CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE BLOOD STAINS

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Abstract

A variety of investigative techniques by several experienced independent investigators has been applied to the study of the blood images found accompanying the frontal and dorsal body images found on the Shroud of Turin. These investigations include forensic analysis of the images, photographic studies (reflection, transmission, infrared thermography and ultraviolet emission), computer image analysis, spectroscopic studies (electron microprobe, X-ray transmission and fluorescence, reflection ultraviolet-visible spectroscopy, infrared spectroscopy, and micro-FTIR spectroscopy), electron and optical microscopy (reflection, transmission, phase contrast, fluorescence, and polarisation), and wet chemical testing (including enzymatic and immunochemical analysis) of materials removed from the surface of the Shroud. These studies at both the macroscopic and microscopic level confirm, complement, and supplement one another so as to provide us with a consistent set of observed physical and chemical characteristics for the various types of blood images found on the Shroud of Turin. The images are forensically correct for a Semitic adult male human showing all the wounds historically associated with the descriptions of the crucifixion of Jesus of Nazareth. All the blood images appear to be impressions of the exudates from clotted wounds of a man who died a traumatic death. The chemical, microscopic, and spectroscopic investigations are all consistent with this forensic conclusion, including evidence for the elevated bilirubin levels indicative of trauma. The mechanisms to form the body (non-contact) and blood images can be demonstrated to be different, with the blood images going onto the cloth first by contact and out of stereoregister with the body images. The forensic studies are in agreement with the computer analysis conclusions that this cloth enfolded a three-dimensional wounded human male body. The blood images penetrate the weave of the cloth, show cementation of fibers to one another, and show capillary flow under the crossing threads of the weave. The blood images show a non-uniformity of color at the microscopic level and have the appearance of a material applied to the cloth showing signs of erosive wear. This is a matter of great concern in the efforts to conserve these images. All the blood images show evidence of clot retraction rings of serum about each wound, these being easily seen in the fluorescence photographic study. The presence of these serum rings makes the notion of a forger painting in the blood images with the correct chemical composition before the image forming process and properly out of stereoregister virtually impossible. This criterion alone is sufficient to dismiss many of the proposed methods for creating the images on the Shroud of Turin by some type

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of artistic rendition technique. It should be noted that many of these proposed techniques have also been ruled out by computer analysis investigations. Image analysis studies have also shown that some of the patterns of blood marks on the Shroud can be linked to similar patterns on the Cloth of Oviedo and on the Holy Tunic. This provides direct evidence against the accuracy of the reported radiocarbon date and supports the historic arguments for the authenticity of these relics.

Introduction

The 4.3 x 1.1 meter image-bearing linen cloth known as the Shroud of Turin became an object of religious and historical dispute following its display in the mid-1350s at Lirey by the de Charny family, as they declared it to be the authentic burial cloth of Jesus. It first became scientifically polemical after the 1898 Exposition in Turin, when for the first time it was photographed by Secondo Pia.[1] Scientific interest was aroused due to the observation that the plate bearing the photographic negative of the body showed details more clearly and gave a more natural appearance to the body images than the visually observed image on the cloth, although the blood images behaved as expected. As its known history predated the invention of photography, this fact stimulated scientific inquiry, hypothesis, and controversy.[2-6] A large variety of investigative techniques carried out by many types of independent investigators has created a large corpus of scientific information on the Shroud. This has been reported in many general and specialized reviews, monographs, conference proceedings and professional journal publications.[7-21]

Science and the Shroud

The validation of scientific conclusions differs from the criteria used in historical arguments in that hypotheses must be testable by a reproducible experiment. For example, determining the chemical structures comprising the blood marks on the cloth can test whether or not they are really blood derived materials. On the other hand, scientific studies cannot establish the authenticity of the Shroud as Christ's burial cloth, but only its disauthenticity, as no acceptable laboratory experiment exists yielding the identity of the human image seen on the cloth. However, evidence that the blood marks are painted mineral pigments would disauthenticate it. Furthermore, initial test results supporting a hypothesis do not necessarily prove it. An alternative hypothesis equally well supported by the observations is always possible, e.g., the presence of interferences can lead to false positive or negative conclusions. The test may not be sensitive enough to draw a proper conclusion or it may be so sensitive that it will give misleading conclusions. There also exists the problem of errors, both random (affecting precision) and systematic (affecting accuracy). The investigator must carry out enough measurements to establish precision and enough control experiments to distinguish which of all reasonable possible testable explanations best fits all the data. One must not simply select or delete data that favors what might seem to be an obvious acceptable conclusion. Scientific truth becomes a matter of relative probabilities to which one approximates by continued application of the scientific method utilizing further testable hypotheses and reproducible experiments.

Forensic Investigations

Vignon[2, 3] and Barbet[7] were the first to examine the Shroud from a forensic viewpoint. They both noted that all the blood marks depicted were in agreement with the historic descriptions of Christ's crucifixion: for example, the presence of flog marks, the fact that the blood flow on the arms demonstrated that they were in an elevated and extended position at the time the wounds were bleeding, the presence of what appeared to be a lance wound, marks on the head that could be attributed to a crown of thorns, etc. Moreover, they also noted that the blood images were forensically consistent with those of clotted blood and not a freshly flowing wound in that they appear thickened on the edges. As blood forms a scab it contracts, thickening the edge of the scab and exuding serum onto the surface and edges of the contracting clot. This phenomenon is simply termed clot retraction and they noted that the major blood wounds even showed to the eye what appeared to be forensically correct serum contraction rings.

Since these pioneering studies, many other physicians have studied the Shroud and extended our knowledge of these matters.[5, 8, 9, 22, 23, 24] For example, by carrying out experiments on corpses and severed arms with attached weights, it was demonstrated that nails driven through the palms as shown in most artistic renditions of a crucifixion will not support the weight of a suspended human body. Rather such nails must be driven through the wrists as it is clearly seen on the Shroud, showing a correct depiction unlike that in older artistic renditions. In addition the conditions and timing of the clotting process have been experimentally investigated to demonstrate that only clotted blood will give the type of clear unsmeared blood images seen on the cloth and therefore these wound marks are really images of clot exudates gotten onto the cloth by contact and not whole blood images.[23] Although these modern studies differ over some details, these investigators all agree that the blood marks are consistent with the historic description of the Crucifixion, are clot images, and have gotten onto the cloth by actual contact transfer with a wounded human body.

Image Investigations

Several different types of photographic studies have been conducted and in several regions of the electromagnetic spectrum. These images have then been further subjected to several forms of analysis by different types of computer algorithms. These studies have then been compared to the microchemical and spectroscopic investigations to test the consistency of the conclusions drawn.

Using appropriate light sources and filters a series of ultraviolet fluorescent photographic images were made of the Shroud and compared to color reflectance photographic images taken of the same areas.[25] The blood marks appear as red brown to brown yellow images of approximately the same intensity range of variation in the color reflectance photographs, as do all the other image types on the cloth. However, these images all appear quite differently in the ultraviolet emission and absorption photographs. The blood marks are all now highly absorbing, as would be expected if hemoglobin were present, as the porphyrin structure in this chromophore is a very strong near ultraviolet absorber. Also the border of every blood mark shows the typical yellowish fluorescence of the

serum ring exudate about scabs as expected for clot retraction transfer marks, thus confirming the medical forensic analysis and observations previously noted for the major blood wounds. Further, all the scourge marks now show a pattern of scratches on the narrow ends, not seen in reflectance, that would be expected for wounds produced by a typical Roman scourge. Therefore an artist painting the blood marks would not only require a 20th century knowledge of the physiology of clot retraction, but would have to produce images of serum rings and scratches that are only obviously evident under ultraviolet excitation. Note he would also require a constantly fresh supply of clot exudate and finally serum to add the rings after the wound marks themselves were dry, as the proper appearance cannot be achieved by painting with whole blood itself which would be in the process of clotting.

A series of color microphotographs of the different types of image areas were made at magnifications ranging from 3.6x to 36x.[26] Utilizing the microscope employed in making these image area microphotographs, a visual mechanical examination of these same image areas was conducted with the aid of a probing needle.[12, 13] The blood area photographs show all the characteristics that one would expect for a clot retraction transfer to a fabric. As confirmed by the probing needle, the fibers are cemented together by the applied chromophore and show capillarity in that they penetrate to the back of the cloth and also can be seen under the crossing threads of the weave. There is evidence of abrasion of the chromophore from the more exposed surfaces as would be expected if this were an applied material with different mechanical properties from the cloth substrate. There is a variation in the color of the adherent particles from orange yellow to deep red as would be expected for clotted blood, as the formation of a clot leads to some separation and aggregation of the blood and serum components. However, this type of color variation would not be anticipated for an artist's uniformly ground and mixed pigments in some type of protein binder for application to the cloth surface.[27] The borders of the blood marks show the diffusive character and color gradient expected for the appearance of serum clot retraction rings. These characteristics are distinctly different from those seen on the other types of image areas. A transmission photograph of the whole Shroud with the light source behind the cloth confirms that the blood marks penetrate the cloth, unlike the behavior of the body images, which are not seen in transmission.[12]

X-ray radiographs of the Shroud were taken employing a medical type diagnostic instrument.[28] Only the waterstain margins, burns, and details of the cloth weave were seen in this study, as would be expected due to their calcium content, an element expected to absorb strongly under these conditions. No evidence for blood marks or body images were seen as might be expected if they were painted with mineral pigments, especially if the blood marks contained a mercuric sulfide pigment as has been proposed. However, as the metallic element content of a blood exudate is comparatively low, the absence of these marks in this examination is consistent with the forensic conclusions. Clearly the blood marks do not behave as if they were painted images.[12]

Infrared thermographs of the Shroud were also taken.[29] They showed no evidence for any underlying paint structures or image outlines as would be expected

for a painting. However, all the different types of images could be distinguished from one another indicating differences in their infrared spectral characteristics.

The computer studies of the Shroud images indicate that the cloth actually enfolded a correct three dimensional human body shape and that the blood marks and body images got onto the cloth by two different processes, the blood marks by contact and the body images by some type of energy projection process.[30] This is well illustrated by another interesting study utilizing a full size photograph of the frontal image of the Shroud face and blood marks.[23, 31] The blood marks were traced onto a piece of cloth then carefully cut out. This mask was then applied to the face of a bearded man whose facial features were dimensionally in good agreement with those of the face on the cloth and the blood marks were then applied to his face by filling in the cut-outs with a marker. When the mask was removed a long standing apparent anomaly was resolved. The blood marks that appear in the hair of the Shroud body image without matting it together are seen to be actually on the sides of the cheeks, where bloodflows from the area of wounds to the temples would be expected to be. The apparent discrepancy arises from the fact that the blood marks have been transferred to the cloth by direct contact with clot exudates from a wounded man. However, the facial features have been projected onto this cloth by a non-contact mechanism of a radiational type and as the sides of the cheeks are in the projection plane they are not seen in the body image, but the hair adjacent to the sides of the cheeks is seen and therefore is superimposed on the blood images. Therefore the two types of images are not in stereoregister.

Chemical Investigations

The chemical testing of the Shroud has been conducted on material removed by various sampling techniques, mainly 'sticky tape' sampling.[12, 13, 32] STURP investigation samples were removed from designated and documented locations by means of a special tape holder supplied with a mylar tape coated with an inert adhesive. Microchemical testing was then carried out on specimens removed from these tapes at off-site chemical laboratories. Methods were devised to deal with a number of sampling problems, sample identification, and contamination problems. The most serious of these types of contaminants was the occasional appearance of materials that could clearly be identified as artistic pigments, e.g., cinnabar. Historical studies have established that over four dozen painted copies of the Shroud have been produced and that almost all these finished copies were sanctified by pressing the copied image to the original and thus unwittingly providing some contact transfer of materials between the two cloths.[33] Therefore unless such materials can be shown to be present in predominating amounts, they cannot be construed as evidence that the Shroud itself is a painting, but only that the cloth has been contaminated by artists copying the image and then sanctifying it.[11, 32] Cinnabar can be taken as a case in point as mercury was not detected in the X-radiographs.

An important class of particles ranging in color from red to orange were found predominantly in the blood areas, although some are seen also in displaced locations, as would be expected from the abraded appearance of the blood marks as shown in microphotographs. These tested as blood derived residues

giving positive tests for the presence of protein, hemin, bilirubin, and albumin; giving positive hemochromagen and cyanmethemoglobin responses; after chemical generation displaying the characteristic red fluorescence of porphyries; testing negatively for the presence of mercury compounds or for the presence of iron oxides, etc.

In traumatic shock as would be experienced under flogging and crucifixion, red blood cells lyse and the released hemoglobin is both bound up in haptoglobin-hemoglobin aggregates (a brownish denatured me themoglobin color) and also degraded by enzymatic action in the liver to bilirubin which is also bound up in protein complexes, mainly with albumin (a yellow orange color). When such blood is shed and then clots, the exudate will contain these protein bound complexes and aggregates with an expected range in non-uniform color from red to orange, while most intact cells will remain in the clot. A simulation of such a traumatic blood exudate prepared from laboratory chemicals as a control matches the appearance and properties of this class of test objects. However, a simulated artistic paint pigment mixture of iron oxide and mercuric sulfide in a gelatin binder does not make such a match. Thus the chemical testing not only supports the forensic conclusion that the blood marks are derived from contact of the cloth with clotted wound exudates, but that the shed blood was from someone who suffered a traumatic death as depicted in the images.

Confirmation of these blood mark conclusions has also been further provided by immunological testing by two independent investigators.[9, 12] One of the investigators obtained positive tests with antibodies for the blood type and human globulin, both oligosaccharide type antigens; while the other investigator obtained positive tests with antibodies for whole human serum and human albumin, a polypeptide type antigen. This pattern of results ensures against false positive results, as for example from bacterial cell wall debris, and allows one to identify the blood as human with a high degree of certainty even though chimpanzee antibody employed as one of several controls also gave the expected positive cross serological reaction. It should be noted that at the time of the de Charny display, no one had the medical knowledge of the details of blood clotting, nor access to a supply of traumatic clotted blood exudates from humans or non-human primates to have considered painting the blood mark images in the forensically correct manner in which we see them displayed on the Shroud of Turin. The blood marks are not painted images.

A series of microchemical tests for the detection of the presence of proteins was also carried out on appropriate test objects. Control studies revealed that many of the basic dyes usually employed for these purposes e.g., amido black, etc., also stain oxidized cellulosic structures whose presence therefore constitutes an interference. However, the use of fluorescamine tested against controls not only proved to be specific but was sensitive under the test conditions to the picogram level. The blood particles and the serum coated fibers from the margins of the blood marks all gave positive responses. All the other test objects, such as body image fibers, gave negative results. Proteases were employed to confirm these results and yielded the same conclusions. While treatment of body image fibers with proteases yielded no changes after several hours of treatment, in less than

20 minutes it removed the coating of the serum coated fibers to reveal a smooth and uncorroded surface. These same results have been obtained in a more recent enzymatic study.[34] This interesting observation suggests that the blood marks were on the cloth before the image producing process took place and protected the blood mark areas from this process. This further confirms that there were two separate processes involved in generating the blood and body images seen on the Shroud of Turin.

Spectroscopic Investigations

An X-ray fluorescence investigation[35] was carried out on the Shroud to complement the X-radiographic study.[28] The blood marks show an element distribution consistent with a blood clot exudate, including an iron value elevated over the cloth background value, but no evidence for the presence of mercury or any of the trace elements associated with the mineral forms of iron oxides. This observation supports blood mark identification as blood derived, but does not support the proposal that the blood marks are painted mineral mixtures of iron oxide and mercuric sulfide (vermilion). This is in agreement with the chemical and X-radiographic conclusions. An independent scanning electron microprobe study compared one of the blood particles with a control sample of human blood and found an excellent match,[36] further supporting these conclusions.

Ultraviolet and visible reflectance studies were carried out on the Shroud and also some fluorescence spectroscopy.[37, 38] In general these observations complemented those seen in the fluorescence photographic study.[25] The reflectance spectra of the blood marks from the whole cloth and also a near UV-visible microspectrophotometric study of blood particles from the sticky tapes,[39] are consistent with the spectra expected for a traumatic blood clot exudate. In a more recent study,[11, 40] transmission UV-visible spectra of a simulacrum of a traumatic clot exudate prepared from laboratory chemicals matches these observed Shroud spectra, but the spectra of a simulacrum of a mineral pigment 'blood' composed of iron oxide, mercuric sulfide, and gelatin is a complete mismatch. This confirms the previous chemical and forensic conclusions. It is of interest to note that the position of the near UV peak for albumin bound bilirubin is indicative of the type of species from which the blood originates and in these observed spectra is consistent with a primate origin,[32] in agreement with the immunological studies identifying a human source for the blood marks.

An infrared reflectance examination of the Shroud accompanied the thermographic study.[29] Although these spectra were of low resolution some high and low frequency peaks typical of amide absorptions associated with proteins could be detected in the blood mark spectra, but not in those of the body images. These results were confirmed and extended in a more recent high resolution microspectrophotometric FTIR investigation of fibers and particles extracted from the STURP sticky tape samples.[11, 40] Again, protein amide bands were only observed in the blood particles and the serum coated fibers associated with the blood marks. Utilizing the spectral analysis capabilities of the computer program accompanying this instrument which allows one to add or subtract standard spectra of a known molecule to the observed spectrum, testing its presence, it

was demonstrated that the observed spectra of the blood particles contained both methemoglobin and albumin bound bilirubin. Infrared spectra of the traumatic blood clot simulacrum cited above agreed with that of the observed blood particle samples, but the mineral pigment simulacrum did not. These results again confirm the chemical and forensic conclusions and demonstrate that the blood marks are not paintings.

Relationships to Other Cloth Relics

The Oviedo Cloth[41] and the Holy Tunic[42] are also blood mark bearing cloths alleged to be associated with the, crucifixion of Christ. There is good evidence that these blood marks like those on the Shroud are derived from blood clot exudates.[43] Further imaging studies of photographs of the blood marks on these cloths show very high pattern congruence to corresponding marks on the Shroud.[43] In particular, the complex non-simple pattern of the dorsal head wounds on the Shroud is in excellent congruence with a corresponding pattern on the Oviedo Cloth.[11, 44] As the edges of the Oviedo marks are all slightly darker than those on the Shroud one can even deduce that the Oviedo cloth was in contact with the clots first while the serum contraction rings were still more liquid. As these correspondences confirm the inaccuracy of the reported radiodate of the Shroud and support the historical arguments for authenticity,[11] these types of studies should be extended. An attempt to match the DNA on the Shroud with that on the Oviedo Cloth failed due to contamination problems.[45] Considering the problems associated with DNA analysis on blood samples[34] and that the Shroud has been through several fires, this is not surprising. These problems might be overcome by utilizing mitochondrial DNA analysis techniques.

Conservation Issues

Nothing lasts forever and this includes the Shroud of Turin. There is already evidence of degradative processes taking place, as for example in the observed abrasion of the blood marks. The various conservation and preservation issues and their possible resolution have been analyzed and reported in some detail.[46] As the need for action is paramount and pressing, a Conservation Commission has been appointed and a program to actively carry out their recommendations has been initiated and pursued.

Recommendations for Further Testing

A biological assay for the microflora and microfauna actually resident on the cloth is imperative. Information leading to a better understanding of the chemical structures found in the blood marks is also imperative. Using some of the new types of fiber optics probe spectroscopic instruments and new spectral imaging methods are recommended. However, some spectroscopic techniques of high potential information value such as various forms of electron spin resonance spectroscopy would have to be performed on off-site instruments. The samples for these purposes should be removed from documented sites with tweezers and definitely not by sticky tape sampling. Such samples could also be used for mitochondrial DNA studies.

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