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Evaluation of the potential role of skin contact and transfer in the Maillard hypothesis of image formation on the Shroud of Turin

Abstract

The mechanism for creation of the faint ventral and dorsal images of a man on the Shroud of Turin is unknown. It has been suggested that image formation involves oxidation and dehydration of the cellulose present in the linen, or, alternatively, that a superficial residue on the surface of the cloth is responsible for creating colored products through the Maillard reaction. One of the major criticisms of the latter hypothesis is that gaseous diffusion is not sufficient to result in the detailed bodily structures present on the cloth. Recently, it was proposed that additional substrates might also participate in the Maillard reaction, specifically proteins and amino acids present in skin, helping to increase the detail of any image produced. Understanding more fully the properties of the Shroud image is important for the preservation of this cloth for future generations. In the current report the potential contribution of skin proteins and amino acids in the Maillard hypothesis of image formation was evaluated. Using a variety of systems, these data show that detailed imaging of skin imprints may be obtained through contact transfer and interaction with reducing sugars. These results importantly extend the scope of this hypothesis and provide additional, relevant information concerning possible mechanisms for the formation of the Shroud of Turin image.

Keywords: Shroud of Turin, Maillard, carbohydrates, image

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Abbreviations: ALA, alanine; ARAB, arabinose; DHA, dihydroxyacetone; ERT, erythrulose; FUC, fucose; GAL, galactose; GLC, glucose; GLUC, glucuronic acid; GLY, glycerol; GLYC, glyceraldehyde; HIS, histidine; LYS, lysine; MAN, mannose; RHAM, rhamnose; SAP, saponin; SER, serine; SUCR, sucrose; UV, ultraviolet light; VL, visible light; XYL, xylose;

Introduction

The Shroud of Turin is an approximately 14 ft. x 3.5 ft. linen cloth bearing the faint frontal and dorsal images of a man with reddish areas corresponding to wounds consistent with scourging and crucifixion. Various explanations exist regarding the creation of the image, including both natural and supernatural mechanisms. During the last public exhibition of the Shroud in 2015, approximately 2 million visitors traveled to Turin to have the opportunity for even a brief observation of the cloth. Figure 1 shows the viewing perspective from both a distance and close up (Figure 1A and Figure 1B). A black and white negative of an original photograph enhances the detail of the image, a property that was first documented by Secundo Pia in 1898.[1,2]

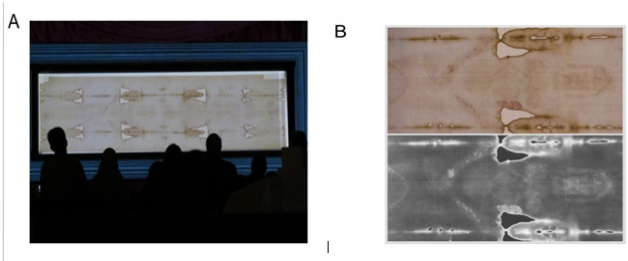

Figure 1 Photographs of the Shroud of Turin during the 2015 public exhibition made by the author. (A) Taken at a distance of approximately 25 feet, both the ventral and dorsal images are visible. (B) Taken at a distance of approximately 15 feet using a zoom lens; the top panel shows the original photograph of a close up of the ventral portion of the image, including the facial region. In the bottom panel, a black and white negative of the original photograph was created using the Photoshop express app. Note the enhanced detail in the negative version.

In 1978, a multidisciplinary group of scientists known as the Shroud of Turin research project team (STURP) performed a one-of-a-kind, hands-on evaluation of the Shroud for approximately 5 days. Much of what is known about the basic characteristics of the cloth results from the samples and data that were collected during this examination some forty years ago. Image formation is not consistent with creation via scorching methods using a hot statue, acid etching, painting, a bas-relief rubbing, or photographic techniques.[2–5] The image formation mechanism remains enigmatic, although the STURP team concluded that the cloth once wrapped a real human body.[3]

One of the most interesting aspects of the Shroud image is that it is extremely superficial. Microscopy studies indicate that the image is approximately 100-200 nm thick and resides on the very topmost portion of the cloth.[2,3] A density correlation appears to exist between the distance the cloth was proposed to be from the body and the darkness of the image; the darker portions are those believed to be closest, the lighter further away. The coloration of individual fibers is similar, indicating that the darker regions result from more colored threads being present as opposed to more color added to certain threads.[2-4,6] The extent to which the cloth was in contact with the body is controversial. John Jackson, the co-founder and leader of the original STURP team, suggested that the majority of the body was in contact, with the left knee being slightly raised.[6] In this model, the bloodstains on the Shroud align very well with their proposed positions on the body. (A video describing the proposed wrapping may be viewed here: Shroud of Turin - How the Shroud wrapped the body (Part One) - Bing video). In contrast, another model has the head positioned significantly forward and both knees at an enhanced incline.[7]

It remains to be determined if the image results from the oxidation and dehydration of the cellulose itself, catalyzed by an undefined energy source, or alternatively, if the image resides in a superficial impurity on the surface of the fibers. Interestingly, the coloration of the image and the background portion of the cloth are not completely uniform, showing a banding effect due to different hanks of linen that were used in the weaving the cloth. Regions that are lighter in color show a relatively lighter image in that area. This is somewhat difficult to explain if dehydration of cellulose is solely involved in image formation; however, it is consistent with the idea that different amounts of impurities may have coated varying batches of linen due to slightly different processing (bleaching?) conditions.[2] Notably, it was found that for image fibers that had been collected on sticky tapes and then pressed onto glass slides, the colored coating could be stripped from the fiber and remain in the adhesive, referred to as "ghosts".[2,3] Such "ghosts" showed identical chemical properties as intact image fibers taken from the Shroud. One STURP scientist in particular, Ray Rogers, was adamant that a change in cellulose was not responsible for formation of the image.[2] Shortly before his death in 2005, Rogers proposed a natural mechanism for creation of the image on the Shroud of Turin, involving release of amine-bearing gases from a dead body. Such gases would then react with a residue of reducing sugars present on the cloth (the Maillard reaction), resulting in the formation of a faint image corresponding to the body which it wrapped. Based on the somewhat equivocal writings of an ancient historian, Pliny the Elder,[2,8] Rogers suggested that reducing sugars were present in a thin superficial layer on the cloth due to processing of ancient linen which involved soaking in soapweed solution to make the cloth more supple. Soapweed extract contains Saponaria, which contains multiple reducing sugars, including arabinose, fucose, xylose, glucose, rhamnose, galactose, and glucuronic acid.[2,9] He theorized that soaking the cloth in such a solution and allowing it to evaporate would create a thin, superficial layer across the top of the cloth. As shown in microscopic experiments using a solution of diluted dye, this impurity layer would not be evenly distributed at the surface, accounting for the observation that colored image fibers reside next to uncolored ones on the Shroud.[2] In the Maillard hypothesis, a colored product is only formed if both components are present within the same nano-area to react. Rogers reported that Shroud fibers tested positive for reducing sugars and believed that the weak yellow-greenish fluorescence observed under ultraviolet on the background (non-image portions) of the cloth was characteristic of their presence.[2]

In 2020, Rogers' original idea was extended to include the potential contribution of amine-containing skin proteins, peptides, and amino acids that would also be expected to participate in Maillard reactions on the surface of the cloth. This "composite" mechanism would involve the reaction of both gases and proteins/amino acids with reducing sugars present on the cloth, providing additional detail in the image that would not be achieved through gaseous diffusion alone.[10] Indeed, the outer layer of skin contains both intact proteins and free amino acids liberated from proteins that have undergone degradation, providing an abundant source of substrates for the Maillard reaction. The modern-day method of spray tanning is based on this concept.[11–15]

In this report, the potential role of skin proteins/amino acids in the creation of the Shroud of Turin image was evaluated. It should be emphasized that the goal of the current research was not to attempt to create a full-length (or even miniature) copy of the Shroud. Rather, the focus of this study was to determine if consideration of the Maillard hypothesis as a possible mechanism of image formation should be expanded to include participation of this additional group of amine-bearing compounds via contact transfer.

Materials and methods

Chemicals

Benedict's solution was obtained from Flinn Scientific company (Batavia, IL). Purified carbohydrates were obtained from Sigma Aldrich (Merck, Burlington, MA) and Hi Media (Groningen, Netherlands). Amino acids were obtained from Nutricost (Vineyard, UT). All solutions were used at a concentration of 10% unless otherwise noted. Saponin was obtained on applicator sticks from In His Hands Birth Supply (Liberty Hill, TX); saponin solution was created by soaking six sticks in 100 microliters of distilled water. For mixing studies with amino acids and carbohydrates, equivalent amounts of each solution (typically 5-10 microliters) was added to filter paper successively and dried. Alternatively, solutions were added together in a microcentrifuge tube, mixed, and 5-10 microliters were spotted onto filter paper. Heating was performed using a Four E's Scientific (Guangzhou, China) dry bath incubator for 10 minutes at 80°C.

Ammonia was created through mild heating of ammonium carbonate from Oasis Supply (Bensalem, PA) at 80°C for 10 min. For mixing studies with dried carbohydrate solutions, approximately 50-100 mg of ammonium carbonate was placed at the bottom of a 1.5 ml microcentrifuge tube, filter paper containing carbohydrates was added to the upper portion, and the lid closed. Samples were then heated in a Four E's Scientific (Guangzhou, China) dry bath incubator for 10 minutes at 80°C.

Filter paper and fabrics

Whatman® 1 mm filter paper (Whatman, Maidstone, Kent, UK) was used. Linen was obtained from a local fabric shop (Joanne Fabrics, Knoxville, TN) or from Rawganique (Blaine, WA).

Ultraviolet light sources and photography

For ultraviolet photography two LED portable 3-watt UV light bulbs, 365 nm, were used, UV-3W-365UV-E27-AC (Golden Gadgets, South El Monte, CA) positioned at a ~ 45° angle from the subject and fitted with a NUV M55.0 x 0.75 filter (Edmunds Optics, Barrington, NJ). All ultraviolet photographs were taken using a Sony RX100 digital camera, and a 425 nm long pass filter (Edmunds Optics, Barrington, NJ) was placed in front of the camera lens. Photographs of samples under visible light were taken using a Sony RX100 digital camera or a Sony 6500 digital camera fitted to a Unitron ZST stereomicroscope.

Human skin and feeder mice

Human skin was exfoliated from the heel using a Rikans foot file (Ontario, CA). Newborn mice approximately 1-2 weeks of age were obtained from Rodent Pro LLC (Inglefield, IN).

Imprinting studies

The systems used for imprinting experiments were system 1, in which samples were added to filter paper or linen that was either dry or very slightly damp and transfer performed in the presence or absence of increased temperature; system 2, where samples were added to filter paper or linen that was either dry or very slightly damp and transfer performed in the absence of increased temperature. Following transfer, samples were briefly exposed to increased temperature to visualize coloration of products; and system 3, where increased temperature was absent throughout the entire procedure.

For imprinting, approximately 25 microliters of solution were added to a 2 x 2 cm^2 sized piece of material and allowed to dry for 10 minutes. The filter paper felt barely damp to the touch, hence the

term very slightly damp is used as a descriptor. Filter paper that felt wet (damp) typically did not yield successful results. Filter paper or linen was placed onto a glass microscope slide or parafilm and snipped tails transferred with a pair of forceps. The use of glass slides made handling of samples easier when a heating block was used or when whole body transfers were performed (even without heating). For experiments involving dry filter paper or linen, the added solution was allowed to dry overnight. With whole body experiments, frozen mouse cadavers were thawed at room temperature for approximately 50 minutes and then transferred to dry filter paper. Samples were either left at room temperature or heated for 5 minutes at 80°C, and then incubated for an additional 15 minutes at room temperature (system 1). For experiments involving post-transfer heating (system 2), material was placed in an oven at 150°C for 1-5 minutes until the sample was darkened enough to visualize the coloration. For most experiments, less than 1 minute was sufficient.

In experiments involving human skin imprints on linen, samples were heated for several minutes at 150°C post-transfer, which was necessary to visualize human imprints above the background fluorescence under ultraviolet light. A similar experience occurred in recent studies on human blood serum using this particular fabric (Rawganique).[16]

Results

A positive reaction with Benedict's solution, indicated by a color change from light blue to reddish brown upon heating, is distinctive of a reducing sugar. As shown in Figure 2, the majority of carbohydrates used in this study gave a positive reaction as expected (Figure 2). Numerous sugars gave a strong response, including DHA, erythrulose, glyceraldehyde, and xylose. Saponin and rhamnose reacted less strongly but were clearly positive. As expected, the non-reducing compounds sucrose and glycerol were negative (Figure 2).

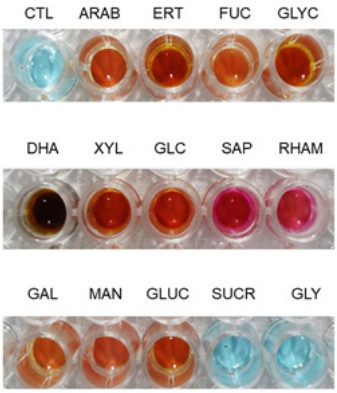

Figure 2 Reactivity of saccharides with Benedict's solution. Equivalent volumes of saccharide solutions and Benedict's reagent were mixed and heated for five minutes at 100°C. A positive reaction (color change) indicates the presence of reducing sugar. CTL (negative control, distilled water), ARAB (arabinose), ERT (erythrulose), FUC (fucose), GLYC (glyceraldehyde), DHA (dihydroxyacetone), XYL (xylose), GLC (glucose), SAP (saponin), RHAM (rhamnose), GAL (galactose), MAN (mannose), GLUC (glucuronic acid), SUCR (sucrose), GLY (glycerol).

The Shroud image has been described as light brown or sepia under most lighting conditions and individual threads and fibers have been scored as light brown or yellowish (Figure 1).[2,3] Similar to Rogers' previous observations, it was found that ammonia reacted with saponin solution to yield a light-yellow color with mild heating (Figure 3A, right-hand side).[2,8] A brief exposure to increased temperature resulted in darker color formation for both amino acids and ammonia reacting with the reducing sugars saponin and DHA. Color displays ranged from yellow to dark brown (Figure 3A and Figure 3B). When the amino acid alanine was mixed with various reducing sugars, color development from dark yellow to brown was noticeable within 24 hours at room temperature (Figure 4A, left-hand side). Coloring was more apparent in samples that had been initially exposed to brief heating (Figure 4A, right-hand side).

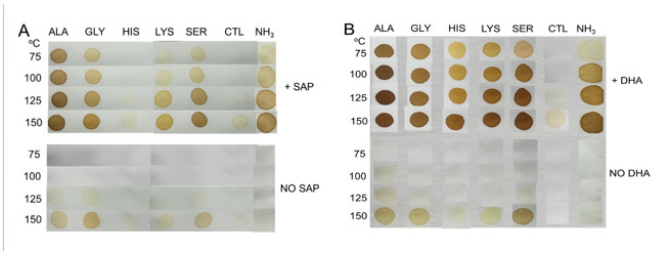

Figure 3 Reactivity of amino acids, ammonia with saponin and DHA. (A) Solutions of amino acids mixed with saponin were spotted onto filter paper and heated for five minutes at the indicated temperature; for ammonia-treated groups, saponin-treated filter paper was mixed with ammonia gas (see Materials and Methods for details). The resultant coloration is indicative of a positive Maillard reaction. (B). Same as in (A) except that DHA was used.

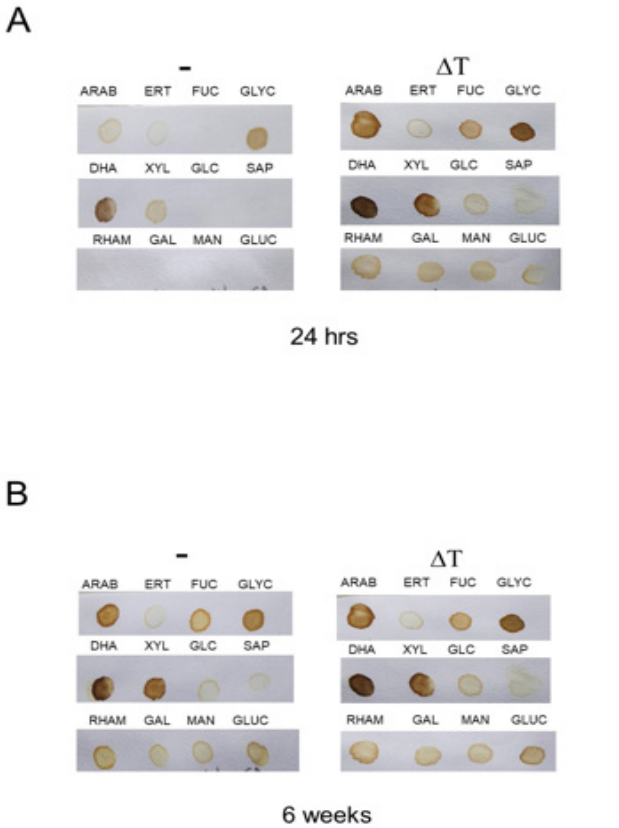

Figure 4 Reactivity of alanine with reducing sugars. (A) Solutions of alanine and reducing sugars were spotted onto filter paper and dried. Samples on the right-hand side were heated for 5 minutes at 80°C (ΔT); both sets were photographed after a 24-hour incubation period. (B) Same groups shown in (A) photographed 6 weeks later.

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With extended time, unheated samples reached a similar coloration as that of the heated samples (Figure 4B, left-hand side). Samples that were initially heated did not significantly change during this time period (Figure 4B, right-hand side). Taken together, these results indicate that both ammonia and amino acids may function as substrates for reaction with reducing sugars, and depending upon the particular combination and conditions, yield colorations that span from yellow to brown. Additionally, these data show that latent coloration in the reaction of amino acids with reducing sugars may be visualized by applying heat or with extended time.

Next, the reactivity of a panel of reducing sugars with human skin was evaluated. As was the case with free amino acids, coloration of skin was enhanced in the presence of mild heating (Figure 5A & Figure 5B), although with extended time, unheated samples showed a similar result (Figure 5C & Figure 5D). No color change was observed with the nonreducing compounds sucrose or glycerol as expected (Figures 5A- Figure D), and the results were specific in that no coloration occurred in the absence of skin (Figures 5E and Figure 5F). Species that appeared particularly reactive were arabinose, erythrulose, glyceraldehyde, and DHA; and to a lesser extent, xylose, saponin, and rhamnose (Figures 5A- Figure D, see figure legend for abbreviations). Related experiments were performed using human hair; the salt and pepper variety was used to allow for detection of any color change that occurred. As demonstrated, a relatively similar reactivity pattern seen with skin was also observed for hair (Figure 6A & Figure 6B); extended time gave similar results as with heated samples (Figures 6C & Figure 6D).

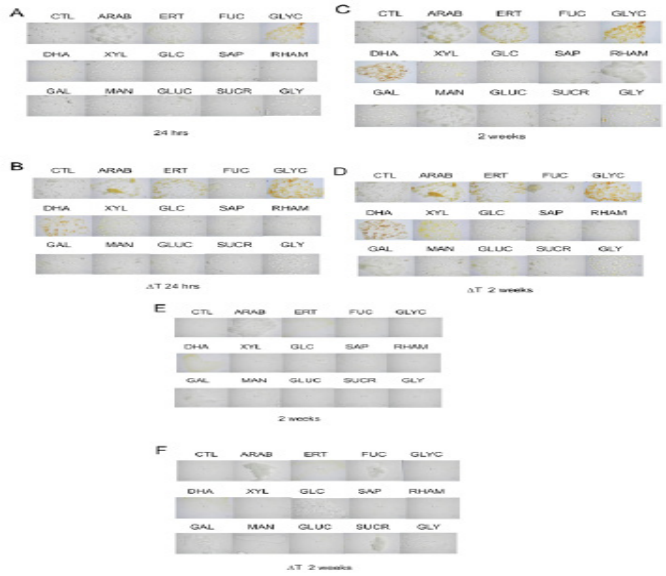

Figure 5 Reactivity of human skin with reducing sugars. Solutions of reducing sugars were added to exfoliated human skin and after drying were either untreated (A) or heated for 5 minutes at 80°C (ΔT). (B). Samples were then incubated for 24 hours and photographed. (A) and (B) samples were photographed 2 weeks later in (C) and (D), respectively. (E) and (F) are control solutions containing no skin. CTL (negative control, distilled water), ARAB (arabinose), ERT (erythrulose), FUC (fucose), GLYC (glyceraldehyde), DHA (dihydroxyacetone), XYL (xylose), GLC (glucose), SAP (saponin), RHAM (rhamnose), GAL (galactose), MAN (mannose), GLUC (glucuronic acid), SUCR (sucrose), GLY (glycerol).

Studies were extended to the murine system; young mouse pups (feeder mice) were used, which are devoid of hair at this stage of development (see below). As demonstrated in Figure 7A, addition of reducing sugar to mouse tails resulted in a color change, which was especially noticeable for glyceraldehyde, DHA, and xylose (Figure 7A). Reactivity with isolated mouse paws was also observed, which was easily detectable within 20 hours at room temperature (Figure 7B). Analogous to above studies with human skin, latent coloration could be observed rapidly by applying heat (Figure 7C, middle panels) or visualized more slowly with increasing time (Figure 7C right-hand panels), (see Figure legend for details).

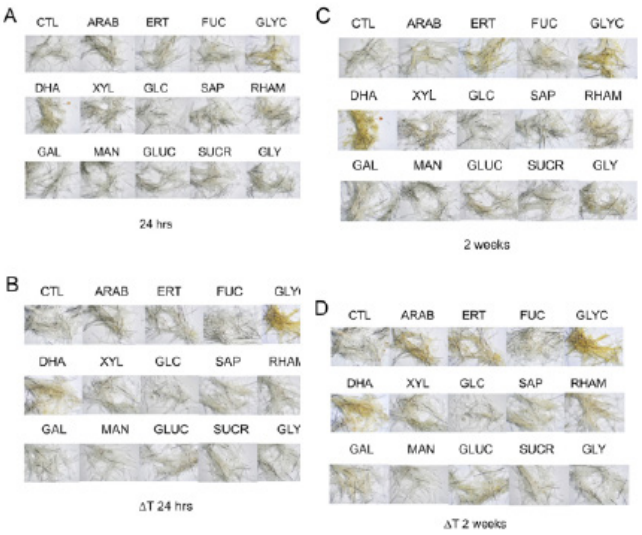

Figure 6 Reactivity of human hair with reducing sugars. Solutions of reducing sugars were added to human hair and after drying were either untreated (A) or heated for 5 minutes at 80°C (ΔT). (B). Samples were then incubated for 24 hours and photographed. (A) and (B) samples were photographed 2 weeks later in (C) and (D), respectively. CTL (negative control, distilled water), ARAB (arabinose), ERT (erythrulose), FUC (fucose), GLYC (glyceraldehyde), DHA (dihydroxyacetone), XYL (xylose), GLC (glucose), SAP (saponin), RHAM (rhamnose), GAL (galactose), MAN (mannose), GLUC (glucuronic acid), SUCR (sucrose), GLY (glycerol).

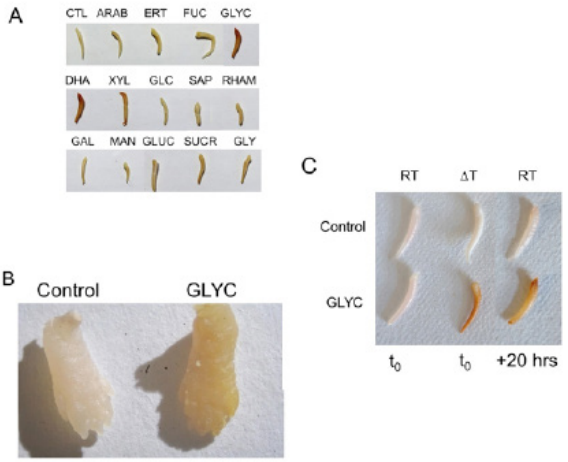

Figure 7 Reactivity of murine tails and paws with reducing sugars. (A) Solutions of reducing sugars were added to mouse tails and allowed to dry. Samples were then heated for 5 minutes at 80°C (ΔT) and photographed. CTL (negative control, distilled water), ARAB (arabinose), ERT (erythrulose), FUC (fucose), GLYC (glyceraldehyde), DHA (dihydroxyacetone), XYL (xylose), GLC (glucose), SAP (saponin), RHAM (rhamnose), GAL (galactose), MAN (mannose), GLUC (glucuronic acid), SUCR (sucrose), GLY (glycerol). (B) A solution of distilled water (Control) or glyceraldehyde (GLYC) was added to mouse paws and photographed after a 20-hour incubation period at room

temperature. (C). Solutions of distilled water (Control) or glyceraldehyde (GLYC) were added to mouse tails and incubated at room temperature (RT) or heated for 5 minutes at 80°C (ΔT). Samples were then photographed at this time (t_0), left-hand and middle panels. A third group was left unheated and incubated at room temperature for 20 hours, then photographed (right-hand panels).

Having established that murine tails show similar coloration with certain reducing sugars as human skin, the next set of experiments assessed the efficacy of mouse tail skin transfer under various conditions. These studies were based on the property that skin fluoresces under ultraviolet light,[17] (Figure 8A). Interestingly, it was noted that similar to what was recently found with blood serum,[16] the fluorescence intensity of mouse tails increased after a brief exposure to heat (Figure 8A). Initially, the importance of moisture was examined, with dry mouse tails added to filter paper that was dry or very slightly dampened (see Materials and Methods for details). As demonstrated, tail imprints were visualized when very slightly dampened filter paper was used, but not with dry filter paper (Figure 8B, top). Longer incubation times (hours) did not affect the results (data not shown). When exposed to heat during transfer, tails imprints were visible in both dry and slightly dampened groups (Figure 8B, top), with increased fluorescence relative to unheated transfers. As it was observed that fluorescence of mouse tails increased upon heating (Figure 8A), samples were also heated post-transfer to see if any increase in fluorescence might be observed, which was not the case, even for samples that were originally unheated (Figure 8B, bottom).

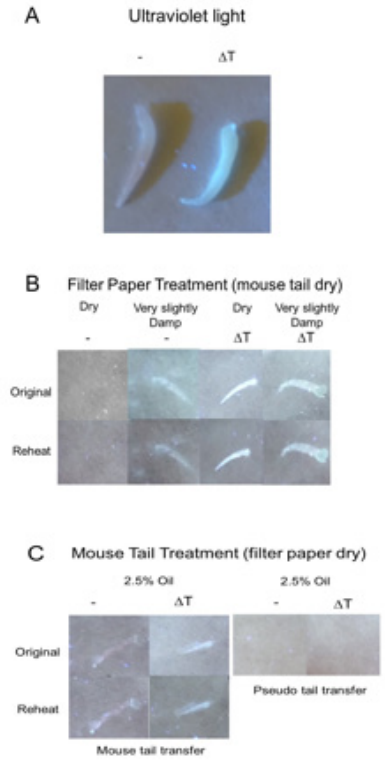

Figure 8 Mouse tail transfer experiments. All of these experiments were done using system 1. (A) Mouse tails were either untreated or heated for 5 minutes at 80°C (ΔT) and photographed under ultraviolet light (UV). (B) Mouse tails were added to filter paper that was dry or very slightly dampened (see Materials and Methods for details) and incubated for 20 minutes at room temperature. Alternatively, mouse tails were added to filter paper that was dry or very slightly dampened, heated for 5 minutes at 80°C (ΔT), and then incubated for an additional15 minutes at room temperature. Tails were removed from the paper and 60 minutes later samples were photographed under ultraviolet light (top panels). All samples were then heated for 5 minutes at 80°C (ΔT) and rephotographed (bottom panels). (C) Mouse tails were swabbed with a 2.5% oil solution, added to dry filter paper, and incubated for 20 minutes at room temperature. Alternatively, mouse tails were swabbed with 2.5 % oil solution, added to dry filter paper, heated for 5 minutes at 80°C (ΔT), and then incubated for an additional 15 minutes at room temperature. Tails were removed from the paper and 60 minutes later samples were photographed under ultraviolet light (top panels). Samples were then heated for 5 minutes at 80°C (ΔT) and rephotographed (bottom panels). As a control to show that fluorescence did not result from carryover of oil solution, portions of toothpicks the approximate size and shape of mouse tails were swabbed and subjected to similar treatment (pseudo tail transfer).

Converse experiments were attempted in which mouse tails were moistened and transferred to dry filter paper without much success, as mouse tails are essentially waterproof and quite repellent to water (data not shown). It was possible, however, to apply a thin coating of dilute oil solution (2.5%) to tails prior to transfer and have positive imprints (Figure 8C). It was noted, however, that oil-facilitated imprints were slightly less defined than those presented above. Control transfers using toothpicks of similar size that were swabbed with oil solution showed that the observed fluorescence was not simply due to oil transfer (Figure 8C). These results collectively show that mouse skin transfer was affected by both moisture and temperature, with moisture being essential for imprinting in the absence of heating. As the most successful results were found using material that was very slightly dampened, these conditions were used in subsequent experiments unless specifically noted (see below).

Similar experiments were performed as those above using material that had been pre-treated with various reducing sugars. As shown, coloration of tail imprints was effectively visualized when transfer occurred in the presence of increased temperature (Figures 9A-Figure 9C). Tail imprints that were performed without heating during transfer were effectively visualized when a brief exposure to higher temperatures was used afterwards to develop the color (Figure 10A), (see Figure legend for details). Imprints were also performed on a piece of linen pre-treated with reducing sugar that was sufficiently long such that it could be folded to allow contact with both sides of the tail; no external pressure was applied during the experiment (see Figure legend for details). As demonstrated, relatively equivalent imprinting of both tail sides was observed, showing that contact transfer occurred rather efficiently under these conditions (Figure 10C).

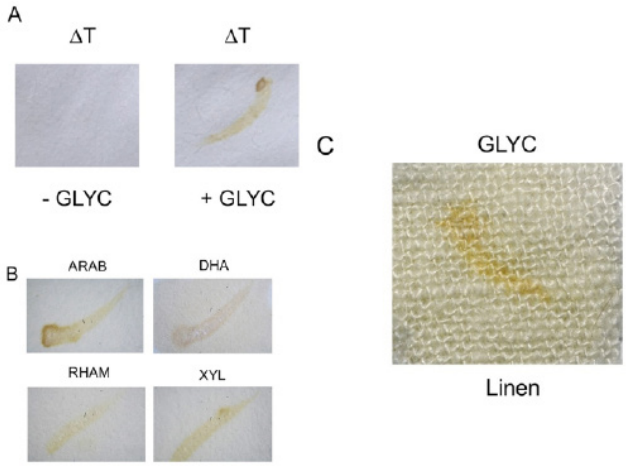

Figure 9 Mouse tail imprints with reducing sugars. These experiments were done using system 1. (A & B) Mouse tails were added to filter paper containing reducing sugar, heated for 5 minutes at 80°C (ΔT), and then incubated for

an additional 15 minutes at room temperature. Tails were removed from the paper and samples were photographed under visible light. ARAB (arabinose), DHA (dihydroxyacetone), GLYC (glyceraldehyde), RHAM (rhamnose), XYL (xylose). (C) Same as in A & B except that modern white linen was used instead of filter paper.

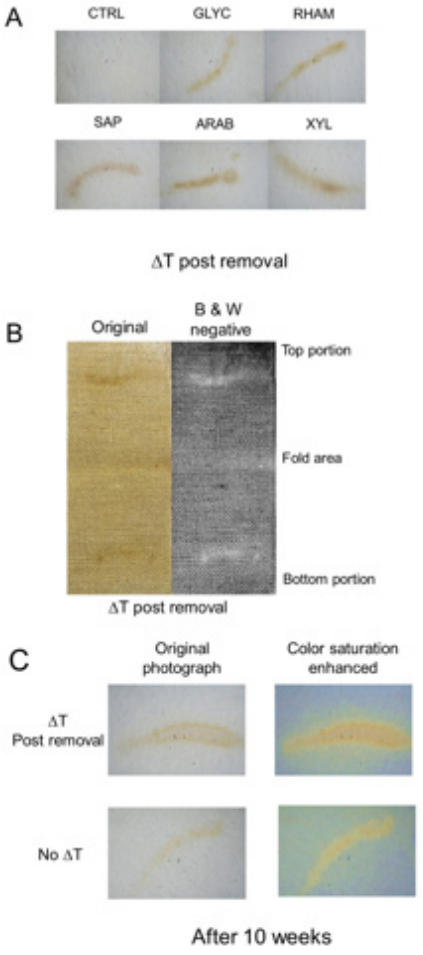

Figure 10 Mouse tail imprints with reducing sugars with no heating during transfer. These experiments were done using systems 2 (A & B) and systems 2 and 3 (C). In (A), mouse tails were added to filter paper containing reducing sugar and incubated for 20 minutes at room temperature. Tails were removed from the paper samples heated for several minutes at 150°C until color was visualized, and then photographed under visible light. CTRL (control), ARAB (arabinose), GLYC (glyceraldehyde), RHAM (rhamnose), SAP (saponin), XYL (xylose). (B). Mouse tails were added to a piece of natural linen pre-treated with DHA that was sufficiently long to be folded over, so that the top portion was resting on the opposing side of the tail as well. Linen was held in place at the edges using two glass microscope slides on either side, but no pressure was applied to the sample. Samples were incubated for 20 minutes at room temperature, tails removed, and heating applied post-transfer. Both the original photograph and a black and white negative version are shown. (C) The top panel was performed similar to (A), with heating applied post-transfer. The bottom panel used an extended incubation time without any heating; filter papers were pre-treated with GLYC. On the right-hand side, the original photograph was post-processed in the power point program to enhance color saturation making the transferred regions more visible.

Lastly, it was noted that analogous to previous findings, latent coloration of tail imprints was revealed using a longer incubation time for imaging to develop, in samples that were never heated (Figure 10C). Together, these data demonstrate that mouse tail imprints can be successfully visualized with sufficient detail under various conditions, including those with and without increased temperature.

Transfer of whole-body imprints was examined, although these studies were somewhat logistically difficult as mice at this stage of development are considerably fragile; the skin is quite thin and discharge from the nose and mouth was relatively common. Unlike what was observed for isolated tails, the most effective conditions for whole-body transfers were using dry filter paper with the body being very slightly damp, although transfers could be performed under completely dry conditions with heating (see below). Representative transfers are shown in Figure 11. In (A), the top panel shows a photograph of a feeder mouse for reference; note that internal organs were visible through the very thin skin (Figure 11A, top left). The middle and bottom panels show imprints that were analyzed under ultraviolet to monitor skin transfer; the positions of legs and the tail are indicated (Figure 11A, left-hand side). Transfers done in the absence or presence of reducing sugar (top and middle/bottom, respectively) are shown on the right-hand side. Heat was used post-transfer to develop the coloration and samples analyzed under visible light (VL), (Figure 11A, right-hand side). The resulting imprints corresponded to the major contact points of the mouse body with sufficient resolution to identify the original object (Figure 11A), which was emphasized in black and white negatives of original photographs (Figures 11B and Figure 11C). Similar to what was observed with isolated tails, whole body transfers conducted in the presence of heating were slightly more defined, with eyes, mouth, ears, and even wrinkling of the skin apparent (Figure11D).

Finally, a few limited transfer experiments were performed using human skin. As none of the reducing sugars used in this study have been approved by the FDA for human skin contact (some, such as saponin, actually carry a warning), caution was used to limit the amount of exposure. Unlike mouse skin, human imprints occurred effectively under dry or damp conditions on both filter paper and linen (Figures 12A and Figure 12B, left-hand sides) and reacted efficiently with DHA-treated material to form a colored product (Figures 12A and Figure 12B, right-hand sides).

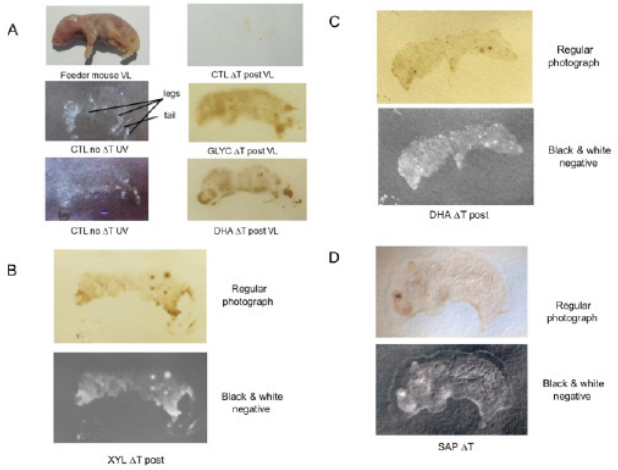

Figure 11 Whole body mouse imprints. These experiments were done using systems 1 and 2. (A) Mice were added to filter paper that had been pretreated with dH_20 (left-hand side) or reducing sugar (right-hand side) and incubated for 20 minutes at room temperature. The bodies were removed and samples with reducing sugar were heated in an oven for 1-2 minutes at 150°C to stimulate color development. Samples were photographed under ultraviolet (left-hand side) or visible light (right hand side). Control (CTL)

samples contained no reducing sugar; the ultraviolet fluorescent material near the head and mouth area in the experimental groups in Figure 10A is a small amount of fluid discharge. Samples on the right-hand side were added to filter paper that had been pretreated with either glyceraldehyde (GLYC) or DHA; after removal, filter papers were heated in an oven for 1-2 minutes at 150°C to stimulate color development and samples were photographed under visible light. For (B) & (C) samples were treated like those in (A), right-hand side, and a black and white negative version of the original photograph was created using the Photoshop express app. (D) Mice were added to filter paper that had been pretreated with saponin and heated at 95°C for 7 minutes. The bodies were removed, and the samples were photographed under visible light (VL). A black and white negative version of the original photograph was created using the Photoshop express app.

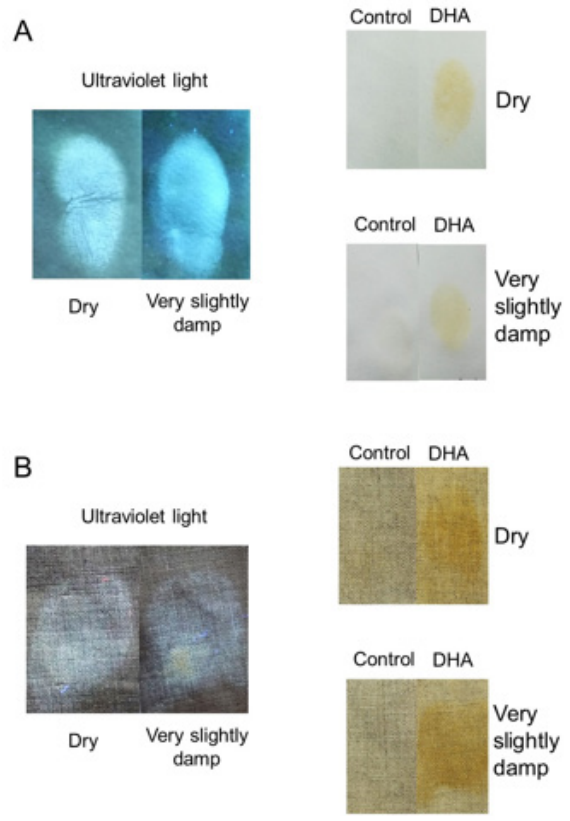

Figure 12 Human skin imprints. These experiments were done using systems 1, 2, and 3. (A) A piece of filter paper was held between the thumb and index finger with minimal pressure for 20 minutes at room temperature. Samples were photographed under ultraviolet light (ultraviolet) to monitor skin transfer (left-hand side). A piece of filter paper pretreated with distilled water (Control), or DHA was held between the thumb and index finger with minimal pressure for 20 minutes at room temperature. Samples were incubated at room temperature for 45 minutes to allow color development and photographed under visible light (right-hand side). (B) Same as (A) except that linen was used and linen was heated in an oven for 2-3 minutes at 150°C post transfer, which was necessary to visualize skin imprints under ultraviolet light above the linen background (see Materials and Methods).

Initial investigations of the Shroud image indicated that it is non-fluorescent under ultraviolet light (absorbing).[18] As Maillard products have been reported to exist as both fluorescent and non-fluorescent species, it was of interest to examine this property, particularly regarding reactivity of reducing sugars with human proteins. For these experiments, skin imprints transferred to filter paper that had been pre-treated with or without reducing sugar were briefly exposed to increasing temperature and examined under visible and ultraviolet light. As seen in Figure 13A, control imprints became nonfluorescent only at extremely high temperatures (Figure 13A), unlike imprints reacting with DHA, which were evident at much lower temperatures (Figure 12B). Comparable results were observed when similar experiments were conducted using exfoliated human skin (Figure 14A). Given extended time, a similar finding occurred without the requirement of heating (Figure 14B). Taken together, these results show that human skin mixed with reducing sugars form colored products that were non-fluorescent under ultraviolet, a characteristic that is similar to what has been described for the Shroud image.

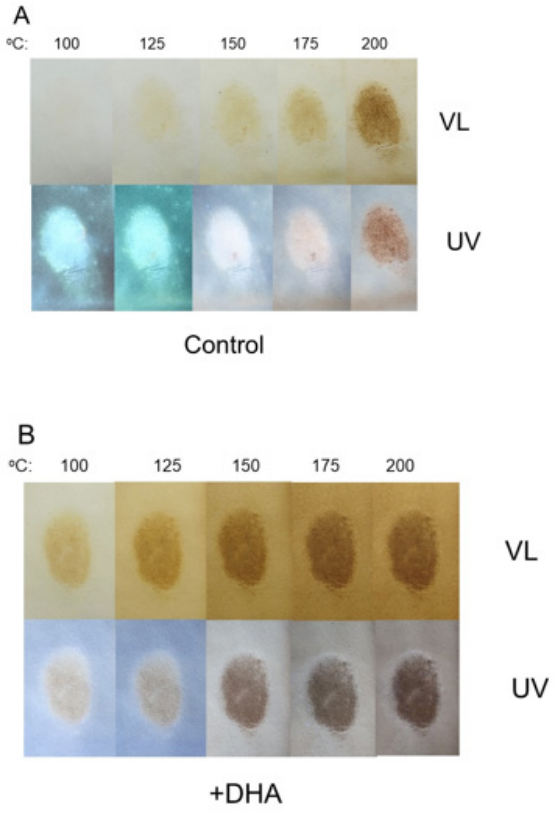

Figure 13 Ultraviolet fluorescence of human skin imprints. These experiments were done using system 2. A piece of filter paper pretreated with distilled water (A), or DHA (B) was held between the thumb and index finger with minimal pressure for 20 minutes at room temperature. Samples were then heated successively for 5 minutes at the indicated temperature and photographed under visible (VL) and ultraviolet light (UV).

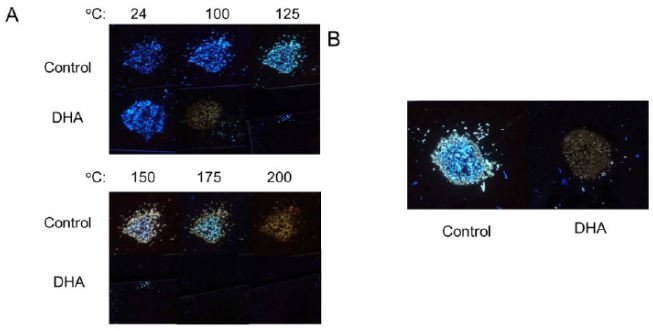

Figure 14 Ultraviolet fluorescence of exfoliated human skin. These experiments were done using systems 2 and 3. (A) Human skin was either

pretreated with distilled water (Control) or DHA, allowed to dry, and the same samples heated successively for 5 minutes at the indicated temperature. Samples were photographed under ultraviolet light. (B) Control or DHA treated skin samples were incubated at room temperature for 10 weeks and then photographed under ultraviolet light.

Discussion

The current report has examined the prospect that skin proteins/amino acids might function in the Maillard hypothesis of image formation for the Shroud of Turin, first proposed by Rogers. Unlike Rogers' original idea, which was limited to diffusion of amine-bearing gases reacting with a cloth containing reducing sugars, these results support the concept that contact transfer of amine-bearing components from skin would not be restricted from participating in such a system. One of the major criticisms of Rogers' original idea was that diffusion alone was not adequate to provide sufficient detail in the creation of the image. Inclusion of proteins, peptides, and amino acids by contact transfer provides an additional mechanism to utilize the same chemical pathway originally proposed by Rogers for image formation. Clearly, this could provide additional detail and resolution in areas where contact was believed to occur.

One of the major conclusions put forth by the STURP team following its collaborative investigation of the Shroud is that the cloth once wrapped a body.[1,3] However, as noted in the introduction, different models exist regarding the position of the corpse and the proposed extent to which the cloth was in contact with certain regions. In either model, some areas will exist where direct contact is prohibited due to anatomical features (portions of the face, for example). The Maillard hypothesis would argue that diffusion is sufficient for delivery of amine-bearing substrates to those areas of the cloth. One objection to contact transfer mechanisms is that if the cloth were wrapped tightly across the face, significant distortion would be observed, with artificial flattening such that the face appears much too wide and disproportionate. This is certainly true if the cloth is "vacuum sealed" and tightly wrapped, but need not be the case if the cloth is secured relatively loosely. As the exact position that the man of the Shroud was believed to exist in is unsettled, it is fair to voice that there is room for interpretation as to exactly how the body may have been situated. Regardless, the results in the current study indicate that the Maillard hypothesis should no longer be considered as operating via gaseous diffusion alone: proteins and amino acids from the extracellular layers of skin transferred by contact must also be included when discussing the potential reaction of amines with reducing sugars residing on the topmost fibers of the cloth.

The mouse cadavers used in this study were kept frozen until use; little to no information exists regarding the release of volatile amines from recently deceased mice at such early stages of development, let alone from mice that have been frozen after their death. Extended studies were not possible in this system as mouse bodies began to dehydrate after a day or so at room temperature. Additionally, as notable differences exist between murine and human skin, including the lack of apocrine sweat glands in mice, and that mouse skin is four times thinner than human skin,[19,20] this system is not particularly suitable for evaluation of the relative roles of volatile amines versus those transferred by contact. Most notably, this data set has established important parameters and conditions for further exploration into this possible imaging mechanism.

Various reducing sugars were employed in this study with the aim of finding candidates suitable for studies to extend these initial observations. As numerous species of soapwort plants exist, and different preparation methods may be used for saponin extraction, it was deemed prudent to examine multiple options. Several of the smaller triose sugars were among the most effective, including DHA and glyceraldehyde, which are breakdown products of glycerin. Glycerin (glycerol) is abundantly present in plants, and it is conceivable that certain reducing sugars may exist on the cloth due to fermentation of substrates during the retting procedure in the early stages of flax processing. Interestingly, treatment of linen with glycerol was found to maintain the reddish color of blood for up to several weeks;[21] the reddish color of the Shroud bloodstains is a characteristic of the cloth which has been noted for hundreds of years.[2] Experiments are currently in progress to evaluate if glycerol-related products, including several reducing sugars used in this study, are able to maintain the reddish color of bloodstains for an extended time period.

Finally, the data in the current report describe a potential role for heat in the formation of Maillard products from skin components. These results show that while increased temperature facilitates the rapid visualization of the results, increased temperature is not requisite, as latent coloration was visualized with extended time. Thus, heat may enhance the result, but is not entirely necessary for it to occur. While it is beyond the scope and intent of this report to speculate on the potential use of heat/energy in the creation of the Shroud image, it is interesting to note that Rogers reasoned that the image formation may have been delayed and was not instantaneous.[2] The current results also indicate an involvement of moisture in efficient transfer of skin components under certain conditions. Moisture was not absolutely required, either, however, as skin transfer occurred effectively in its absence, both with and without heat, particularly with human skin. The contact times in this study were quite brief, with a maximum of only 20 minutes before removal of the skin substrate in both murine and human systems. Very little information exists in the literature regarding transfer of human skin cells or their products as the focus is typically on DNA as the readout. Stanciu et al. reported that up to approximately 100,000 skin cells could be transferred by holding a plastic laboratory tube for only five minutes.[22]

In summary, the current report has evaluated the possible contribution of skin proteins and amino acids in the Maillard hypothesis of image formation of the Shroud or Turin. Using a variety of systems, these data show that detailed imaging of skin imprints may be obtained through contact transfer and interaction with reducing sugars. These results importantly extend previous concepts of the Maillard hypothesis of image formation and indicate that this mechanism could potentially contribute to the creation of the image on the Shroud.

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Conflicts of Interest

Author declares there are no conflicts of interests.

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