heller and Adler[10] acknowledge the existence of Fe_2O_3 and HgS in blood-image areas. Pellicori[9] found high concentrations of iron in blood-image areas, but he attributed this to blood. However, Accetta and Baumgart[10] state that "Shroud blood comparisons with known bloodstains show marked differences." As I stand accused of "misinterpreting [my] otherwise good data", I see that, at least, I am not alone.

(9) Pellicori, S. F. *Appl. Opt.* **1980**, *19*, 1913.
(10) Heller, J. H.; Adler, A. D. *J.—Can. Soc. Forensic Sci.* **1981**, *14*, 81.
(11) Accetta, J. S.; Baumgart, J. S. *Appl. Opt.* **1980**, *19*, 1921.

Conclusion

Sensitive mass spectrometric methods have revolutionized carbon-14 dating in the last decade, so that high accuracy can be achieved on 1-mg samples. The three independent measurements recently reported[8] (October 1988) show that the Shroud of Turin dates from 1325 ± 65 years. This is in gratifying support of our evidence that an artist's image is contemporary to that time and was probably painted just before its first exhibition in 1356. This conclusion, in 1980,[3] was based solely on observations made with a polarized light microscope, though confirmed later by X-ray, electron diffraction, and electron microprobe analyses.[4]

Very few chemistry students, undergraduate or graduate, have ever looked through a microscope except, perhaps, in high school biology. The direct microscopical approach to the solution of chemical problems has been largely lost during the past three decades. This project on the authenticity of the Shroud of Turin has been an excellent opportunity to gain wide publicity for the unique capabilities of polarized light microscopy, providing a real impetus to its deserved renascence.

I acknowledge with gratitude the help of Ray Rogers of Los Alamos, who took the excellent Turin Shroud tapes; Christine Skirius, one of my students, who patiently made the meticulous color evaluation of more than 8000 linen fibers; McCrone Associates microscopists, including Anna Teetsov, Mark Andersen, Ralph Hinch, Howard Humecki, Betty Majewski, and Deborah Piper, who made careful X-ray and electron optical studies of individual fibers and image components; and my wife, Lucy, who helped to confirm and interpret my observations as well as making important suggestions for presenting the written reports. I am also grateful to all of the reviewers of this paper for their constructive suggestions.

Registry No. Red ochre, 37338-85-5; vermilion, 1344-48-5.