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Developing a Quantitative Method to Assess the Decomposition of Embalmed Human Cadavers

Short Communication

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Abstract

Embalmed human cadavers are an essential educational tool in forensic science and medicine. Cadavers are often embalmed to extend the period they can be used. Qualitative observations such as odours, tissue texture and colour are the only methods currently used by anatomists to assess the decomposition progress of embalmed cadavers. The aim of this study was to provide a first proof-of-concept to determine whether methylamine, putrescine, and cadaverine could be detected and monitored over time from embalmed human tissues. The hypothesis was that these three compounds would exhibit temporal trends to quantitate progress of decomposition in embalmed cadavers. Two human cadavers were embalmed using McGown solution and liver samples were analysed over 35 days. Liver samples were extracted, homogenised and derivatised to quantify the presence of methylamine, cadaverine and putrescine by gas chromatography - mass spectrometry. All three amines were detected in the tissue samples throughout the duration of the study. Both cadavers had elevated methylamine levels over putrescine and cadaverine at early stages postmortem. This was followed by peaking and reducing in different patterns by the two cadavers; however, the three compounds from a single cadaver changed in a similar pattern. The proposed experimental procedure provides a foundation for further development of quantitative biogenic amine methods to determine decomposition progress in embalmed human cadavers.

Keywords: Forensic Chemistry; Forensic Taphonomy; Anatomy; Methylamine; Cadaverine; Putrescine

1. Introduction

The use of cadavers to study human anatomy can be traced back to Greek and Egyptian civilizations [1, 2]. From a scientific approach, dissections of a human cadaver performed by the Greek physician, Herophilus of Chalcedon were the first ever recorded public dissections in history [1]. This was followed by Andreas Vesalius who was described as the father of anatomy as he was the first to describe the human body based on the dissection of the human cadaver [3]. Dissection is conducted for teaching and research purposes in most anatomy departments worldwide. Moreover, cadavers are also used to train clinicians on different clinical interventions, such as ultrasound guided anaesthesia and surgical procedures [4, 5]. In addition to their use by clinicians, cadavers are used by medical companies to develop new technologies that improve the practice of medicine. The use of human cadavers in anatomy departments across Ireland and the United Kingdom is governed by the Anatomy Act of 1832 in Ireland and the Human Tissue Act in the United Kingdom 2004 [6, 7].

While fresh (i.e. unembalmed) cadavers are ideal as they are the closest representation of live tissue, the handling of unembalmed cadavers is a potential biological hazard as the tissue has not been treated with preservatives or antimicrobial agents [8]. One of the most commonly used embalming chemicals is formaldehyde, yet studies report that its use leads to a rigid, malodorous and discoloured cadaver [9, 10]. Moreover, formaldehyde was recently classified as a Category 1b carcinogen and is currently under review for potential banning within the European Union [11, 12]. For these reasons, researchers have started to develop new chemical solutions that produce a cadaver that more closely resembles live tissue such as Thiel, Genelyn and Imperial College London- Soft Preserving solution [13-15]. Embalming within academic institutions is distinct because it is done with the goal of preserving the body for a long period of time, while embalming for the funeral service is intended for a shorter period of time [8].

Several criteria are considered by anatomists when identifying a new chemical solution such as the quality of the tissue post embalming, its antimicrobial abilities, and the period over which the solution can preserve the cadaver [8]. While there have been a few attempts to compare the efficacy of different embalming solutions, no standard quantitative method has been established. In order to

quantify the progress of decomposition, anatomists refer only to qualitative factors such as odour, tissue texture and colour [16-18].

Decomposition of the human body commences almost immediately after death [19]. At early stages, autolysis causes the digestion of cell membranes in soft tissue, which releases many enzymes that begin to break down macromolecules [20]. This leads to the secretion of degraded carbohydrates, proteins and fat into the surrounding tissue. Aerobic bacteria from the intestines and respiratory tract then invade body tissues as they exploit the nutrient-rich media. These putrefactive processes start approximately 48-72 hours after death. The breakdown products of proteins have been studied in several contexts related to the taphonomic processes of human remains. When putrefaction occurs, proteins become decarboxylated, leading to the production of many biogenic amines. Methylamine, putrescine and cadaverine are three biogenic amines that have been previously investigated from human tissues [21]. The decarboxylation of the amino acids lysine and ornithine leads to the formation of cadaverine and putrescine respectively [22-24]. These two biogenic amines were also studied to detect decomposition in food such as mutton, cheese, shrimp and wines [25-28]. Moreover, cadaverine and putrescine were also detected in low levels in the living human oral cavity and were related to oral malodour [29, 30]. Methylamine is a derivative of ammonia with one nitrogen atom being replaced with a methyl group [31].

Several studies have reported the importance of putrescine and cadaverine as tissue decomposition biomarkers [21, 32, 33], but because of their low volatility they are not frequently reported in literature. In order to overcome this property, several studies have chemically derivatised these diamines to be detected by analytical instruments such as gas chromatography – mass spectrometry (GC-MS) [19]. Subsequently, further research [34] has refined the method developed by Ngim et al. (2000) to use pentafluorobenzaldehyde (PFB) to derivatise a number of biogenic amines, including cadaverine and putrescine [24].

Many studies have looked at decomposition biomarkers in soil, liquids and the air around human cadavers [35-37]. However, the study of methylamine, cadaverine and putrescine as chemical

markers in human tissues has received little attention until recently. A study by Pelletti et al. performed the first validation and application on putrescine and cadaverine as postmortem interval (PMI) estimation biomarkers from unembalmed brain tissue [38].

The aim of the current study was to perform an initial proof-of-concept on liver samples from two human cadavers to demonstrate the feasibility of using biogenic amines to estimate PMI from embalmed cadavers. In order to justify further study, two points needed to be demonstrated. First, a suitable method for detection and quantification of these three compounds was required. Second, the ability to monitor trends over time had to be established. The demonstration of these two criteria would provide justification for commitment of cadaveric resources for additional studies that would allow the establishment of mathematical relationships between biogenic amines and PMI in embalmed cadavers. In addition, such information would assist in establishing trends in embalmed tissues to further support current research on unembalmed tissues for comparison purposes. Further development of this work in the long-term could have the potential to perform quantification of biogenic amines as PMI biomarkers from human liver samples as an alternative to brain tissues that was previously presented by other studies [38]. Moreover, this work will help embalmers within anatomy departments to determine whether a donated human body is far too decomposed to warrant embalming.

2. Materials and Methods

2.1. Ethical Considerations

The study was carried out under the auspices of the ‘Licence to Practise Anatomy’ in University College Cork (UCC), granted by the Irish Medical Council to Prof. John Cryan under the Anatomy Act of 1832. The study was also approved by the Ethics Committee of the Faculty of Computing, Engineering and Sciences at Staffordshire University (SU) and ethical approval was granted by the Faculty Research Ethics Committee at SU as per guidelines of the Human Tissue Act 2004 in the United Kingdom.

2.2. Donor Information

For the purpose of this proof-of-concept study, two human cadavers were embalmed using a local funeral industry solution (McGown). Donors premorbidly signed written consent to use their bodies for education and research by the Department of Anatomy and Neuroscience at UCC. All cadavers were admitted into the department 24–48 h after death and stored at -20°C . The freezing process was conducted for logistical purposes to prepare and follow the same embalming protocol, and to maintain a similar timeline for both donors. Cadavers were thawed for 5 days after which embalming was completed on the same day. Chemical were injected into the body through the femoral artery following similar protocols to that of regular formalin-phenol based embalming within anatomy departments. Table 1 includes information on the two the donors used in the study.

Table 1. Information on donors 1 and 2 used as part of this study.

Donor	Gender	Race	Age	Cause of Death	Date of Death	Date of Admission	Date of Embalming
1	M	Caucasian	82	liver cancer	20/09/2013	22/09/2013	28/08/2014
2	F	Caucasian	80	Breast cancer, secondary in liver	14/03/2014	16/03/2014	28/08/2014

2.3. Extraction of Liver Tissue

After four days of embalming where femoral infusion was used to introduce the solution into the body, both cadavers developed signs of decomposition (odour and dark patches covering the body). This could be due to the fact that the McGown solution is primarily used in the funeral industry which follows a different embalming protocol to that used in embalming protocols within anatomy departments. The presence of microorganisms within the abdominal cavity along with the high enzyme content within the liver indicates that it could be the quickest to decompose [19]. Due to the size of the liver as a relatively homogenous organ within the abdomen, the ease of access to it and to avoid the hazard of allowing a full body to decompose in the morgue, a decision was made to only study the decomposition of the liver in a controlled environment. For this reason, an incision was performed along the right lower costal margin. Equal parts of the right anatomical lobe of the liver were removed and stored in a sealed container. No macroscopic differences could be observed between the two samples. Livers from both cadavers were stored at 8 °C and samples were taken on days 7, 10, 14, 17, 21, 28 and 35 post-embalming.

Human liver samples (5 ± 0.1 g) were sliced using the McILwain tissue chopper (Stoelting Europe, Dublin, Ireland) and homogenised using a tissue homogeniser (Polytron Kinematica homogenizer PT2100, Kinematica, Lucerne, Switzerland) in 15 mL of distilled water for 3 min and then immersed in an ultrasonic bath for 10 min. The solution was centrifuged at 2500 rpm at 24 °C for 3 min. The supernatant was transferred to a 1.5 mL Eppendorf tube and centrifuged at 10000 g at 4 °C for 10 min. The remaining solution was filtered using 20 µm, non-pyrogenic, sterile filters (Sartorius, Dublin, Ireland) and transferred into 5 mL screw-cap glass vials. A sample of 10 µL was mounted on a microscope slide and examined under a microscope at 40x magnification to ensure the absence of cellular debris. This process was conducted at University College Cork and samples were transferred on frozen ice packs in a Styrofoam box to Staffordshire University for chemical analysis.

2.4. Chemical Derivatisation of Liver Tissue

Standard solutions of putrescine, cadaverine and methylamine were prepared at 0.1 M in distilled water and then diluted to a 1 mM combined amine solution that was used as positive control

for the derivatisation of the liver samples. The method used to derivatise the samples was based on Ngim et al., 2000 and Blom et al., 2012 [24, 34]. These samples were derivatised by pipetting 1 mL of each liver sample into separate clean 4 mL vials; the pH of the solutions was adjusted to pH 11 using 1.0 M sodium hydroxide, 0.1 M sodium hydroxide and 0.1 M hydrochloric acid (Fisher Scientific, Leicestershire, U.K.) as appropriate. Next, 0.5 mL of 10 mg/mL pentafluorobenzaldehyde in acetonitrile (>99.8%, Fisher Scientific, UK) was added to the vials. Aluminium foil and plastic caps were used to seal the tops and the vials were shaken gently before placing them into an oven to incubate for 1 h at 60 °C. After incubation, the vials were placed in an ice bath for 1-2 min, then 1 mL of a 0.5% undecane in hexane, 100 mg sodium sulphate and 1 mL 0.1 M sodium hydroxide were added. The vials were resealed, shaken using a vortex for 10-15 s and the top layer was pipetted into 2.0 mL autosampler vials. This process was performed in duplicate to test the reproducibility of the derivatisation process. The derivatised samples were analysed using a Clarus 500 GC-MS (Perkin Elmer, Waltham, MA, USA). The column used was an SLB-5MS (Sigma-Aldrich Company Ltd., Gillingham, U.K.) with a length of 30 m, inner diameter of 0.32 mm, and film thickness of 0.25 µm. The injector temperature was 250 °C. Helium was used as the carrier gas (Fisher Scientific, Leicestershire, U.K.) with a constant flow of 1.5 mL/min. The oven temperature started at 45 °C and held for 4 min, was increased to 280 °C at 15 °C/min, and held at 280 °C for 15 min. The MS transfer line was held at 300 °C and the ion source temperature was held at 200 °C. The MS was operated in electron ionization mode with a mass range of 30-500 *m/z*. Identification of the biogenic amines was conducted using *m/z* fragments 117, 208 and 209 (methylamine), 181, 222 and 263 (cadaverine) and 181, 208 and 263 (putrescine).

2.5. Calculation of Concentrations

Quantification was performed using a six-point external calibration, ranging from 1 to 500 µM. The slope and intercept were calculated using the least square method and the limit of quantification (LOQ) was determined to be the lowest calibration standard using the following formula: $LOQ=10s_a/b$. Where ' s_a ' is the standard deviation of the intercept and ' b ' is the slope of the calibration curve [39].

All samples were primarily stored in 2 vials (a and b) except for samples 17, 21, 28 and 35 from donor 1 and samples 21 and 28 from donor 2. After storing the samples at 4 °C, unidentified microorganisms were observed on samples 7a, 10b and 17b of donor 2.

3. Results

Relative standard deviation (RSD) was calculated to assess the accuracy of the derivatisation process where samples volumes permitted two aliquots to be taken from the same vial. To assess the effect of storage on the samples, the RSD was calculated for the combined results from vials a and b. The RSD values for the derivatisation process were generally low, with higher RSD values where concentrations were near the limits of quantification as shown in Table 2. The exception was the RSD value for putrescine in sample 10b. With regard to storage, higher RSD were observed on days 7, 10 and 17 where microorganisms were noted as present in one of vials in each set. For days 14 and 35 where no growth was observed, RSD values for storage were below 10%.

Table 2. Relative standard deviation (RSD) of the derivatisation process and the effect of storage for donor 2.

Donor 2	Putrescine RSD		Methylamine RSD		Cadaverine RSD	
Day	Derivatisation	Storage	Derivatisation	Storage	Derivatisation	Storage
7	a- 8%	24%	a- 19%	13%	a- 36% *	153%
	b- N/A		b- N/A		b- N/A	
10	a- 17%	80%	a- 8%	10%	a- 7%	12%
	b- 22%		b- 15%		b-10%	
14	a- 3%	4%	a- 2%	9%	a- <1%	8%
	b- 6%		b- 1%		b- 6%	
17	a- 3%	35%	a- 3%	22%	a- 6%	96%
	b- 9%		b- 7%		b- 16%	
21	N/A	N/A	a-11%	N/A	N/A	N/A
28	b-22% *	N/A	b-11%	N/A	b-27% *	N/A
35	a- 4%	5%	a- 2%	5%	a- 14%	9%
	b- 5%		b- 2%		b- 4%	

* denotes the concentration was near the limit of quantification. A & B are the two different vials used.

3.1. Donor 1

For Donor 1, no cadaverine or putrescine was detected initially at day seven whilst a concentration of 107.65 ng/g of methylamine was detected on day seven. Figure 1 represents the average concentration (ng/g) of methylamine, putrescine and cadaverine in donor 1 of vials a and b, on days 7, 10, 14, 17, 21, 28 and 35. The concentration of methylamine slightly increased on days 10 and 14, it then decreased on day 17 and stayed the same on day 21. A spike in the concentration was detected on day 28 followed by a sharp drop from 326.5 ng/g to 84.17 ng/g. Both putrescine and cadaverine showed a spike in their concentrations from day 7 to day 10. The concentration of putrescine remained similar then spiked on day 28 followed by a sharp decrease on day 35. Meanwhile, cadaverine gradually decreased up to day 21 followed by a sharper decrease on 28 and slight increase on day 35.

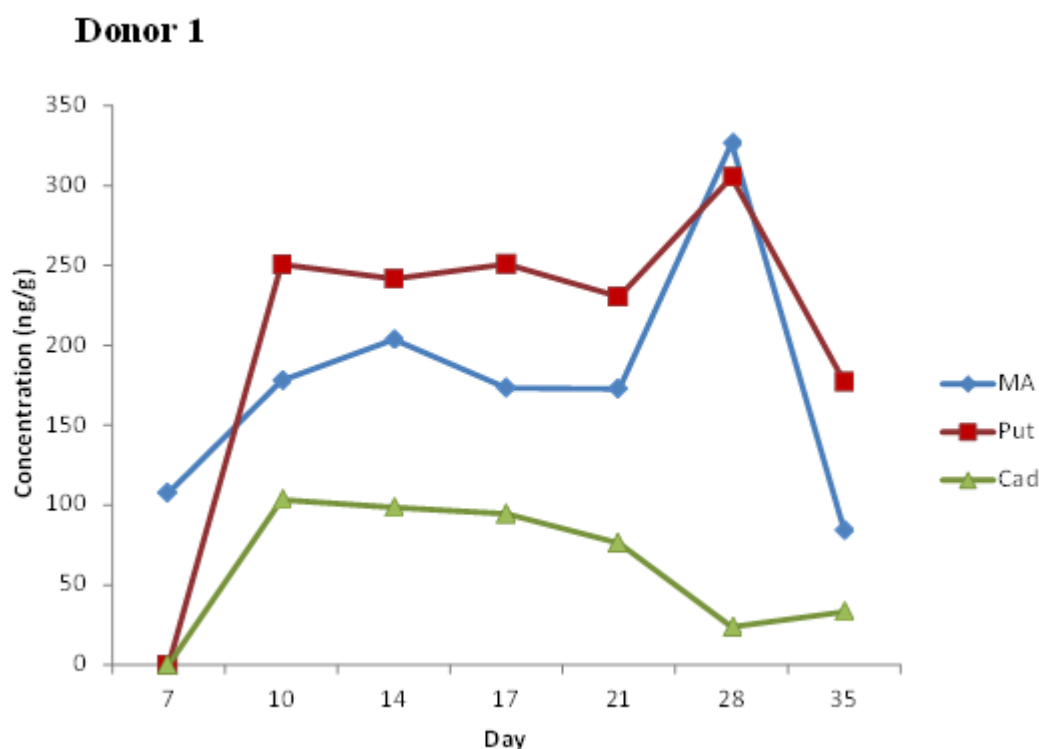


Figure 1. The average concentration (ng/g) of methylamine, putrescine and cadaverine in donor 1 from both vials a and b on days 7, 10, 14, 17, 21, 28 and 35.

3.2. Donor 2

For donor 2, in contrast to what was observed in donor 1, all three biogenic amines were initially detected on day 7. Figure 2 represents the average concentration (ng/g) of methylamine, putrescine and cadaverine in donor 1 of vials a and b, on days 7, 10, 14, 17, 21, 28 and 35. However, as with donor 1 the concentration of methylamine remained highest of the three compounds (349.07 ng/g) on day 7. Methylamine gradually increased on days 10, 14, and 17. A sharp decrease was observed from 884.27 ng/g on day 17 to 20.80 ng/g on day 21, followed by a gradual decrease on days 28 and 35.

Both cadaverine and putrescine gradually increased across days 7, 10 and 14 as noted in Figure 2. This was followed by a decrease on day 17 and concentrations below detection limits on day 21 for both amines. While cadaverine gradually increased on day 35, the abundance of putrescine spiked to 214.47 ng/g.

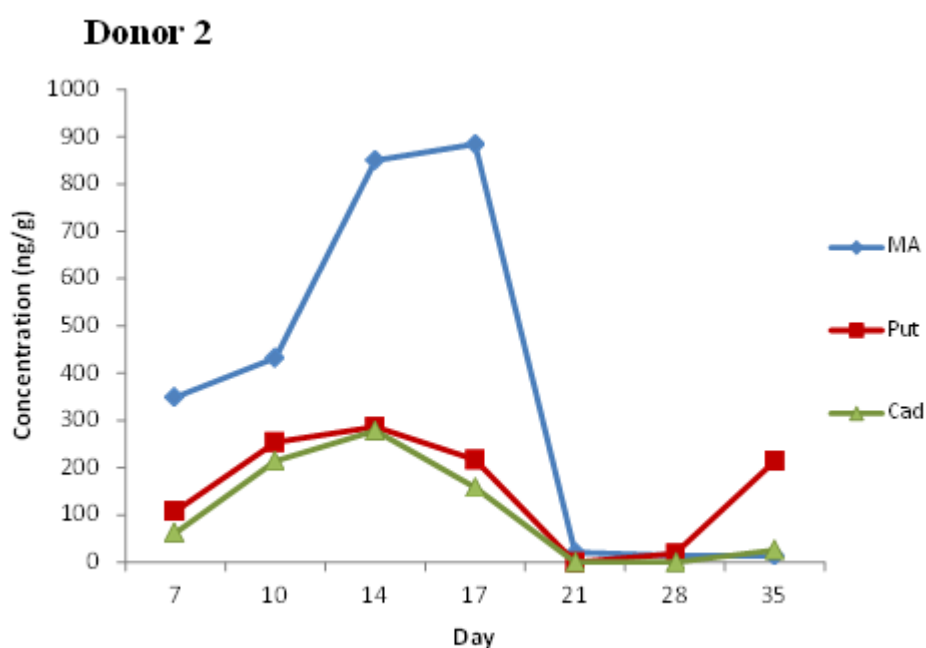


Figure 2. The average concentration (ng/g) of methylamine, putrescine and cadaverine in donor 2 of vials a and b on days 7, 10, 14, 17, 21, 28 and 35.

4. Discussion

4.1. *Process Experimentation and Effect of Storage*

Microorganisms have the ability to break down biogenic amines as reported in literature [21]; hence affecting their concentrations. This was observed by the growth of microorganisms in samples 7a, 10b and 17b. This growth appeared to result in a change in the concentration of methylamine, putrescine and cadaverine compared to the corresponding samples taken on the same day that did not have microorganism growth (vials a and b). This can also be seen in the different RSD values observed for cadaverine, putrescine and methylamine in table 2. These findings highlight the impact of microorganisms on the concentrations of methylamine, putrescine and cadaverine, and therefore providing a possible explanation to the changes in concentrations over the 35 studied days.

Most RSD values from duplicate samples showed percentages under 15% except for those samples where the concentration was close to the limit of quantification. This showed that the derivatisation process was reproducible and reliable to measure the quantity of cadaverine, putrescine and methylamine in the sample.

4.2. *Methylamine*

While methylamine was the most prominent compound at early stages of decomposition in both cadavers, cadaver 2 showed higher concentrations on day seven (349.07 ng/g) compared to cadaver 1 (107.67 ng/g). This could be related to the more prominent signs of decomposition that were observed on cadaver 2 five days after embalming, such as a strong putrid smell and skin discolouration. Different reasons could explain the faster decomposition in cadaver 2, such as the larger burden of disease from metastatic cancer where biogenic amines are highly secreted as described in literature [40]. Donor 1 died from isolated primary liver cancer, whereas donor 2 had breast cancer with distant metastasis indicating a more widespread systemic involvement of disease. Another reason could be an interaction with the embalming process and the individual's vascular pathology where chemicals did not perfuse into the tissue to prevent its decay.

While donor 1 showed a relative plateau concentrations of methylamine on days 10, 14, 17, and 21; donor 2 showed a gradual increase that peaked on day 17 matched by a peak on day 28 for donor 1. This supports the faster decomposition of donor 2 compared to donor 1, however, additional work should verify this trend. These peaks were followed by a sharp decrease in concentration on day 21 in cadaver 2 and day 35 in cadaver 1.

4.3. Cadaverine and Putrescine

The higher concentrations of both cadaverine and putrescine on day 7 in cadaver 2 support the previous results that decomposition occurred faster than in cadaver 1. This was followed by the highest peaks of cadaverine and putrescine at day 14 in cadaver 2, and days 28 and 10 in cadaver 1 for putrescine and cadaverine respectively. This was followed by a sharp decrease on day 21 for donor 2, and day 35 for putrescine in donor 1. Meanwhile, the gradual decrease in cadaverine and the highest peak being at day 10 does not follow the trend established for the amines in donor 1 or in comparison with that of donor 2. Methylamine and putrescine in cadaver 1 both peaked at day 28 followed by a sharp decrease in both amines.

4.4. Stages of Decomposition

The embalming solution used to preserve cadavers 1 and 2 is based on a funeral industry solution. While embalming in the funeral industry includes the injection of chemicals into the abdominal cavity along with femoral infusion, this is not practised in anatomy and therefore likely led to faster decomposition of both cadavers [41]. This solution was enhanced by the manufacturer by increasing its preservative capacity in order to be used for anatomical embalming which still was not strong enough to fix the tissue for a longer period of time as demonstrated in this study. The more rapid decomposition of cadaver 2 may be explained by the cause of death and medical history. Literature describes that decomposition starts by the autolysis of cells [20]. This leads to the production of biogenic amines, which reached its highest peak on day 28 for cadaver 1 (except for cadaverine) and approximately on day 14 for cadaver 2.

This peak was followed by a sharp decrease that could be explained by the start of putrefaction. This stage of decomposition is characterised by the destruction of soft tissue within the body by the action of microorganisms [19]. Microorganisms have the ability to consume the biogenic amines as building blocks for their multiplication, which could explain the sharp decrease after the peak. This could also explain the abrupt and exponential flourishing growth of microorganisms that covered the entire surface of the liver and could be observed by naked eye on day 28 of donor 2 liver. Indeed, the microbiome has been also used as a surrogate measure of decomposition as reported in literature [42]. It is hypothesised that this stage could be potentially followed by a period of microorganism death, causing the increase in the concentration of the biogenic amines putrescine and cadaverine on day 35 of donor 2.

The embalming solution utilised in this study (McGown solution) is typically used in the funeral industry and is administrated into the body using a different protocol to that practised within anatomy departments. This enables the use of the developed method to quantify the decomposition of cadavers embalmed within Anatomy Departments. With the increasing demand for the use of human cadavers in education and research within academic institutions, the need for a quantitative way to measure decomposition has become inevitable. This is due to the link between decomposition and the growth of microorganisms, which raise concerns associated with the health and safety of students, educators and researchers. Moreover, the developed protocol that was used in this study could be utilised within forensic investigations when studying a human cadaver that has been previously treated by chemicals for different reasons.

In order to validate the findings of this study, a larger number of cadavers must be investigated in future studies. This project focused on the demonstration that biogenic amines could be detected in embalmed cadaveric tissues, and that longitudinal trends develop over time. These points were supported by the data and justify the commitment of further resources to develop longitudinal trends that reflect different cadavers from a larger cohort study. While several factors could affect the decomposition of the human body, having access to the full medical background of the donors would assist in interpreting the experimental results and building models of biogenic amine production related

to status of decomposition. These details were not provided in this study as the Anatomy Act of 1832 does not allow anatomy departments any access to donor's medical history.

5. Conclusion

This study supports the effort of moving embalming towards a scientific discipline with quantitative research at its core. While anatomists currently use qualitative methods to assess the decomposition of embalmed human cadavers, this study shows the potential of a reliable and relatively simple and cheap method to quantify the stages of decomposition with additional studies. In this study, a suitable method was described for the detection and quantification of biogenic amines, as a potential method of measuring decomposition, while detecting those amines over a period of time providing a longitudinal trend.

While all three biogenic amines (methylamine, putrescine and cadaverine) were detected, a higher concentration of methylamine was detected at early stages of decomposition. This trend may be important to investigate in future studies to provide support that this may indicate a fresh cadaver. The findings of this study support further research into the use of biogenic amines as decomposition biomarkers in embalmed human cadavers and within a forensic context of criminal investigations.

Conflicts of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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