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ORIGINAL ARTICLE

Detection of Mercury in the 411-year-old Beard Hairs of the Astronomer Tycho Brahe by Elemental Analysis in Electron Microscopy

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ABSTRACT

Hairs more than 400 years old of the famous astronomer Tycho Brahe were studied by electron microscopy to evaluate the hypothesis that Johannes Kepler murdered his teacher Brahe by mercury intoxication. The beard hairs showed a well-preserved ultrastructure with typical hair scales and melanosomes. The authors detected an accumulation of electron-dense granules of about 10 nm inside the outer hair scales, but not in the hair shaft and roots. At the places of these heavy-metal-containing granules they detected mercury besides other elements by energy dispersive X-ray analysis (EDX, Oxford, UK) in a field cathode scanning electron microscope (SEM, Gemini, Zeiss). The mercury-containing granules were found over the whole length of hairs, but only in the outer hair scales. Nevertheless, surface coatings of hairs were free of mercury. This distribution of mercury does not support the murder hypothesis, but could be related to precipitation of mercury dust from the air during long-term alchemistic activities.

Keywords: beard hairs, electron microscopy, elemental analysis, forensic medicine, mercury intoxication

Tycho Brahe (1546–1601) was one of the famous astronomers during the Renaissance. He observed the movement of stars and planets very precisely with his naked eyes and recorded the angles using self-constructed instruments. His protocols about the positions of stars and planets over a period of nearly 40 years served as the basis for the three laws of planetary motion formulated by Johannes Kepler. However, there was a strong distrust between Brahe and his student Kepler, so Brahe kept his protocols under lock and key. Therefore, the sudden death of Brahe in 1601 after a banquet at Baron Peter Rosenberg's palace in Prague led to speculations about an unnatural cause of death [1–3]. The pain and symptoms of Brahe's death throes were described precisely in the funeral speech by the imperial physician Jan Jessenius and by Kepler himself in a notice written at the end of the astronomical protocols and published some years later. A rumor about a possible poison attack was raised after Brahe's death, but after some decades, this rumor was completely forgotten by most people and scarcely mentioned by the historians for the next 390 years. After the Velvet Revolution, things changed. Hair and beard samples extracted from the tomb in 1901 were offered by the Czech authorities to Denmark, where they were examined twice. In 1992, the Danish forensicist Bent Kæmpe was the first to detect mercury in the hairs of the beard by AAS analysis [4,5]. In 1996, the Swedish physicist Jan Pallon confirmed these results, when he discovered the same poison in the astronomer's beard hairs by PIXE analyses [6].

In Scandinavia, these discoveries led to reinforced speculations about murder, but the rest of the world did not take notice of the scientific results, until the journalists Joshua and Anne-Lee Gilder published *Heavenly Intrigue* in 2004 [7]. In this book, they accused Kepler of having murdered his master with

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mercury in order to obtain the astronomical protocols. This American best seller was translated into German in 2006, but it was fiercely criticized by serious historians and upset Keplerians. Recently, Andersen found indications in the Royal Library of Copenhagen and in Stockholm that seem to support a possible murder attack on Tycho Brahe by his Swedish cousin Erik Brahe on the behalf of the Danish king [8].

All these murder hypotheses are seriously weakened and perhaps definitely disproved by electron microscopic studies and measurements on 10 hairs from Brahe by a singular new instrument and a very sensitive analytical method. Tycho Brahe was buried in 1601 in a tomb under the floor of the Teyn church in Prague. On the occasion of the 300th anniversary of his death, the tomb was opened [7]. The investigators found two well-preserved skeletons, a female (Tycho´s wife) and a male with residues of Tycho´s beard as well as some parts of the shroud. The authenticity of Tycho´s skeleton and beard was given by traces of copper at the skull. Tycho lost his nose during a duel in 1566 at the University of Rostock (Germany) and probably used different nose prostheses made out of copper or gold. Furthermore, the residues of beard were red, as pictured in oil paintings of Tycho Brahe.

MATERIALS AND METHODS

Hairs

We received in all 10 hairs of the beard of Tycho Brahe, which were authorized by E. K. Jessberger from the Institute for Planetology, University of Münster, Germany. They came from the first exhumation in 1901 in Prague, where the beard and the rest of the shroud were placed in a glass box and saved in the National Museum at the Wenzel Place until 1989. The museum (Martin Solc, director) donated the beard as a quasi-relic to the government of Denmark, and some hairs were given to Jessberger. Four hairs were without roots and about 10–15 mm long. Six hairs were still located inside the residue of skin and contained roots. The hairs were red, which is the color known from oil paintings of Tycho Brahe's beard. Figure 1 shows the hairs before they were embedded in epoxy resin. We received the hairs in a sterile glass flask and handled them under sterile conditions with rubber gloves, filters of the exhalation air, and sterile instruments. Nevertheless, the hairs were contaminated with foreign DNA in the long period between Brahe's death in 1601 and the exhumation, as well as during the 100 years of non-sterile exhibition in the museum in Prague. Therefore, studies with the isolated mDNA after PCR breeding in the Department of Forensic Medicine of the University of Rostock, Germany (V. Weirich, J. Nowotnik) were without value. Furthermore, no authorized comparative DNA of Tycho Brahe from the second exhumation in 2010 is available.

FIGURE 1. Wisp of beard hair with a residue of Tycho Brahe's skin; hairs of red color with some pollution material at the surface.

Light Microscopy

The hairs were documented before being embedded in epoxy resin under the light microscope Axiophot 40 (Zeiss) and the CCD camera ProgRes C 10 (Jenoptic, Jena, Germany).

Scanning Electron Microscopy

For scanning electron microscopic (SEM) studies within a Gemini (Zeiss) as well as DSM 960 (Zeiss), hairs were mounted on carbon tabs longitudinally. Furthermore, we used the field cathode scanning electron microscope (FI-SEM, Gemini, Zeiss) and the backscatter mode (BSA).

Transmission Electron Microscopy

For transmission electron microscopic (TEM) studies, pieces of dry hairs were embedded in epoxy resin araldite without any previous dehydration in acetone or alcohol and cross sectioned after polymerization with an ultramicrotome Ultracut (Leica). Ultrathin sections were mounted on gold or copper grids. For simple ultrastructural studies, we used the TEM Libra 120 with a LaB_6 cathode and ultrathin sections on copper grids after staining with uranyl acetate and lead citrate. The 300-mesh grids were covered with a carbon film before the placing of the ultrathin sections (100–300 nm). To increase the contrast, we used the HCI mode. For scanning transmission electron microscopy (STEM), the FE-SEM Gemini Ultra (Zeiss) was used from the Zeiss SMT Company, Oberkochen, Germany (H. Jaksch).

Elemental Analysis

For elemental analysis in TEM, we used unstained epoxy resin ultrathin sections of about 120 nm thickness on 300-mesh copper or gold grids. The analytical system consisted of the Libra 120 (Carl Zeiss) and an EDX system (KEVEX). We were not able to detect mercury in the hairs by this method. Additionally, we studied the elemental composition of hairs in the SEM DSM 960 A (Carl Zeiss) with an EDX system (KEVEX), but we were also not able to detect mercury with our local system (EMZ Rostock, Germany).

Therefore, we used the Oxford Inca EDX system in combination with the FE-SEM Gemini Ultra (Carl

Zeiss). To localize the analysis at different places we used its special STEM and BSE mode. Special deconvoluting software was used to separate overlapping elemental signals (Oxford Inca 300). The analysis was performed on uncontrasted ultrathin sections at carbon film-coated 400-mesh gold or copper grids (Plano, Germany).

Some attempts at elemental analysis were also made by time-of-flight secondary ion mass spectrometry (TOF-SIMS-IV) in Münster, Germany at the Department of Planetology (Kerstin Klemm) with a machine of ION-TOF GmbH/Cameca, Münster, Germany. For this method cross and longitudinal slips of one hair were used after it was embedded in epoxy resin and mounted on glass slides. This method was able to detect Mg, Al, Si, K, and Ca, but unable to demonstrate Hg. Therefore, we do not present these results here.

RESULTS

Figure 1 shows the over 400-year-old beard hairs of Tycho Brahe with residues of the skin. Furthermore, there is some visible pollution or coatings on the surface of hairs. In TEM, the hairs showed an amazingly good status of preservation of their ultrastructure (Figures 2, 3). Cross sections of the hairs showed the typical structure of native hairs (Figure 2). There was no destruction of the fine structure during the 410 years. Four or five sheets of scales were oriented around the hair axis. In the hair shaft there were detectable pigment granules with the typical bulb-like structure of red hairs, so-called melanosome stages II and III (Figure 3). Mature melanosomes at stage IV, as typical for black and deep brownish hairs, were not detectable in Tycho's hairs [9–11].

The hair scales were limited by typical membranes, as is visible at higher magnifications (Figures 4, 5). Inside the homogenous content of scales, we detected electron-dense globules of about 10-nm size. In uncontrasted sections, these granules were also visible (Figure 4), which means that they consist of heavy elements that do not originate from contrasting by uranyl acetate and lead citrate. Much better ultrastructure was seen in ultrathin sections in TEM after contrasting with uranyl acetate and lead citrate (Figure 5). These granules were numerous in the outer layers and decreased to the more inner sheets (Figures 3–5, 7, 8). Within one scale the globules were mostly concentrated near the inner plasma membrane. The inner sheet of scales at the hair shaft was almost free of electron-dense globules.

In SEM the hairs showed the well-known structure at the surface with typical hair scales, but with partial amorphous depositions (Figure 6). Figure 6a shows the hair in secondary electron mode (SE), and Figure 6b shows the same hair in the backscattered electron mode (BSE). These amorphous coatings appeared in EDX signals of silica and carbon, but not in those of mercury and other heavy metals. Cross sections of the

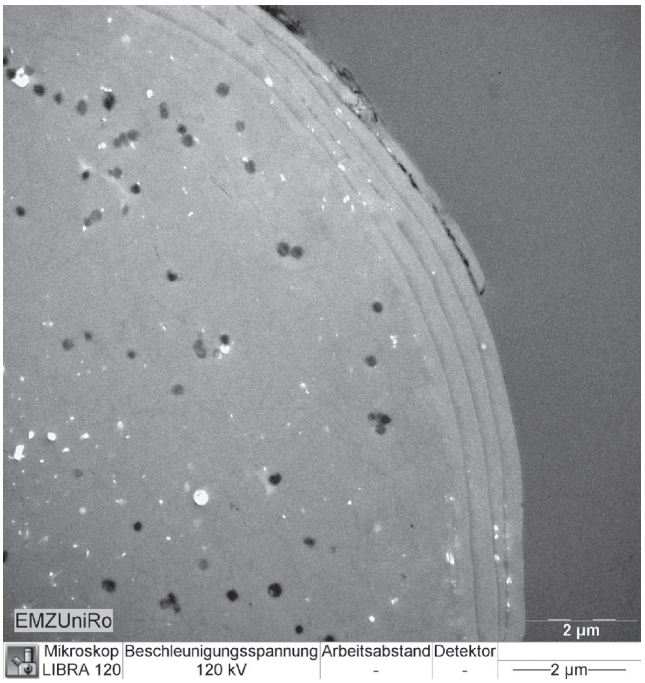

FIGURE 2. Cross section of hair in TEM with 6 typical hair scales and a hair shaft (axis) with elongated pigment bodies (melanosomes).

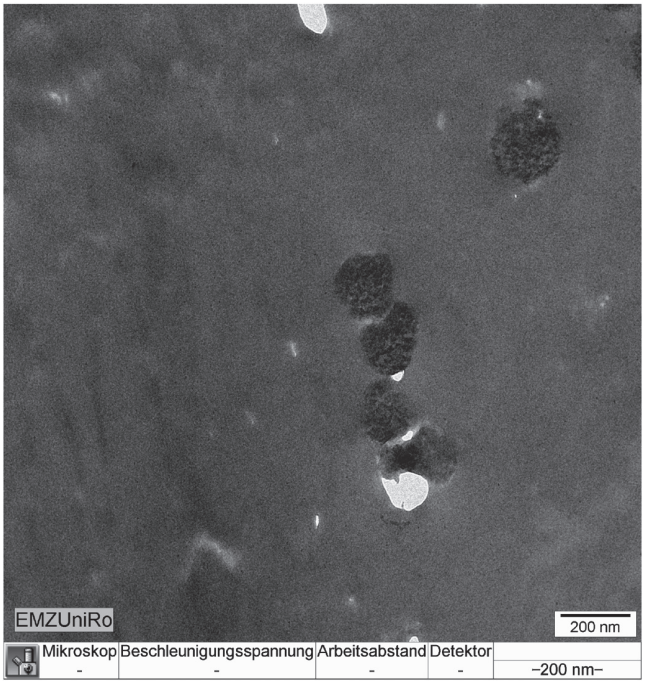

FIGURE 3. Stronger magnification of melanosomes with typical onion-like sheets of pigment.

hairs with a razor blade (not presented here) showed also the typical structure of native hair, with 4–6 sheets of hair scales around the hair shaft.

In STEM and BSE mode we studied unstained cross and longitudinal sections in the FT-SEM Gemini Ultra (Carl Zeiss). Figures 7–9 show the cross section. The ultrastructure and resolution are similar to that in TEM.

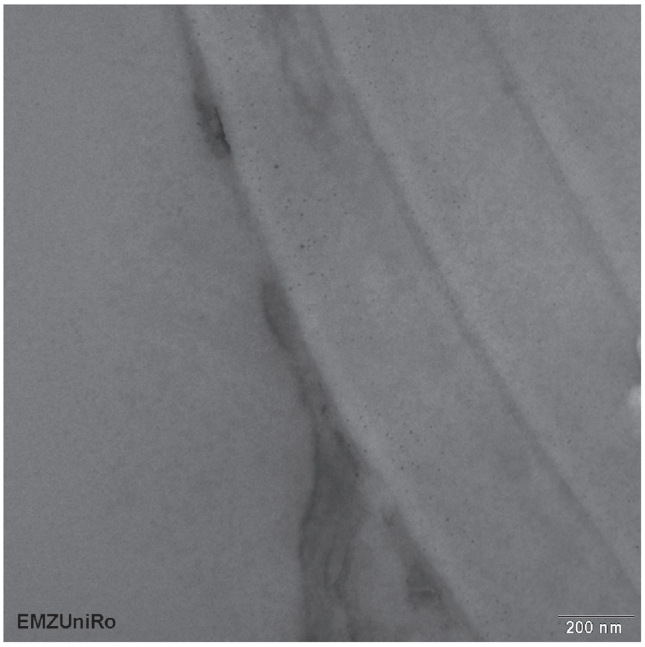

FIGURE 4. Ultrathin section of hair scales in TEM without contrast. Electron-dense globules in the outer three scales and a pollution coating of the hair surface.

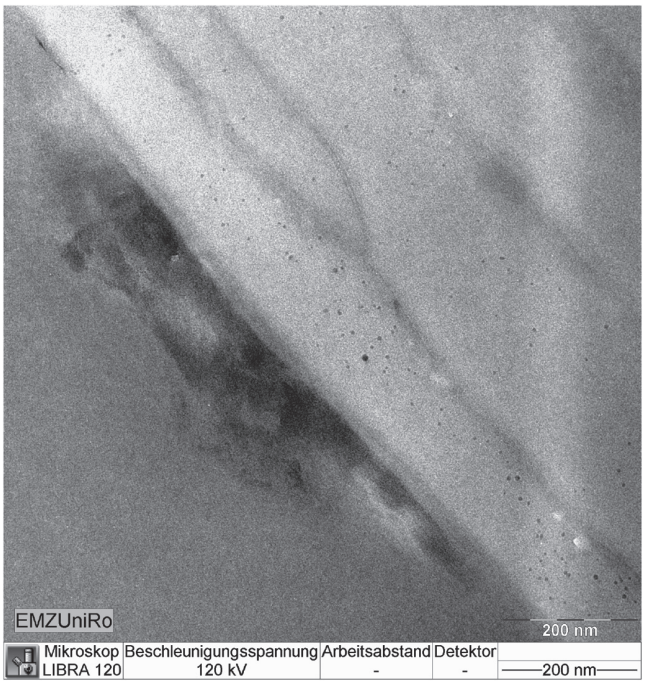

FIGURE 5. Ultrathin section in TEM after contrast. Electron-dense granules in the outer two scales. Note the well-preserved membrane structures even after more than 400 years.

Figure 7 shows 6 sheets of hair scales with light granules in the outer 2 scales and elongated pigment granules (melanosomes) in the hair shaft. At higher magnification (Figure 8), a layer of pollution at the surface of hair is visible. The outer scale contains numerous electron-dense granules, which were demonstrated in BSE mode as light globules. In Figure 9 two fields were labeled for following the measurement of elemental composition

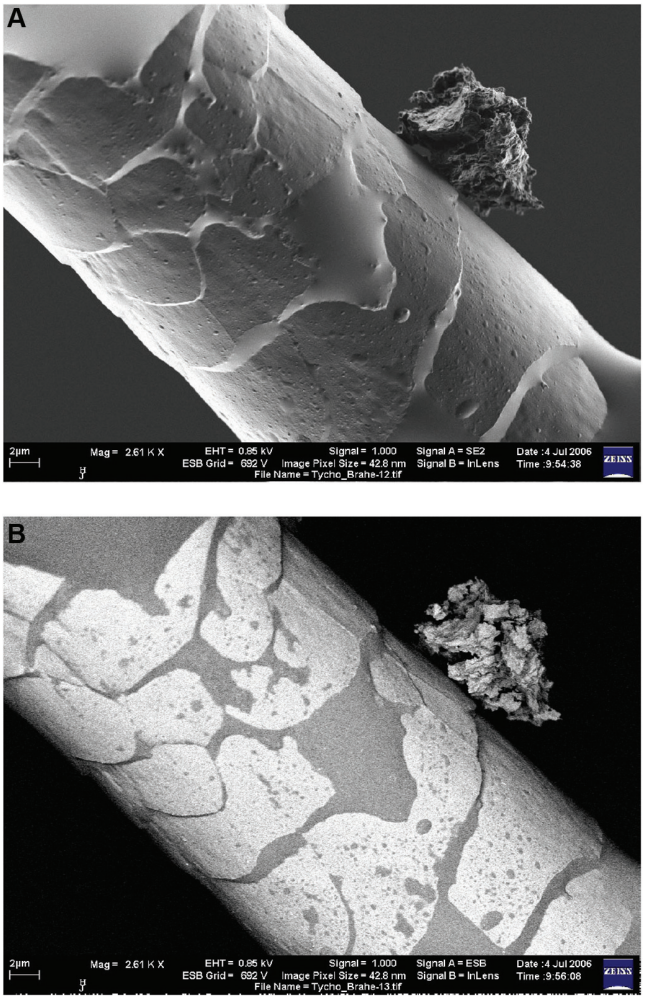

FIGURE 6. (a) FI-SEM: hair in SE mode; hair scales with some amorphous coating as well as a pollution granule at the surface. (b) The same hair in BSE mode.

by EDX. Area 1 contains the coating material at the surface of the hair, whereas area 2 contains the material of the first scale with the electron-dense globules.

Figure 10 demonstrates the EDX spectrum of the area 1. It contains no signals for mercury (Hg), whereas Figure 11 shows the spectrum of area 2, containing the numerous light granules within the outer scale. There were specific signals for Hg besides those for Al, S, Ti, Fe, Ca, Si, and V.

In a hair root, some light inclusions were visible (Figure 12, green arrows), but the areas of these materials were free of mercury, as seen in the EDX spectrum in Figure 13. X-ray microanalysis in the root detected specific signals for Al, Fe, Ca, Pb, and Bi, but none for Hg.

DISCUSSION

The hairs of Tycho Brahe have been amazingly well preserved during the 300 years the body was entombed under the floor of the Teyn [Týn] church in a moist atmosphere. The skeletons of both Tycho Brahe and

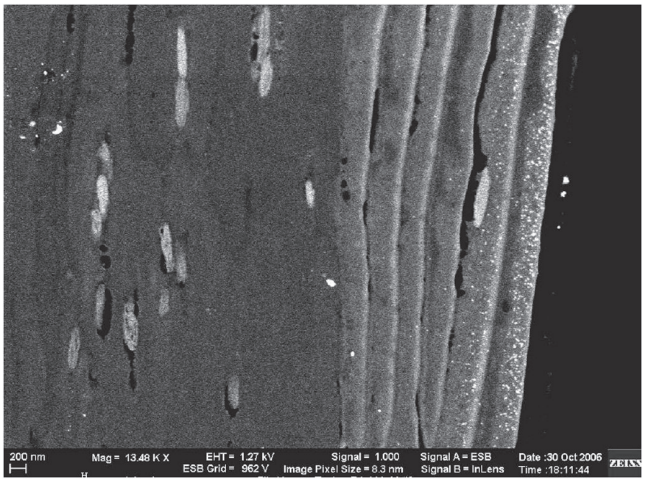

FIGURE 7. FI-STEM: uncontrasted ultrathin cross section of the hair in low-voltage BSE mode (1.27 kV). Six sheets of hair scales are evident. In the outer two scales the typical highly contrasted heavy elements (light granules) are detectable as well as elongated pigment granules in the hair shaft (axis). Note the different densities from proteins: keratin A, B, C, melanin, shaft proteins.

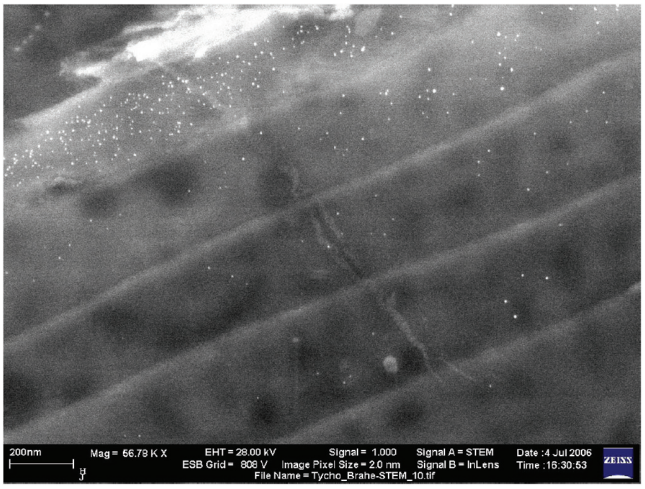

FIGURE 8. FI-STEM: uncontrasted section in BSE mode. Light granules in the outer scales with a pollution layer on the surface of hair.

his wife, who died in 1604, were well preserved. More spectacular is the preservation of mercury inside the hairs. The wreath of hairs around the skull, the hairs of both eyebrows, and Brahe's typical red moustache were preserved. It could be that a high concentration of mercury was the reason the hairs were not destroyed by bacteria (rottenness).

When Kæmpe detected mercury in Tycho Brahe's beard after solving the beard pieces by a strong acid, the AAS method did not enable him to determine whether the measured mercury was placed along the whole length of hairs. Kæmpe was nevertheless the first to be convinced that a murder had been committed. He suspected Kepler's wife who was in very bad terms with her husband's master. [new line] In 1996, Jan Pallon in Lund detected mercury inside the hairs of Brahe with the

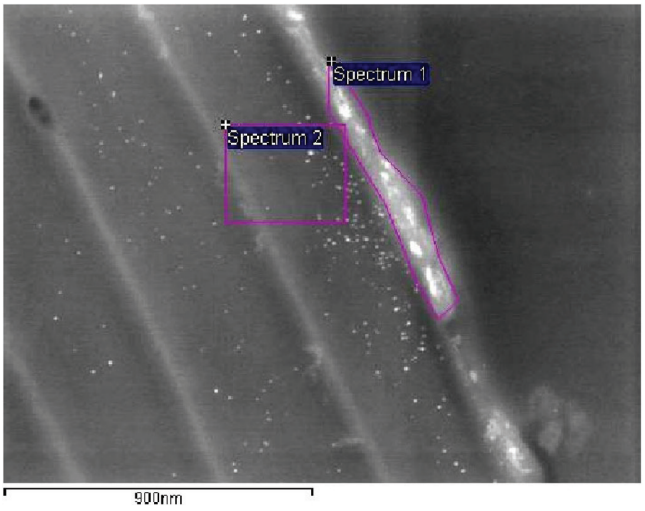

FIGURE 9. FI-STEM: uncontrasted section in BSE mode. Two labeled fields for measurements with EDX. Spectra 1 and 2 are shown in Figures 10 and 11.

proton-induced X-ray emission method PIXE [6, see also 7]. He described mercury only in the roots of hairs, but not in the higher parts. Therefore, his results supported the hypothesis of the murder of Tycho Brahe by Johannes Kepler. That would mean that mercury was incorporated into the hairs inside the roots from the blood. The origin of mercury could therefore have been a poisoned drink.

Our data support the results of positive detection of mercury of Pallon [6,7] and of Kæmpe [4,5], but with the structure-related detection of elements by STEM, BSE, and EDX we found the poison only inside the hair scales and not in the hair roots or hair axis. Therefore, the origin of mercury seems to be that of a long-term accumulation of mercury vapor at the surface of hairs by alchemistic activities. For example, Tycho Brahe described procedures to clean mercury and to detoxicate the poison by several chemical reactions in 1597 [12]. He gave his elixir to friends against diseases affecting skin and blood, such as scabies and chronic venereal infections. Syphilis was a widespread disease during the late Middle Ages and the Renaissance. Tycho Brahe believed that his elixir containing mercury oxide and mercury sulfate was no longer dangerous. Nevertheless, the exposure to mercury inside his laboratory without any exhaust mechanism could have led to a chronic intoxication of him within weeks. Additionally, the uptake of alcohol at the banquet before his death could have liberated a critical concentration of mercury and altered its pharmacokinetics.

We can exclude the origin of mercury in the hairs by a mercury-containing embalming of Brahe's body because of the mercury-free amorphous coatings at the surface of the hairs.

There are different possibilities to detect mercury in biomedical samples by chemical and physical methods. AAS and ICP-MS are more sensitive than EDX and PIXE, but the first two methods are

Analysis type B

Element	Weight%	Atomic%
S K	29.07	44.94
Cl K	3.86	5.40
Fe K	2.22	1.97
Cu K	59.41	46.35
Hg L	4.88	1.21
Pb M	0.57	0.14
Totals	100.00	

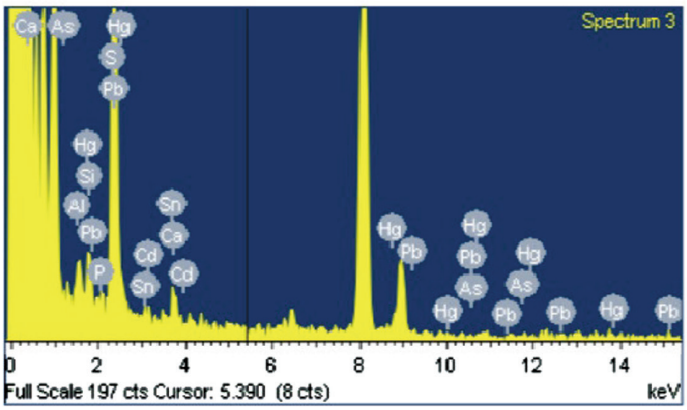

FIGURE 10. EDX spectrum of area 1 (coating at the surface of hear) without signals for Hg.

Tycho Brahe section 120nm XRF

Analysis type A

Element	Weight%	Atomic%
Al K	16.97	35.69
S K	1.70	3.01
Ti K	14.80	17.54
V K	0.38	0.43
Fe K	0.75	0.77
Cu K	39.87	35.62
Hg L	0.00	0.00
Pb L	7.86	2.15
Bi L	17.67	4.80
Totals	100.00	

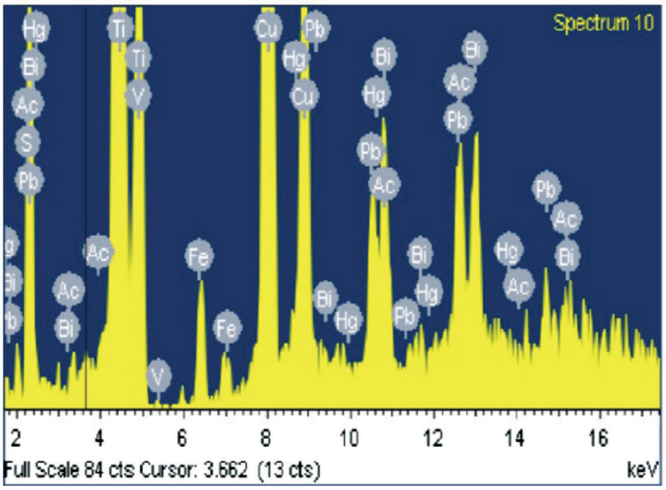

FIGURE 11. EDX spectrum of area 2 (first outer scale with numerous light, electron-dense granules). Positive detection of mercury (Hg) besides signals for Al, S, Ti, Fe, Ca, Si.

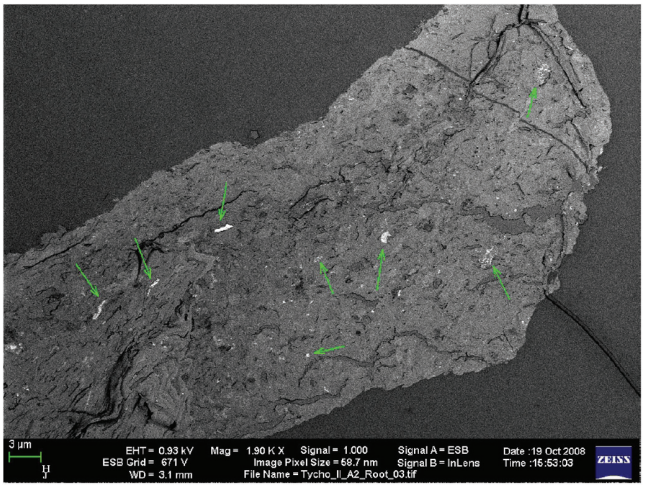

FIGURE 12. FI-STEM: hair root in BSE mode. Some light inclusions (green arrows), but not from mercury, as demonstrated in the corresponding EDX spectrum in Figure 13.

destructive, so the analysis cannot be repeated with the same volume at several places and we get only a mean value of concentration of the estimated element or substance. Many biomedical samples are very heterogeneous, such as bile and urinary stones or hair. In such cases, the structure-related analysis by EDX, EELS, and PIXE gives additional information that is helpful for interpretation of results. The EDX and PIXE methods were sensitive enough to detect the mercury inside the hairs of Tycho Brahe. The TOP-SIMS method was not able to detect mercury in the same samples of hairs (Klemm, unpublished data). A combination of AAS/ICP-MS and X-ray microanalysis would be optimal. In preceding papers we published different examples for the validity of electron microscopy in combination with elemental analysis by EDX or EELS [13–20].

The general problem for the analysis was to minimize artefacts from preparation, such as staining or coating. For all analyzed samples, we did not use any staining or coating. It would have been easier to analyze under low vacuum conditions, but the fine structural imaging did not allow this. Also, the absorption from the gas would have absorbed the extremely weak and low signals from mercury. To detect fine structures in the surface of low-density materials like proteins, low-voltage conditions are mandatory. On the other hand, the analysis of elements

Analysis type A
Tycho Brahe section 120 nm XRF

Element	Weight%	Atomic%
Al K	11.21	26.48
Fe K	2.10	2.39
Cu K	63.94	64.16
Pb L	9.03	2.78
Bi L	13.73	4.19
Totals	100.00	

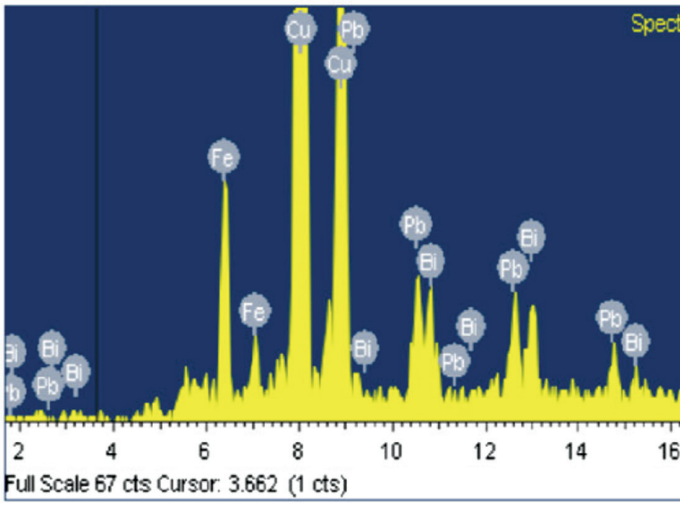

FIGURE 13. EDX spectrum of the hair root with the inclusions, as seen in Figure 12. No signals for Hg, but detection of Al, Fe, Ca, Pb, Bi.

like Cd, Pb, Hg, Sb, and Bi require higher voltages (5–7 kV at minimum for L–M shells), to ionize the interesting elemental K–L–M shells. To have significant separation and easier deconvolution conditions from coincidence of lines (e.g., PbL and sulfur K), high landing energies were required. Also the thin sections (~120 nm thickness) required at least 20 kV, until they showed good contrast and the fine 5- to 10-nm metal inclusions (Figures 5, 7, 8). For high-sensitivity survey of the samples, XRF radiation was used (analysis type A), to have very low white noise (bremsstrahlung) background and so good detection efficiency for low (trace) elemental concentration. With this analysis method, remarkably high concentrations of Pb, Bi, Ti, vanadium, and aluminium were detected. The presence of copper (Cu) in the analysis comes from the supporting Cu grid, where the samples were attached.

Mercury was not detected with the XRF technique, where an area of about 2 μm^2 was ionized for analysis. To find the mercury, a finer analysis technique was necessary. On the thin section, EDX analysis was used. Due to the fact that we had only relative thin samples of about 120 nm thickness, the ionization volume was reduced down to 20–30 nm. This volume was still too big to analyze the individual particles, so we chose areas (Figure 9, spectra 1 and 2) to get better detection efficiency and higher count rates. The sample thickness, one of the limiting factors responsible for the size of the ionization volume, had to be kept relative high (120 nm) to stabilize the sample while analyzing with the relative high electron current density at the analysis area. With 30 kV of landing energy, most of the electron dose (>90%) is transferred into heat and absorbed in the sample, which finally damages the sample at the analyzed area. Due to the fact, that mercury could probably be in the methylated form (CH_3–Hg) in the outer keratin layers, the analysis had to be very fast, to minimize the vaporization of mercury. Mercury shows very high affinity to sulfur, and so HgS (α–HgS, which is red!) could also be a possible elemental binding form in the hair, due to the fact that in the scales of the hair normally a high sulfur content was detectable.

The results in the EDX analysis revealed sometimes very high elemental concentrations, but these results are not absolute concentrations. They are just relative values, because the mathematical quantification routines of the analysis method assume homogeneous distributions in a homogeneous *thick* matrix. Neither condition is given in our analyzed samples. First of all, the elements were not homogeneously distributed and, second, the sample is relative thin. Nevertheless, we get an idea of the presence of some elements (Hg, Bi, Pb), because some spectral lines of elements are "hidden" below other spectral lines from other elements. So mathematical quantitative deconvolution routines had to be used for their visualization.

In Figures 12 and 13 we can see a completely different situation of the elemental distribution and their localization. High amounts of mercury should be expected in the root (and only there) to validate the murder hypothesis. However, we found only aluminium, titanium, silicon, and calcium at these locations. None of these elements indicates a criminal background. In contrast, we see in Figures 10 and 11 a totally different distribution of the elements mercury, lead, and sometimes bismuth. These elements (fine bright dots) are found in the outer scale regions and their concentration reduces toward the inner shaft of the hair. This indicates an application from *outside*!

The concentration of the analyzed elements in the scales of the hairs is amazing, but we have to consider the scales as very permeable layers. Diffusion from outside into the highly permeable scales has to be considered as a transportation mechanism. Even the "relative" amount of elemental concentration in the scales suggests a high exposure dose from *outside* (environment) and not from *inside* (incorporation). Since mercury was below the detection limit in the shaft protein and in the roots of the hair—incorporated mercury would be visible also in the shaft and especially in the roots—we assume that mercury was not the cause of the sudden death of Tycho Brahe.

So we have to relieve Johannes Kepler from the murder of his teacher Tycho Brahe.

SUMMARY

The more than 400-year-old beard hairs of Tycho Brahe were amazingly well preserved. Three to six layers of hair scales protected the hair shaft, where typical melanosomes for red hairs were visible in the TEM and FI-SEM BSA modes. In the outer hair scales numerous electron-dense globules were detected of about 10 nm in diameter. These globules were not detectable in the hair shaft and roots. At the areas of small electron-dense globules, we were able to detect mercury beside other elements. The roots and shafts of hairs were free of mercury. Therefore, the origin of mercury accumulation seems to be of the environment, perhaps from the air, and not originating from an intoxication attack via the blood after ingestion of a mercury-contaminated drink.

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