

# **THE IMAGE ON THE TURIN SHROUD: ANALYSIS OF THE MAILLARD REACTION HYPOTHESIS PART (I): THE ORIGIN OF THE REACTIVE AMINES**

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The Maillard reaction postulated by Ray Rogers (1) appears to be one of the most reliable hypothesis to explain the chemical as well as the microscopic characteristics of the image fibers of the Turin Shroud.

It occurs (necessary and sufficient condition) between reactive amines (i.e.: molecules with a free  $-NH_2$  group) and sugar fractions to produce finally a large number of stable colored polymerized compounds in/on the thin layer of sugar impurities found on the surface fibers. Ammonia ( $NH_3$ ), although it is not usually considered as an amine, has 2 free electrons and reacts like amines.

While the presence of sugar reactive molecules on the shroud is a fact (starch impurities found with iodine microchemical test, low stability polysaccharides spectra in the pyrolysis-ms data), the origin of the reactive amines is not obvious.

With the assumption and in the context of a corpse lying under the shroud, Ray Rogers postulated reactive volatile amines (particularly Putrescin (P) and Cadaverin (C): see his messages in ShroudScience Yahoo Group) coming from the early decomposition of the corpse as the amines involved in the reaction. Ammonia coming from the lungs through the mouth and the nose in the first minutes/hours after the death might also explain the high general luminance level in the face area.

In fact, with the hypothesis of diffusion or contact/diffusion coupled with the Maillard reaction at least 2 mechanisms (eventually combined) can be supposed:

- Hypothesis 1 (Rogers' hypothesis): the amines come from the inside of a decaying corpse. Amines produced in the corpse as soluble molecules, transported by some mechanism onto the surface, appear in the liquid phase at the surface of the skin, evaporate and diffuse in the air in the non-contact areas or react directly in the contact areas.
- Hypothesis 2 ("modified vaporography hypothesis"): the amines come from the surface of the body and are not related with decomposition.

The body was widely covered with sweat before the death by crucifixion. The sweat contains large amounts of urea that was hydrolized in ammonia. Ammonia (with water vapor) evaporates and diffuses in the air or reacts directly with the sugar fractions on the sheet.

In any case the following conditions must be satisfied :

- spatial condition: the diffusing substance must come roughly equally from all parts of the corpse, including the face, the extremities and the hair.
- time/quantity condition: the diffusing substance must reach the sheet in a sufficient amount within a certain time.

**HYPOTHESIS 1**: the amines are produced in the corpse within the 36-72 hours after the death by the early decomposition process.

The question of the production and the development of amines belongs to forensic science. We are here only interested in the very early stages of the decay of human corpses. According to Ray Rogers and all the forensic literature, the lack of evidence of body fluid on the shroud, the signs of rigor mortis and the lack of distension of tissues (except perhaps for the abdomen) imply that the corpse can not have been in contact with the sheet after 36-72 hours, depending on the temperature.

In order to test this hypothesis it is of paramount importance to understand the decomposition process.

Vass and all. (2) describe it as follows:

*“Human decomposition begins approximately 4 min after death. The onset of decomposition is governed by a process called autolysis or self digestion. As cells of the body are deprived of oxygen, carbon dioxide in the blood increases, pH decreases and wastes accumulate, which poison the cells; concomitantly, cellular enzymes (lipases, proteases, amylases, etc.) begin to dissolve the cells from the inside out, eventually causing them to rupture, releasing nutrient-rich fluids.(...).*

*Autolysis usually does not become visually apparent for a few days. It is first observed by the appearance of fluid filled blisters on the skin and skin slippage where large sheets of skin slough off the body. Meanwhile, the body has acclimated to ambient temperature (algor mortis), blood has settled in the body causing discoloration of the skin (livor mortis), and cellular cytoplasm has gelled due to increased acidity (rigor mortis). After enough cells have ruptured, nutrient-rich fluids become available and the process of putrefaction can begin. Putrefaction is the destruction of the soft tissues of the body by the action of microorganisms (bacteria, fungi, and protozoa) and results in the catabolism of tissue into gases, liquids, and simple molecules. Usually the first visible sign of putrefaction is a greenish discoloration of the skin due to the formation of sulfhemoglobin in settled blood. The process progresses into distension of tissues due to the formation of various gases (hydrogen sulfide, carbon dioxide, methane, ammonia, sulfur dioxide, and hydrogen), especially in the bowels. This is associated with anaerobic fermentation, primarily in the gut, releasing by-products rich in volatile fatty acids, primarily butyric and propionic acids. Gas and fluid accumulation in the intestines usually purge from the rectum, but can be severe enough to rip apart the skin causing additional post-mortem injuries. Shortly after the purging of gases due to putrefaction, active decay begins. Muscle, composed of protein, which in turn is composed of amino acids, readily decomposes to form additional volatile fatty acids through bacterial action. Further protein and fat decomposition yields phenolic compounds and glycerols. Compounds including indole, 3-methyl indole (skatole), putrescine, cadaverine, and various fatty acids have been detected and are significant decomposition products. (...).For example, estimating the PMI (Postmortem interval) prior to the onset of putrefaction (36–72 h) generally involves visual inspection of the body by observing the appearance (i.e., rigor and livor mortis) and determining the core body temperature and gastric contents “*

All the literature agrees that the amines produced during the decomposition result only from the bacterial reactions with amino acids.

For example, Bonte and Bleifuss (3) wrote:

*“After death the further breakdown of the proteogenous amino acids is accomplished by foreign enzymes because the auto-enzymes, after complete exhaustion of the energyspending systems, are blocked (...) Because amino-acid degradation is effectuated exclusively by*

*microbial enzymes, amine production should be suppressed after disposal of the microflora. As expected, this effect did occur when antibiotics were added to the putrefaction trial with liver homogenate. No amines could be identified during the entire test series”.*

In the same paper one can read that at least 16 amines were identified in the putrefaction products, including P and C. Each of these amines is the product of the biological degradation of one amino acid by a bacterial enzyme called decarboxylase. P comes from the decarboxylation of Ornithine and C from the decarboxylation of Lysine.

To summarize the process can be described as follows:

- step 1: immediately after the death begins the process of autolysis as described above. The breakdown of the proteins in their elemental components leads to the appearance and the increase of amino acids in the different tissues at a different rate.

Concomitantly the exponential proliferation of many different species of microorganisms begins. However we must remember that all the internal organs of a healthy living man are sterile, except the natural orifices and cavities, particularly the bronchi, lungs and digestive system.

Therefore the bacterial invasion begins in the abdomen and the chest to extend progressively.

- step 2: **the production of amines appears after a certain time only in the corpse areas where both conditions are combined: 1) presence of free amino acids and 2) presence of microorganisms able to transform those amino acids into amines.**

Understanding that, we can now try to see if the hypothesis can check the previous space/time/amount conditions.

Unfortunately, I was not able to find numerical data about the appearance and the evolution of the amines in or at the surface of a human cadaver during the 2 or 3 first days after the death. However, the two papers cited above are sufficient to conclude.

- In the abstract, Vass and al. described the aim of their work :

*“ This study was conducted to characterize the chemistry associated with the decomposition of human remains with the objective of identifying time-dependent biomarkers of decomposition. The purpose of this work was to develop an accurate and precise method for measuring the postmortem interval (PMI) of human remains. Eighteen subjects were placed within a decay research facility throughout a four-year time period and allowed to decompose naturally.*

*Field autopsies were performed and tissue samples were regularly collected until the tissues decomposed to the point where they were no longer recognizable (encompassing a cumulative degree hour (CDH) range of approximately 1000 (3 weeks)). Analysis of the biomarkers (amino acids, neurotransmitters, and decompositional by-products) in various organs (liver, kidney, heart, brain, muscle) revealed distinct patterns useful for determining the PMI when based on CDHs. Proper use of the methods described herein allow for PMIs so accurate that the estimate is limited by the ability to obtain correct temperature data at a crime scene rather than sample variability “*

Because the ambient temperature is known as the most important parameter of the rate of decomposition of a body, all the results are given in CDHs.

If we assume a mean ambient temperature of 20° C, the CDHs for 36 to 72 hours are 60 to 120 CDHs. This means that we are only interested in the biomarkers appearing in the organs before 120 CDHs (rounded to 150 CDHs).

Unfortunately, P and C as well as their precursors (the amino acids lysine and ornithine) did not appear as “diagnostic” with regard to the aim of their work: *“Initial tissue surveys indicated that the common, odoriferous amine indicators of decomposition, cadaverine, and putrescine would be useful biomarkers. Unfortunately, this was not the case in this study. While the concentrations of these compounds were quite abundant (3000 ng/mg tissue) in some instances, the values (between corpses) were quite inconsistent as were the precursors of these compounds (lysine and ornithine)”*.

For that reason no data about P and C or their precursors are given.

Most of the biomarkers studied were the amino acids, the necessary precursors of the amines. An interesting comparison can be done between the 5 organs studied regarding the date of the appearance of the amino acids: *no* amino acid can be found in the muscle before 350 CDHs, except leucine and isoleucine (2/10). In contrast the number of amino acids or other biomarkers appearing before 150 CDHs is: 7/12 in the liver, 5/10 in the kidney, 6/14 in the heart and 9/9 in the brain.

Notice that most of the muscle samples were mainly obtained from the quadriceps (a muscle of the thigh) and for some others from the biceps, pectorals, abdominal or calf.

This important result confirms logically that the autolysis of the muscles begins much later than the autolysis of the deep organs: **the necessary substrate of the bacterial reactions leading to the formation of amines is quite absent from the human muscles before 350-400 CDHs (+/- 9 to 10 days; 20° C. mean storage temperature).**

- The second paper (Bonte and Bleifuss) is more useful because direct measures of the amines in different tissues are reported. However, except for a human liver, the others come from animal cadavers.

The investigations were carried out on stored blood, liver homogenate, gallbladder bile including extravascular putrefactive transudate, and putrefactive thoracic fluid.

The main result is so described in the summary:

*“ Up to 12 ptomaines\* could be identified. The post-mortem alterations were similar in all substrates under investigation and were characterized by a time-dependent gradation. During the first week, beta-alanine and gamma-aminobutyric acid were observed. At a storage temperature of 20° C., agmatine, etholamine, and tyramine appeared during the second week, cadaverine and phenylethylamine during the third, and putrescine and histamine during the fourth week or later”*.

\* (another name for the amines resulting of the decomposition)

Concerning the temperature they also notice that, with the exception of gamma-aminobutyric acid, all the amines appeared at 3°C. or at 35°C. at lower concentration than at 20°C, which seems to be the optimal temperature for the bacterial decarboxylase enzyme activity.

For P and C, the authors also report previous interesting studies: *“For the thanatologist it is interesting that the appearance of ptomaines in cadaveric material seems to be time-related. For example, Brieger noted that although traces of cadaverine and putrescine could sometimes be identified after 3 or 4 days, a quantity sufficient for analysis could be detected only after 11 to 14 days postmortem”*, or *“The classical putrefaction diamines, cadaverine and putrescine, which never appeared in concentrations higher than 20 mg/litre in our experiments, were never quantitated by previous researchers. Brieger mentioned that the*

*preparation of 10 to 20 kg of raw material was necessary to gain analytically sufficient quantities”.*

In their work, the authors have found that gamma-aminobutyric acid (GABA) and beta-alanine are the only amines appearing before 36-72 h of storage. However, from the curves reported the concentrations are very low before the fifth day (below 10 mg/l). Both amines were fortunately studied in the paper of Vass previously cited: GABA appears actually early (at 0 CDHs) in human livers, kidney and brain, at 166 CDHs in heart, but at 351 CDHs in muscle. Alanine appears at 519 CDHs in kidney, 0 CDHs in heart and 401 CDHs in muscle.

- Discussion and conclusion:

The critical space/time/amount conditions for the Rogers' hypothesis imply that at least one amine must appear everywhere on the skin within 36-72 hours after the death, including the legs and arms which are mainly composed of muscles, blood vessels and tendons.

The two published works studied here, although based on different materials (human and animal cadavers) and different biomarkers, are consistent: no amine can be found in the animal tissues before the fourth-fifth day except GABA and alanine which are not found in human muscles before 9 to 10 days at 20°C.

Concerning putrescine and cadaverine it is clear that they can not appear within the 36-72 hours required but much later.

All these data are easily explained by the mechanisms of early decomposition. In particular the muscles (and tendons) in the legs and arms are not concerned by the autolysis during the first hours as proved by the rigor mortis and the data of Vass. Then the bacteria must reach these areas in a certain time to produce the amines.

Therefore no amine can be produced in these areas before several days.

The characteristic smell of decomposition can be perceived after the first day under normal conditions.

Rogers (as chemist and also having a dog specialized in the retrieve of cadavers) stated that he was able to recognize the smell of the amines and that his dog was able to discover a very fresh cadaver as well as to distinguish between human and animal cadavers.

Even if the earliest gases emitted by a corpse contain amines, it does not mean that they are emitted through the skin. It is much more likely that the (possible) amines come from the very early decomposition of the intestines and lungs through the natural orifices of the body.

The Rogers' hypothesis of amines coming from the early decomposition (36-72 hours) of a human body is clearly not compatible with the critical time/space/amount conditions required and must be excluded as the reactive amines involved in the Maillard reaction hypothesis.

**HYPOTHESIS 2** : the modified vaporography hypothesis: the reactive “amine” is ammonia coming from the hydrolysis of urea contained in the sweat.

In one of his messages to the group (number 3000), Rogers stated: “I have not been able to find any sweat analyses that showed urea as a major component”.

In fact, the data can be found in two very old paper (4).

In the first one the urea was measured in the sweat of 20 subjects during the summer 1925 and 1926: “*In our experiments, we have discovered that the urea nitrogen of the sweat ranged from 0.24 to 1.12 mg per cc*”.

The 0.24 result came from a vegetarian subject. Most of the results range between 0.30 and 0.90 mg/cc.

In the second one, the authors have quantified directly the ammonia present in the sweat and found a minimum of 0.05 mg/cc and a maximum of 0.35 mg/cc. Most of the results range between 0.10 and 0.20 mg/cc.

They also noticed a correlation of 41% between urea and ammonia nitrogen in the sweat.

In the context of the Turin Shroud we must also take account of 3 very important facts:

1) The conditions of a crucifixion as well as the previous events “visible” on the shroud (scourging...) implicate the emission of liters of sweat during the hours before death: the *absolute* quantity of urea on the skin that has been progressively transformed into ammonia would be very important.

2) The inhuman effort caused by the scourging, the carrying of the cross and the crucifixion implies that all the glucose reserves (glycogen in the liver and the muscles) must have been quickly depleted. Then the proteins of the body are used to furnish energy to the cells. It has been shown (5) that in that case the urea nitrogen flow increases dramatically in the sweat from 10 mg/hour at rest, up to 1400 mg/hour (!).

3) The acute renal failure coming from the hypovolemia induced by the sweating and the internal and external hemorrhages also increases the urea level in the blood and in the sweat.

Consequently the following scenario is probable:

During the hours before his death the TS lost liters of sweat on his entire body, and the hair were largely wetted. The urea concentration in the sweat was very high and urea accumulated on the skin as the water of the sweat (99%) flowed on the ground or evaporated.

After the dead, sweating stops and the evaporation of the water goes on slowly. The hydrolysis of urea continues to transform urea into ammonia.

The high quantities of ammonia in liquid phase evaporate quickly (ammonia is very volatile) and diffuse in the air up to the sheet or react directly with the thin surface layer of sugar fractions in contact areas.

## **CONCLUSION:**

Under the assumption that the image of the Turin Shroud has been formed by a chemical reaction between reactive amines and the thin layer of sugar fractions found on the surface fibers (Maillard reaction) it is now clear that:

1) no amine (and particularly putrescine and cadaverine) produced in the corpse during the decomposition can satisfy the time/space/amount necessary conditions.

2) it is probable (although not demonstrated) that the ammonia evaporating from sweat containing large amounts of concentrated urea satisfies the same conditions.

Because ammonia is very volatile, high concentrations of gaseous ammonia could reach the sheet within the first 36-72 hours after the death, diffuse through this porous medium and strongly adsorb on the cellulose and the thin layer of sugar fractions where it begins to react following the complex roads of the Maillard reaction. The reaction then continues slowly at room temperature within several days, months or years until (theoretical) completion, leading to a progressive development of the colour.

The question to know if the laws of diffusion applied to ammonia in the body/shroud configuration allow the very peculiar spatial distribution of the image will be the subject of the second part of this paper.

#### REFERENCES:

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