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The Role of Human Skin Bacteria in the Formation of Photographic-like Images on Linen with Implications for Shroud of Turin Conservation and a Method to Clean Shroud Linen of Microbial Contamination for Reliable Radiocarbon dating

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Thirty or more species of bacteria are known to reside on human skin. Among these are species of *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*. The most common is *Staphylococcus epidermidis* and is estimated to represent about 85% of the total bacteria on human skin. Nutrients for these bacteria are largely obtained from the disintegrating epithelial cells on the surface of the skin and moisture provided by sweat glands. The nutrients and moisture levels are generally present in limiting amounts preventing rapid growth of skin bacteria. The bacteria that are normally found on human skin completely coat the surface and are estimated to range from hundreds to thousands per square centimeter. An important function of these normal flora bacteria is to occupy all sites on the skins thereby preventing more pathogenic micro organisms from gaining a foothold.

One of the properties of *S. epidermidis* that allows it to efficiently coat the skin surface is the ability to produce glue-like polysaccharides, which provide for its strong attachment to receptors on epithelial cells. Our modern hygienic bathing procedures result in the daily removal of accumulated dead epithelial cells along with their attached bacteria without leaving visible signs of their presence on towels or linen surfaces. However, when the skin surface becomes covered with rich nutrients from blood, serum, or a culture medium, *S. epidermidis* present on the skin can grow to extremely high levels. Bacteria present at 1000 colony forming units/cm² on the skin and dividing every 30 minutes can grow to 20 million bacteria/cm² over an 8-hour time period. In order to replicate these high levels, skin bacteria can either be grown on blood agar plates or in a liquid broth medium overnight at 37°C. After collecting the bacteria, a cotton swab is used to apply bacteria over the surface of the skin to be imaged until the skin assumes a shiny surface that is sticky to the touch. The skin surface is then gently bathed in water and uneven areas smoothed with a damp cloth. The evenly coated bacterial skin surface is then wrapped gently in slightly damp linen and allowed to dry. As the linen dries on the skin, the hydrophilic bacterial polysaccharides withdraw moisture from the skin and linen. This dehydration process results in the "gluing" of the linen to the skin surface preventing a blurring of the image. Upon removal of the linen, a straw-yellow image develops due to the instantaneous oxidation of dried bacterial fatty acids and possibly bacterial pigments. Removal of moisture from the skin surface exposes the underlying bone structure and also results in a highly wrinkled appearance of the skin. The image would be more intense in areas that were in direct contact with the skin. Reproduced below are positive and negative images produced by these procedures.

Due to the initially moist linen or subsequent exposure of the linen to moisture, diffusion of water-soluble nutrients (blood, serum and culture medium components) would allow skin

bacteria to grow in areas not in direct contact with the skin surface. However, the extent of diffusion of organic materials would provide a definite boundary to the image. Skin bacteria, such as *S. epidermidis*, require complex nutrients and therefore are not able to grow on linen without nutrients.

The most readily oxidizable organic materials that contribute to a yellowing appearance are lipids (fatty acids) and some pigments that are susceptible to oxidation by molecular oxygen. However, other bacterial components, such as the cell wall peptidoglycan, are not as readily oxidized but will contribute to color development over a period of time. Bacterial cell wall peptidoglycan is the main structural component of the cell wall and comprises as much as 50% or more of the cell by weight. Bacterial peptidoglycan has the same basic structure of that of cellulose, namely, β -1,4-linked glucose polymers. It is only distinguished by the presence of N-acetyl groups and several amino acids. Other cell material would include DNA, RNA, and protein as well as extracellular polymers, such as polysaccharides and other types of organic material. Over long periods of time, periodic increases in moisture content would allow microbial enzymes to break down the larger polymers into smaller molecular weight material, which would be more prone to oxidation, and thereby increase the intensity of the image.

In summary, regarding the mechanism of image formation, the presence of an unusually large number of bacteria on the skin is responsible for image formation on a linen surface that has attributes of a photograph. In regards to the Turin Shroud, the basis for the unusual number of skin bacteria can be attributed to the prolonged period of bleeding from the head area as well as the rest of the body (probably 6-8 hours), which allowed normal skin bacteria (particularly *S. epidermidis*) to continue to grow at 37°C (body temperature) and reach extremely high levels. This is a natural phenomenon but it required specific events to occur in a sequence. Under these defined conditions, an image will always form and to deny this mechanism of image formation requires a microbiological explanation that specifically rules out the role of skin bacteria in image formation.

In regard to microorganisms and Turin Shroud conservation, the Turin Shroud is completely coated with both live and dead microorganisms. It is not necessary to examine the Shroud linen to make this observation. All surface objects on the earth are coated with microorganisms, both living and dead. In fact, we now know that microorganisms are found everywhere on earth including deep within the earth's rocks and in superheated thermal vents deep within the oceans. Bacteria that contributed to image formation on the Turin Shroud have long ago died as nutrients from the skin and body became exhausted. However, other bacteria with fewer nutritional demands grew on the skin bacteria utilizing some of their disintegrating macromolecules. In time, bacteria and fungi completely covered the entire Turin Shroud and have colonized every available site on the linen. A few of these bacteria have produced intracellular and extracellular polymers, one with the properties of a transparent plastic-like material. The most common plastic-like polymer produced by some bacteria is a poly- β -hydroxyalkanoate, which has the tensile strength of ordinary plastic and is widely used in Europe as biodegradable plastic. Studies with Garza-Valdez indicated that at least one type of bacteria produced a plastic-like material that appears to have completely coated both image and non-image areas of the Turin Shroud. It is our hypothesis that the bacterial plastic material or bioplastic coating actually has served to protect the linen from decomposition. However, oxidative processes due to live microorganisms

and directly from oxygen itself will continue and over a period of time, it will become increasingly more difficult to distinguish between image and non-image areas. All microorganisms require several factors for growth including an energy source, specific nutrients, and moisture. In some cases, an energy source can also serve as a nutrient source. Atmospheric gases, such as nitrogen, carbon dioxide, carbon monoxide and others can serve, in some cases, as energy sources and sole sources of nitrogen and carbon for bacteria, which are referred to as autotrophs. Heterotrophs are bacteria that utilize preformed carbon, such as that found in dead bacteria. Some bacteria are aerobic and require molecular oxygen for metabolism. Molecular oxygen is well known to directly oxidize organic molecules over a period of time and thus contribute to a yellowing appearance of the linen. Because of all these biological and chemical processes, moisture and oxygen must be excluded from contact with the Turin Shroud. Although some bacteria can grow in the absence of oxygen, they still have a requirement for moisture, so the absence of both moisture and oxygen is necessary. The choices for exclusion of oxygen include either using a vacuum or an inert gas, such as argon. A number of techniques are available for excluding moisture. The continued exclusion of moisture over a long period of time will eventually result in the death of all vegetative microbial life forms with the exception of spores, which may exist for an undefined period of time, but causing no further damage. Exclusion of water will result in a loss of luster in both the image and non-image areas. This is due to removal of water from microbial polymers, such as polysaccharides, which have a high affinity for water, and contribute to the glistening or moist appearance. This removal of moisture should not be considered detrimental to the Shroud's integrity.

In regard to obtaining a scientifically valid radiocarbon date for the Turin Shroud, the issue of linen contamination must be considered as a necessary requirement before a date can be assigned. A radiocarbon date of a linen sample demonstrated by chemical analysis to be 95% or greater by weight as linen cannot be disputed. Unfortunately, this was not the case with the 1988 radiocarbon dating of the Turin Shroud. A detailed chemical analysis was not performed and therefore the purity of the linen samples was not known. The requirement of a chemical analysis is the first and most fundamental step in the examination of any biological material. Therefore, the radiocarbon date of the Turin Shroud remains unknown.

In order to remove microbial contamination from the Shroud linen, a series of enzymatic treatment steps are required. A fundamental requirement for any enzyme treatment is that the enzyme preparations must be demonstrated in preliminary studies not to have cellulase activity, which would result in breakdown of the Shroud cellulose fibers. Bacterial cell wall peptidoglycan can be solubilized with lysozyme or similar enzymes. Likewise, fungal cell walls can be treated with chitinases. A large variety of DNases, RNases, proteases, lipases and enzymes that solubilize the bioplastic-like polymers are available to help remove microbial polymers and products from the linen. To assess cleaning of the linen, several milligrams of the linen must be examined before enzymatic cleaning and after cleaning. Because linen is composed of macromolecular cellulose polymers, which are in turn composed of glucose units, an analysis must measure the glucose content. This can be achieved in several standard assays. A direct chemical assay for hexose (glucose) content can be achieved by an anthrone-like procedure and compared to an enzymatic procedure that utilizes cellulase to solubilize the linen cellulose followed by glucose oxidase treatment of resulting glucose. Other assays are available for quantitative analysis of glucose, but the goal is to obtain a final linen sample that is >95%

cellulose by weight. When this is achieved, an amino acid analysis should be performed that demonstrates removal of essentially all amino acids that are components of protein and peptidoglycan, the major macromolecules of bacterial cells. Radiocarbon dating of such purified linen will reveal the actual date of the Turin Shroud.