ROGERS' MAILLARD REACTION HYPOTHESIS EXPLAINED IN DETAIL BY ROGERS HIMSELF.

Thibault Heimburger – August 2012.

The basic statements of Rogers' Maillard reaction hypothesis (MRH) are well known and published in many papers including a peer-reviewed paper (The Shroud of Turin: an amino-carbonyl reaction (Maillard reaction) may explain the image formation. Melanoidins vol.4, 106-113 (2003)).

However the many details of the thought of Rogers are generally not known but are available in several messages sent to the members of the private Shroud Science Group.

I have gathered here most of them. My hope is that this could help to better understand the MRH and hopefully to discuss step by step all the aspects on a more sound basis.

I have divided the messages in different subjects for clarity. In some messages I have replaced the name of a SSG member by X or Y.

PYROLYSIS, CARAMELIZATION AND MAILLARD REACTIONS

There are three somewhat overlapping types of non-enzymatic browning reactions,
1. Pyrolysis
2. Caramelization
3. Maillard reactions.

Pyrolysis is a high-temperature process that ultimately involves the total loss of water from the sugar molecules and the breaking of carbon-carbon linkages; i.e. the end product is amorphous black carbon. Attempts to accelerate color-formation processes too much will lead to pyrolysis: the cloth will turn black. Incidentally, cellulose melts with decomposition at about 260C. The end product is black char.

Caramelization is a heat induced dehydration of sugars by way of "anhydro sugars." Many series and parallel reactions cause sugars to lose water molecules from their structure through "1:2 and 2:3-enolization." These processes are influenced by pH and appear in both caramelization and Maillard reactions. For example, through many intermediates, and in the pH 2-7 range, D-fructose will produce furans, isomaltol, and maltol (a major reaction in bread browning).
I am not an expert on Maillard reactions: they are much too complex for a physical chemist to follow. For more authoritative information, I would suggest contacting Prof. Anna Arnoldi. But I will try to give a short summary.

Maillard browning reactions involve simple sugars and amines, amino acids, and simple peptides (all containing the -NH2 group). They begin to occur at lower temperatures and at higher dilutions than

caramelization. [Don't misunderstand: they can occur at very high concentrations even better.] The rate can increase 2-3 times for each 10C rise in temperature. However even long term storage of malt extract will Maillard-brown at room temperature. The reactions have a finite rate cold.

Maillard reactions have three basic phases.
1) The initial reaction is the condensation of an amino group with a simple sugar, which loses a molecule of water to form N-substituted aldosylamine. This is unstable and undergoes the "Amadori rearrangement" to form 1-amino-1-deoxy-2-ketoses (known as "ketosamines"). You may think of some of these reactions as being caramelization reactions that have been catalyzed by amino groups.

2) The ketosamine products of the Amadori rearrangement can then react three ways in the second phase. One is simply further dehydration (loss of two water molecules) into reductones and dehydro reductones.
These are essentially caramel products. A second is the production of short chain hydrolytic fission products such as diacetyl, acetol, pyruvaldehyde etc. These then undergo the "Strecker degradation" to aldehydes and aldols. Other series reactions in the cascade form heterocyclic compounds (e.g., furnans, furanones, and pyrones).

3) A third path is the Schiff's base/furfural path. These products also undergo aldol condensation and polymerize further into true melanoids.

The spectral evidence from the Shroud indicates that the image is a complex mixture of melanoids.

The rates of all of the many, many reactions and the structures of the final products depend on the specific amines and/or amino acids and sugars that react, the pH, the temperature, and the concentrations of all reactants.

As for complexity, simple dextrose and proline produce over 120 different melanoid products. Such complexity explains the featureless uv/visible spectrum of the image.

High concentration favors both caramel and Maillard reactions, but dilution eliminates caramel reactions.

Temperatures over 100C favor the production of pyrazines.

The evidence supports a low-temperature image-color-formation process.

MAILLARD REACTION IN DETAIL

Dear X and Researchers:

Wow! X , you really hit the hearts of a bunch of problems. The broad outline you presented is pretty close to what I think the evidence supports, but some details need to be filled out. Rather than "pull a Franco" and say nobody can understand, I will try to take the subjects one at a time and give as much detail as seems reasonable. I will leave your numbering system for clarity.

1) "during the linen yarn manufacturing process at one stage or perhaps at multiple stages a very thin layer of foreign material forms very thinly on the surface of the fibers/threads composed of the residue of evaporating liquids. This layer is rich in saccahrides which I gather are "sugars""

Film formation depends on the surface tension of the liquid and the surface energy of the solid. A wetting agent (soap or detergent) reduces the surface tension of the liquid, making it spread into a thin film. A drop of water forms a ball on a newly waxed car, but a drop of soapy water spreads out. The very thin layer indicates use of some wetting agent in the last process used on the cloth. The cloth would have already been woven at this stage. The probable "detergent" 2000 years ago would have been S. officinalis.

In order to protect and lubricate the warp yarn during weaving, starch was used until the recent past. Ancient starch was just extracted from ground grains; and it contained a complex mixture of simple sugars (like glucose, a monosaccharide, $C_6H_{12}O_6$), disaccharides (two sugar molecules linked together), and more complex linkages of sugars up to very high molecular weights (starches). Cellulose is the same kind of thing but bigger and hooked together a little differently.
You expect to see some members of all possible combinations. Modern starch has had all of the light stuff (simple reducing sugars) extracted, and the high-molecular-weight starches are left behind, because they are too hard to get into water. As you can see, ancient starch would have the probability of producing some very complicated reaction products. Apparently it did on the Shroud.

Some of the saccharides still have enough aldehyde (-CHO) and ketone (=CO) groups to reduce Fehling's solution enough to detect. Those are called the "reducing saccharides." There are lots of them in crude starch. They are the types of saccharides that take part in Maillard reactions. Honey contains levulose, a ketose, and you can make good Maillard colors with it. Table sugar, sucrose, won't do it, because it is a biose - a glucose and a levulose hooked together. Cellulose and high-molecular-weight starch do not give Maillard colors.

2) "amines of various sorts (which from my point of view read "staining vapors") are evolved from the surface of the body due to sweat, initial decomposition or whatever."

Much has been made of ammonia being produced from sweat by hydrolysis of urea (H_2NCONH_2).

CO2 (and some carbamates) is the other final product. I have not been able to find any sweat analyses that showed urea as a major component. In any case, the reaction would be over in a few hours. Ammonia would leave a fog of color on the cloth, because it diffuses quite rapidly. The image was not primarily formed by ammonia. The fog around the nose and mouth looks to me like some ammonia was involved ("I think I see").

The heavier decomposition products begin to appear quite slowly and build up with time. Dr. Fred *[Dr. Zugibe]* distributed a great alignment chart to help estimate body cooling rates, and he has often supplied us with information about the factors affecting body decomposition rates.
Vass published a method for estimating "Cumulative Degree Hours (CDH)," together with analyses of decomposition biomarkers to calculate the time since death. Vass, et al., say that structural degradation of the body is not important until about 36-72 hours (liquid products appear on the surface). As one example, the CDH for a 10C, constant-temperature tomb would be 20 per day. Their analyses showed that muscle tissue decomposition was no longer diagnostic after about 800 CDH. A body should be in pretty bad shape by something less than 40 calendar days, even at 10C. All of the literature and Fred indicate that within about 36 hours, decomposition amines would still be increasing and be nowhere near their maximum rate of appearance.
This is important.

The amines would slowly appear at the surface of the skin during the time in the tomb, and there would be no pools of liquid at the surface within the time assumed for Jesus' burial.

3) "these vapors/gases are transported to the fibers/threads through two processes: 1) contact and 2) gas diffusion where they react with the surface layer to either make color directly or sensitize the locations where they react so that those areas react further with the oxygen in the atmosphere at an accelerated rate relative to the background to create color."

Because the skin is in contact with the cloth in some areas, contact transfer of reactants is inevitable. The concentrations at the surface of the skin will be important, but the reactions will be the same. Because the decomposition products have significant vapor pressures [putrescine boils at 158C, which puts it between regular gasoline and diesel - it evaporates about the same], some reactants will definitely evaporate into the gas layer between skin and cloth.
They will diffuse from the skin to the cloth from the entire surface of the skin (not just a single point). That is important.

Oxygen has nothing to do with Maillard reactions. Maillard reactions have three basic phases. a) The initial reaction is the condensation of an amino group with a reducing saccharide, which loses a molecule of water to form an N-substituted aldosylamine. Notice that this is a dehydration. There is a different product for every specific saccharide and amine. These aldosylamines are colorless, but they are unstable and undergo the b) "Amadori rearrangement" to form 1-amino-1-deoxy-2-ketoses (also known as "ketosamines"). There is a huge mixture of these, but most are not colored. Then c) the

ketosamines undergo complex subsequent dehydration, fission, and polymerization reactions. You end up with what is essentially caramelized, dehydrated saccharides, but it all happened at a much lower temperature than required for caramelization. The reactions will proceed at ice temperature. They are not oxidized. All of the dehydration and polymerization reactions produce the conjugated double bonds that give the non-specific color we see on the Shroud.

4) "where the reaction takes place it runs to completion yielding color of almost uniform density either immediately or over time depending upon the details of the process"

Here is where things get a little difficult. I do not have all of the numbers needed accurately to calculate how close the reaction gets to completion anywhere. I doubt it does for the following reasons. Here comes some non-freshman chemistry.

The reaction is heterogeneous: it occurs between a liquid phase on the skin or a gas phase between the skin and cloth and a solid. Solids do not follow the same rate laws as liquids and gases, and the way they react changes with extent of reaction. [Actually, during my career, I have never studied a "simple" reaction.] I distributed a table of "depletion functions" some time ago. A solid usually starts to react on its outer surface; however, reaction changes the specific volume of the impurity layer (it swells or shrinks). You see the "corrosion" Al talked about, which is what I called the "crackled surface." As the solid reacts, it cracks, creating more reactive surface. [A great book on the subject of reactions in and with solids was written by Garner, The Chemistry of the Solid State. Physicists should love it.]
In other words, the chemical rate *increases* as unaffected solid decreases. This is a definition of an "autocatalytic" type reaction.
Garner called it "topochemical." The rate reaches a maximum and falls off. The depletion function looks something like: x to the power p times (1 - x) to the power q. The rate is very slow when there has been little reaction (x is small). After the maximum, unreacted solid becomes scarce, and the depletion function usually becomes "pseudo first order," (1 - x).

But we haven't said anything about the concentration of the reactants in the gas phase. As mentioned in part 2), the amount of reactive amine at the surface of the skin increases with time, and it does not reach a maximum until long after tissue decay (loss of integrity) starts. The Shroud does not show any signs of tissue liquefaction, so the time for image formation must be less than about 36 hours (Is that OK, Fred?). The amine concentration would still be increasing, but it would not yet be near maximum. We would need analytical data from the "body farm" at U or Tenn. to make calculations. They have such data, but they won't share. Maybe one of our MIs can get the data. I would like to know how many ppm of amines appear directly over a body in still air as a f(t).

Now some "quantitative guesses." Nobody knows the rate constants for all of the reactions involved in the initial formations of all of the hundreds of N-substituted aldosylamines. Nobody even knows what all of the reactions are. When faced with a problem like that a chemist uses "global kinetics" that describes the effects of all of the reactions through a fairly narrow range of conditions. Several

publications have stated that the rate increases by a factor of about 3 for every 10C increase in temperature. This must be qualified to "normal" temperatures. I have measured activation energies for similar reactions at about 19,000 cal/mole. That would indicate a frequency factor of about 10exp8/s.

If the two reactants were nicely in solution or a gas phase at about the same concentration, the reaction would probably be "second order" (bimolecular). The depletion function would be (1 - x)exp2, and 1/(1-x) = kt. It would take 24 days to get to 90% of completion (2,411 days to get to 99.9%). Things slow down a lot as you approach completion. Theoretically, you can never reach 100% completion.

But that's not the whole story, because you have to add some time for the earliest part of the process while the surface of the solid layer of impurity is still unfractured and the concentration of amines in the gas phase is nearly zero. That is called the "induction time."
Given a low concentration of amines, the rate will closely follow a zero-order rate law, depending primarily on the surface area of the solid reactant. There is a large surface area in a woven cloth.
Also, the decomposition products (amines) are going from zero to some finite value in the 30-36 hours in the tomb.

The bottom line is that the most rapid rate will be so slow in the first day or so that the reaction will be "diffusion controlled." But that is not the whole story.

When you are talking about color density, you must consider the "extinction coefficient" of the colored compound, how optical density varies with concentration of chromophore. Some of the Maillard products have very high extinction coefficients - - - and all of them must be somewhat different. Maybe Prof. Anna Arnoldi can tell us how color density varies with % completion of Maillard reactions. I asked a person who does research on brewing, but he hasn't found the data yet. Not many people take spectra of their beer before they drink it.

5) "the "image" is the result of a concentration difference at the surface that encodes cloth to body distance which implies that the process requires a path-length dependent attenuation of the concentration of some kind to make one image area different in average density from another due to fewer fibers/threads being involved in the reaction due to reduced concentration."

The color density will depend on the extent of reaction, but the extent of reaction is a function of depletion functions, reactant concentrations, temperature, surface area, and time. What your eye sees depends on the light-absorbing properties of the specific products that are formed in any area. Contact areas will color first, and they should be closer to completion than non-contact areas at 30-36 hours. I have no idea how long it took to produce a visible image at ambient temperature after the Shroud was removed from the body. It could have been years.

As for "a path-length dependent attenuation of the concentration," that comes out of basic Kinetic Theory of Gases. [Notice that this is a scientific "theory." Actually it is now a "law."]

When gases are diffusing into one another, the concentration at any point (c) depends on the starting concentration (Q) *[concentration at the emitting surface]*, time (t), and the coefficient of diffusion (delta) (also called diffusivity}.

dQ/dt = -delta(dc/dx)dydz

The concentration gradient is dc/dx, the rate of change in concentration with the distance. This is one of the important path-length factors. If there is a large change in concentration in a small distance, as with heavy amines, resolution can be good; otherwise, the image would become "diffuse." The diffusivity of a gas goes down as its molecular weight goes up; for example, the diffusivity of hydrogen (mw = 2) is 0.634 while that of oxygen (mw = 32) is 0.178. Heavy decomposition amines will have very steep concentration gradients as they diffuse into air. The concentration will be much higher at 5 mm than at 1 cm.

Because the rate of evolution of heavy amines is limited during the first few days, and gases can escape from under the cloth by flowing through the pores of the cloth, something approaching a "steady state" can be achieved. The dc/dx will become essentially constant. This is important in rate considerations.

Although the concentration gradient will be a major factor, it will not be the only one in calculating the maximum resolution of an image-formation mechanism that involves diffusion. The depletion function will be even more important. Different parts of the cloth will be at different levels of completion, thus following different rate equations, at the same time. For example, an area that started reacting earlier may be at an autocatalytic stage before a slightly later area is out of its induction time.

Concentration gradients are also formed by depletion of reactant(s) as a result of chemical reactions. When reactants hit the cloth and react, they are not free to diffuse parallel to the cloth. They produce products only where they hit the cloth. The adjoining areas must be hit by other columns of reactants to produce a color.
Remember, diffusion from a surface occurs as a front not as a growing hemispherical volume. You can have collimation normal to the emitting surface.

Other mechanisms leading to loss of reactant and production of a concentration gradient include adsorption on the surface without reaction (there is a lot of surface that does not have a film of impurities on it), diffusion through pores in the cloth, and other series or parallel reactions (most do not produce color). Fibrous or particulate barriers (e.g., matted hair) can inhibit diffusion rates. Barriers increase the concentration of reactive gases in the barrier.
Increased concentrations result in increased reaction rates. Did you ever wonder why the hair showed so much color?

An increased reactant concentration in the hair, beard, and moustache increases flow through the

orifices (pores) of the cloth. This explains the image on the back side of the cloth at the hair, beard, and moustache. Diffusion from a pore would be hemispherical, explaining reduced resolution on the back of the cloth.

6) "The resulting image is due to conjugated carbon bonds which are very stable and can only be removed through the action of strong reducing agents which when applied leave the fibers white."

But the cracked, colored layers of impurities can also be pulled off of the surfaces of the fibers with adhesive, a physical process. The fiber that remains is colorless.

Yes, X you have it close enough for government work (as we say in the government - but who would admit to working for the government these days?). The only problem is a few simplifications that need more detail.

For example, you said: "A key element I think is the notion that the reaction run to exhaustion of the reactants -- which seems to me to be the case of all chemical reactions since if reactive elements remain they react -- the rate may change, but the reaction naturally runs until the reactants on one side or the other of the reaction are exhausted." That is correct, but the rate is an exponential function of depletion: it gets very, very slow as reactant(s) is depleted.

Then you said: "Here it has to be the amines that are exhausted else you'd have to believe that the image was pre-planted on the shroud in the pattern of the saccharides." Not if the areas with the shortest diffusion paths have reached the toe or maximum of the rate curve before the next group of fibers farther from the skin has. It would be probable (at least possible - I wish we had more numbers, but the range is broad) that most of the image fibers would look about the same color when the cloth is removed. However, the statement that all image fibers look the same is not correct: there are some significant differences. The bands of color should be explained by the thickness of the impurity layer and its effect on the order of the reaction (depletion function).

X said: "Now if the gases were generated in great profusion and transported to the cloth then the whole surface would be "flooded" and discolor and there could be no image. Thus the image results from there being a delicate balance in the amount of reactant that proceeded from the body to the cloth which means to me that not much evolved which would be consistent with the scriptural admonition that God did not allow his servant to suffer corruption. I'm not advancing the scriptural though as an argument, only as a cross-disciplinary outside element that this account would seem to corroborate."

I hadn't thought of that, but it would certainly apply as an historical source. The body should not have showed "rotting" in the time assumed. The rate of evolution of amines should have been quite slow. But I'll bet our cadaver-searching dog could have detected the body at about a km. Those amines have a terrible, potent odor. So how many ppm of combined amines is over a 30-36-hr body? I wish we knew.

I hope I have answered this: "Most of the details are a bit fuzzy to me -- for example (5) demands that path-length reduce reactant concentration so that at the extinction distance either the reactant has been exhausted by path-length alone or the concentration has fallen to such an extent that it is below the concentration necessary for the reaction to go forward." There is no extinction distance (see Kinetic Theory). The concentration necessary for a forward reaction depends on the free-energy change. If you want more, please ask again.

For those of you who are experts, please be kind. I realize where I have skimmed over some details that we may think are critical. I think I have hit the most important ones. There are some secondary effects, but experience says they wouldn't be important. If you think things need amplification, please help and I will also try again.

I am too tired now for any more tonight.

Regards,
Ray

SCORCH

When we believed that the image was probably a dehydration (scorch) color, we had trouble explaining the limited penetration of the color into the cloth. In order to make a deeply colored area, heating must be prolonged (or at higher temperature). A concomitant to that is that the color penetrates the cloth.

When we found that all image color resides on the outer surface of colored fibers, it was possible to consider superficial impurities of lower thermal stability than cellulose. Theoretically, it should be possible to coat a cloth with an easily-caramelized sugar (like arabinose, xylose, etc.) and produce color at much lower temperatures than required for coloring linen. However, the lower the temperature the longer the heating time required. Some times become unreasonably long. The table in "Cellulose-high rate" refers to 99% decomposition (I calculated it for estimation of scorch temperatures). At 160C, it would take 7.1 years to get to 99% decomposition; however, it would take 1.3 days to get 10%. That is quite a long time to maintain 160C over a large piece of cloth.

Also, heating changes crystal structures. We get crystal modifications in the cellulose, and we can not detect any signs of heating in areas well removed from the 1532 scorches. Heating at 150-160C would certainly change crystal structures.

The only way I have found at this time to caramelize sugars at low temperatures is the Maillard path. Given the saccharides and amines, the reaction will proceed: color will ultimately be produced.

Your questions:
1) Do EM photographs of the cellulose under the image exist?
Al Adler had several taken (if you mean electron microscopy). I do not have good copies. I did not find anything of primary interest in them or ones Joe Kohlbeck had taken (for different reasons).
2) How could be ruled out image formation by an artist using a "hot model" at a temperature and in a time that does not destroy the linen fibers (and no pyrolysis is produced) but still induces caramelization.
I would like to see such an image. Several things could be tested: e.g., crystal perfection, lignin composition, physical properties of the fibers.
3) For the ones that like to defend the Shroud image as a "byproduct" of the resurrection, the only thing needed is a kind of energy emanating from the body and able to induced rearrangement of the sugar
molecules in the same way that heat does but without affecting the cellulose of the linen at the same time.
Does anyone have suggestions of a kind of energy (not infrared, apparently) that could produce a caramelization without any effect on the polysaccharide structure of flax fibers? I have not been able to find one.

We tested image formation by heat (hot statues, painting with torches, etc.). The darker the image the deeper the penetration of scorch into the cloth.

In developing an hypothesis, I had to search for low-temperature chemical processes that produced the observed type of color. The processes had to involve only impurities. I had to eliminate direct "caramelization" of carbohydrates, because required temperatures were too high and/or times were too long. The low-temperature processes needed some foreign reactants and/or catalysts to dehydrate the impurities. Some reactive foreign material had to have come into contact with the impurities on the Shroud.

Dehydration-rate calculations made direct dehydration of any carbohydrate impurities, even pentose sugars, seem very unlikely.
Sustained temperatures higher than 100C would be required to give a color in any reasonable amount of time (less than months).

A person hoaxing the Shroud image could not have lived long enough to produce a room-temperature image. Using a cloth washed in a low-surface-tension solution containing pentose sugars (e.g., a Saponaria solution) and dried in the sun, a non-metallic statue at a temperature above 100C might have worked, but it probably would have shown some heating effects in the linen. Such a hypothesis could be tested; however, using Occam's Razor, another hypothesis seemed more probable.

A strong candidate for a low-temperature system that could produce the image color is what is called Maillard reactions.

Darker-appearing, pure-image areas did not penetrate significantly more deeply into the cloth than did lighter areas. The effect was much different than that produced by scorching a cloth with a hot statue.

Cellulose is not anxious to get rid of water. According to one of the people who have measured rates as a job (Bruce Waymack at Phillip Morris), pure, microcrystalline cellulose has the following kinetics constants (used in the Arrhenius equation): E = 50.6 kcal/mole and Z = 3.16 X 10exp16/s. It is remarkably stable for an organic compound.
And it is mostly crystalline, which makes a large difference.

Using those kinetics constants, you can calculate how long it would take to dehydrate by 10% (about where your eye can see color forming).
At body temperature (37C), it would take 5.3 X 10exp10 years (that is about 3.8 times older than the universe since the Big Bang). I don't want to wait around to do that experiment. At the boiling point of water (100C), it would take about 49,400 years (don't fidget while waiting). At 500C, it would take about 0.007 seconds. As you can see, it is not a linear relationship.

Linen will be somewhat less stable than pure cellulose, but in order for the image to be a result of the dehydration of the cellulose of the linen, you either need a high temperature for a little while or a lower temperature for a long time. And remember, the image is only on the surface of colored fibers: it does not penetrate into the linen fibers. The thermal conductivity of linen is not low enough to allow that to happen at lower temperatures - or even at quite high temperatures in short times.

A layer of impurity on the linen would almost certainly be less stable than the cellulose. The types of impurities you normally find on cloth are indeed much less stable than cellulose, and you can dehydrate them at much lower temperatures and/or much shorter times.
For example, a common component of crude starch (used on the warp during weaving) has the following kinetics constants: E = 25.99 kcal/mole and Z = 6.3 X 10exp9. It would begin to show color in about 1.2 years at body temperature. It would take about 8.2 hours at 100C, and it would take about half as long as cellulose at 500C.

You could color a surface impurity by heating without causing any coloration in the cellulose, just what we see with Shroud image samples. But it can be even easier than that.

Maillard reactions (e.g., decomposition products from a dead body reacting with crude starch on the cloth) can produce color at room temperature (or even colder).

Does anyone know accurately how long the reliquary was heated in 1532?
The scorches seem to indicate that the main part of the cloth had not been heated enough for any significant reaction. If Baima Bollone was really able to type the blood, and the blood could still be removed by a proteolytic enzyme, the blood must not have been heated very much.
It was still red in most areas of the Shroud.

BLOOD

The reactions occur between *reducing* saccharides and amino groups (-NH2). This means that the aldehyde or ketone groups of the sugars must be free to react. There is very little free sugar in blood. Also, blood tends to form a membrane over its surface as it dries. This would inhibit any potential reactions with the gas phase.

The protein in the blood has not been denatured by heat.

One of the most sensitive chemical tests used on the blood was the "iodine-azide" test. When a sulfur-containing functional group is present, the reagent bubbles vigorously with nitrogen bubbles. Fibers from the major blood spots bubble. Fibers from the non-image/non-blood areas do not. Fibers from the margins of the scorches do not (this fooled McCrone). The Shroud provides its own, irrefutable internal standards for the test.

When a chemist wants to make a little hydrogen sulfide (H2S), he/she gently heats a little elemental sulfur in paraffin (Don't do this without adequate ventilation! H2S is more toxic than cyanide.) Sulfoproteins, as found in blood and tissue, produce H2S on gentle heating. This is why the iodine-azide test gives a negative result on heated blood spots.

The blood on the Shroud was never heated enough to remove the sulfides. The Shroud was never boiled in oil or subjected to intense radiation heating. Please remember the trade-off between temperature and time (the Arrhenius law).

The composition of the blood (at least in 1978) agrees with the observations on defect populations in the flax fibers.

Let me give you a little chronology: 1) In 1977, the chemists in STURP were asked to test the hypothesis that the image was a painting. We planned methods to do this. 2) Working from photographs in 1979, Don Janney and his group reported that the halos around blood spots had

the same color characteristics as the non-image cloth. This would indicate that there was no image under the dried blood serum. 3) In 1978, we found that the image was not painted. Our assumption was that if nothing had been added to the cloth, the color had formed in the linen of the cloth. We began to test that hypothesis. 3) In 1980 Adler and Heller reported that blood could be removed from image fibers with proteolytic enzymes. They found no indication of image under the blood. We found starch fractions as impurities on the cloth. We still assumed that the cellulose of the linen had produced the color. We assumed that the best hypothesis for the lack of image color under the blood would involve protection of the cloth's surface by blood at the time of image formation. This would prove that the image-formation process was not violent enough to change the characteristics of the blood. 4) About 1982, we found that the image color exists only in a thin film on the surface of the image fibers: The cellulose of the linen was NOT involved in color formation. We still had no hypothesis for image formation at that time.

When I developed the hypothesis that reactions between -NH2 products from a dead body and reducing-saccharide impurities on the cloth might have produced the color, I wondered why there was no color under the blood. After all, doesn't blood contain -NH2 groups? It certainly contains a well-known reducing saccharide, glucose. Why doesn't the glucose in blood react with the -NH2 groups of the proteins to produce Maillard products in the blood?

Can any of our physicians or hematologists tell us why proteins in the blood would not react with saccharides on the cloth? Have all of the -NH2 groups been masked (a chemical term) with glucose in the blood? Are there steric problems?

MISCELLANEOUS

Heavy amines appear much more slowly. I sent the CDH data previously, and Fred sent his alignment chart. Most will start to appear when the body has essentially cooled to ambient. There will be no convection cells to mix and/or transport them. The amount that appears at the surface of the skin will increase on a time scale of days. They are liquids at room temperature, but they vaporize more rapidly than they appear (pools do not form, certainly not in less than 30 hours when tissues start to liquefy). They diffuse much more slowly than ammonia, improving resolution. Putrescine boils at 158C; cadaverine boils at 178C. They don't evaporate as rapidly as water, but they smell a lot worse and are a lot more reactive with saccharides. They are also basic and toxic. They have a much harder time diffusing through the pores of the cloth, although I wouldn't be surprised to find that some had made it.

What this says is that the image probably formed over a significant length of time, in at least two main stages. The image of the hair was probably largely a result of ammonia from the lungs. That agrees with the observation of image on the back of the cloth in the hair.
The image of the body surface was almost certainly a result of heavy amines from the tissues. As the heavy amines appear, they will react rapidly with the saccharides, and they will adsorb strongly to the

cloth. When covered with a reactive, high-surface-area cloth, they would stay close to their source.

The rates of appearance of the different body-decomposition products is not "theory." It is based on observations and peer-reviewed publications. The reactions between body-decomposition amines and saccharides are not "theory." Given the composition of the Shroud that we observed, the reactions would have occurred.

You can argue all you want about resolution. The Maillard colors are somewhere on that cloth. Where do you think?

Regards,
Ray

PS: I have published my 1978 observations, probing the cloth with a dissecting needle, in several places. They have been garbled in several other places. Is there image color inside the cloth? If anyone wants to believe there is, feel free. But please recognize the fact that there is at the very least much, much more color on the surface in image areas.

Dear X and Researchers:

X asked: "Can I ask if it can be determined quantitatively how much of starch, polysaccharides of lower stability, dehydrated saccharide, and saccharides involved in Maillard reactions are on the shroud in image and non-image areas?"

The word "quantitative" makes this question difficult to answer. When we were sampling the Shroud in 1978, our primary task was to determine whether the image could be a painting. This even included trying to think up methods that had never before been reported (e.g., painting with a torch). Unfortunately, we did not think of impurities on the fibers; however, our methods can be reevaluated to get some information along those lines.

Our qualitative tests proved the presence of starch on the cloth. It did not seem important at the time. The only method we used that could give quantitative results at the necessary level of sensitivity was the pyrolysis/ms.

The impurity layer varies in thickness, and sample recovery from the tape lost some. The coating layer represents less than 10% the mass of a fiber, and a fiber on the tape is normally less than one millimeter long. We are talking picograms for sample sizes and less than 10% of that for a quantitative analysis. Fortunately, we could see the pyrolysis products from samples of those sizes with the mass spectrometer. Unfortunately, we did not run a valid non-image fiber.
We did not know that the Raes sample was not part of the original cloth, and we used it as our control.

The matrix of the seven samples we ran appears in the attachment.

Figure 3 of the attachment shows how the different pyrolysis products appear as the sample is heated, with the total ion current being the lower graph. There was relatively little polysaccharide of lower stability left on that image fiber. I have assumed that this showed depletion of reducing saccharides as a result of image formation. I would be happy to hear other hypotheses.

There are obvious saccharides of lower molecular weight and lower order than cellulose on the Shroud. We just can't measure them quantitatively.

Crude starch is composed of a very large number of different chain lengths, and they all have different properties. Remember that the image was not soluble in the water that percolated through parts of it in 1532. Many of the components of crude starch are soluble in water; therefore, I assume that image areas are no longer polysaccharides.
The margins of the older water stains (see Aldo Guerreschi's hypothesis) show that soluble fractions are on the cloth (or were at the time the water stains were produced). The intense color you see around 1532 water stains are largely polymerized furfural.

The solubility, spectra, and pyrolysis results make me believe that image formation largely "fixed" the saccharides of lower stability than cellulose on the surface of the fibers.

Regards,
Ray

X quoted Y as saying that the color layer was "nominally 600 nm thick." To be accurate, that is not absolutely true. I have made measurements between 180 and 600 nm. NOTE: the thickness is in the range of a wavelength of light, and measurements are not very accurate with a visible-light microscope. Don't take the measurements as the definitive word.

The uv/visible spectra show that the color is not a pure yellow: your eye sees best in the yellow so that is what you see. The color absorbs nearly all of the blue and reflects nearly all of the near IR.
It is almost a straight line between them. The statement can be misinterpreted as saying that the image color is caused by a yellow chromophore. That is far from the truth. Incidentally, in chemistry a chromophore is not the same as a pigment.

The strings of conjugated carbon-carbon double bonds have great differences in length: the different lengths absorb different colors of light. There are more short strings than long strings.

Reactions that cause double bonds to form shorten distances between carbon atoms. Distances depend

on the rest of the structure, but a normal C-C single bond is about 1.5 Angstroms long, and a normal C=C double bond is about 1.3 Angstroms long. Dehydration and crosslinking in any saccharide causes it to SHRINK. Think of what a mud puddle looks like as it dries, producing a network of little cracks. That is a good analogy for the surface of some of the image color areas – BUT NOT ALL.

The shrunken layer is easier to remove with tape than is the original impurity layer. Incidentally, very light scorches produce the same kind of shrunken coating as did the image-formation process.

A30) "All the colored fibers are uniformly colored, i.e. an exposed fiber is either colored or not colored (Adler 1996, 1999)."
That is a somewhat misleading statement. Some image fibers have thicker coatings than others, consequently a darker color. Many fibers are darker on the ends where pendant drops of washing liquid probably formed (I see the same effect in dye experiments). I can send photomicrographs to illustrate these facts. HOWEVER: all of the image areas show exactly the same visible/uv spectrum.

A32) "All the image shows a uniform straw yellow coloration yielding less than 2% variation in the absorbance of the individual colored body image fibers (Adler 2000, 2002)."
I would like to have asked Al how he made the measurement. The microscope I used in his lab used incandescent illumination, and I assume he used the same exposure meter he used for photomicrography.
X was correct in interpreting the importance of a 2% variation.
But, perhaps Al simply missed the darkest fibers. Anyway, I can't agree with the statement, and I will send anyone who asks some photomicrographs.

The color tells us that there is no single "conjugated carbon bond compound." There are many different ones. The color has the properties of a caramelized saccharide or Maillard products, the two kinds of products being nearly identical in their final configurations. The Maillard reactions can be considered to be catalytic for the dehydration of a saccharide.

Then X asked: "If we are talking about a very thin coating of a carbohydrate material, we must wonder if it is opaque. Would any measure of a fiber's color show through (they vary as in the banding, do they not)? Is the chemical reaction completely through the coating?"
The layer of carbohydrate is not opaque. We have phase-contrast photomicrographs that show it very clearly. The fiber's color does not "show through": it is produced in the layer of carbohydrate. The layer of carbohydrate becomes the image color after reaction. The coating is so thin that I can not see whether the color is all of the way through. I would guess it is all of the way through on the basis of the chemical process. The banding corresponds to the amount of impurity (including dark lignin) that can be seen on the fibers from any band.

Saponaria is hemolytic, which could explain why the old blood stains on the cloth are still red. Diane Soran (deceased) of Los Alamos tested hemolysis on Saponaria-washed cloth before we went to Turin. The blood is still red on those 25-year-old samples [Now 27 years old.]. Controls are black.

Saponaria hydrolyzes to produce some aglycones that are fluorescent [I can supply compositions.], and the non-image part of the Shroud is weakly fluorescent. The image quenches and/or filters that fluorescence.

Saponaria is toxic, and it is a potent preservative. A textile conservator [Anna Maria Donadoni, Museum of Egyptology, Turin] told us that old cloths tend to be better preserved than newer ones. Comparison samples loaned to us by the amazing Museum of Egyptology in Turin were still supple, and several dated to several thousand years BC.

Saponaria produces four glycosidic saponins, all containing gypsogenin. The glycosides hydrolyze to produce sugar chains (10).
The following carbohydrates were identified in those chains: galactose, glucose, arabinose, xylose, fucose, rhamnose, and glucuronic acid.
Pentose sugars with a furanose structure appear to be the most reactive sugars (11). The Saponaria sugars should be quite chemically active. [They could participate in Maillard color-producing reactions.]

Slightly different amounts of impurities on the different batches of linen yarn would cause slightly different surface energies. One major linen impurity is "flax wax," and it produces a hydrophobic surface.
Liquids wet the threads as a function of the difference between the surface tension of the washing solution and the surface energy of the specific linen yarn. This could help explain the "banded" appearance of the Shroud. The original observations and experiments on this phenomenon were done by Benjamin Franklin in 1774 (35).

My point in mentioning surface-tension effects is to show that different amounts of suspended and/or dissolved impurities would deposit during evaporation of the washing liquid, depending on the surface characteristics of the batch of fibers. The soluble and colloidal components of crude starch would be in solution/suspension, and those impurities would be deposited on all parts of the cloth as the liquid evaporated. Differences in deposition would be seen depending on the surface properties and the rate of evaporation. This type of process can also explain the superficial nature of the image color on the weave surface.

The evidence indicates that several different impurities could have had an effect in image formation, but I suspect that starch was the most important as a color-producing reactant.

It is NOT valid to state that "diffusion can not yield good resolution": you must address the laws of diffusion. You must also recognize that thermal anisotropies may be important, but you have to consider body cooling in that context. You must also recognize the fact that cloth is porous: reactants can be lost through the pores (improving resolution). You must also recognize that reactions at the cloth surface use up reactants, reducing their lateral spread (improving resolution). You must also recognize the fact that a cool body and cool cloth do not produce significant convection cells (improving resolution). Think about how long it will take for the different decomposition products to appear. Evaporation concentration at the surface of a cloth limits the depth of penetration of reactions into the cloth: the dorsal image is NOT a problem. Decomposition amines do not all appear at the same time, and they appear gradually.

Little or no low-molecular-weight ammonia would appear at the back of the body. Resolution is better with later, heavy amines, because they do not diffuse so rapidly. If the cloth and body are separated, the dorsal image will not be flooded (subjected to excess vapor concentrations). The cloth does not show any liquid decomposition products. Does this sound like a simple hypothesis? Is vapor diffusion the only factor? Let's be rigorous about all of the factors.