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THANATOMICROBIOME – STATE OF THE ART AND FUTURE DIRECTIONS

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Abstract: Microbiological studies show that there is a possibility of PMI estimation in reference to presence of typical bacteria and fungi on cadaver or in soil beneath. Microbiome after death (thanatomicrobiome) changes and depends on time since death, temperature, seasons and environment- if human remains are covered, buried, placed in ice or left on the surface. To enlarge current knowledge, some of studies are conducted on animal models with further comparison thanatomicrobiome of different animals-pig, rats- to human cadaver thanatomicrobiome. This study collects different branches of thanatomicrobiome studies as a review to summarize current knowledge.

1. Introduction. 2. Living host microbiome and mycobiome. 3. Diseases-related differences. 4. Thanatomicrobiome – human cadavers studies. 5. Fungi presence – thanatomycobiome. 6. Thanatomicrobiome of frozen cadavers. 7. Soil microbial communities changes. 8. Seasons related microbial changes. 9. Thanatomicrobiome and entomology correlation. 10. Conclusions

Keywords: bacterial succession, forensic medicine, microbiome, necrobiome, thanatomicrobiome

1. Introduction

Every human has got their own bacterial flora on their skin, in their gastrointestinal tract, genitourinary system and in the oral cavity, which is called the microbiome [44]. The human microbiome is shaped by many different factors – newborn babies' microbiomes depend on the labor type and way of feeding – natural breast milk or infant formula. Later, the microbiome is related to diet, age, sex, medications taken and diseases. Although microbiome formation varies, in adults, it is relatively stable. The microbiome is characteristic to a living host, but after death, there are specific changes of microbial phyla, genera and families. The microbiome of deceased humans is called the thanatomicrobiome (in Greek mythology Thanatos was the personification of death) [52, 98]. To estimate the PMI (post-mortem interval), a forensic medical examiner uses such indicators as: *pallor mortis*, *algor mortis*, *rigor mortis, livores mortis*, decomposition stages and insect activity – forensic entomology. It is proved that the changes in the thanatomicrobiome are characteristic and repeatable enough to become an additional PMI indicator [98]. Research showed that the sequences of microbial phyla changes are nearly the same among mammals, and thus allow the expansion of the research area to animal models [20, 86].

Microbial communities change not only on cadavers. Burial places and the soil beneath cadavers during decomposition process also undergo microbial phyla changes [31, 88]. Also, like the changes in the thanatomicrobiome, bacteria shifts in soil are characteristic during particular decomposition phases. Different authors distinguish various number of decomposition stages – usually three to five decomposition stages appear in studies: fresh, bloat, active decay, advanced decay and the dry remains stage [1]. For each stage, there is a specified bacterial phyla predominance, and increasing or decreasing bacteria abundance over time [83].

2. Living host microbiome and mycobiome

The skin microbiome consists of four main phyla: *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. The most abundant genera are *Staphylococcus* spp. (mostly *S. epidermidis*), *Corynebacterium*, *Propionibacterium*, *Brevibacterium* and *Micrococcus* [42, 67].

In the oral cavity there is tremendous diversity of bacteria [14], predominantly *Streptococcus*, *Veillonella, Fusobacterium*, *Neisseria*, *Haemophilus*, *Propionibacterium*, *Eikenella*, *Peptostreptococcus* and *Eubacteria* [67]. Nasal bacteria are *Actinobacteria* (*Propionibacterium* and *Corynebacterium*) and *Firmicutes* (*Staphylococcus* spp.) [33, 42].

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The bronchi and lungs are colonized mostly with four phyla: *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Actinobacteria* [67], [Table I]. The most common bacterial taxon in the esophagus is *Streptococcus*. Additionally, *Haemophilus*, *Prevotella*, *Neisseria*, and *Veillonella* may be present [75]. The stomach is inhabited by *Proteobacteria* (*Helicobacter pylori*) and *Firmicutes*. In the intestines, two phyla dominate: *Bacteroidetes* and *Firmicutes*, most of intestinal bacteria are anaerobic: *Bacteroides*, *Bifidobacterium*, *Fusobacterium*, *Eubacterium* and *Ruminococcus* [94]. However, in the intestines, aerobic and obligately anaerobic bacteria are present as well, for instance *Enterobacter* spp., *Escherichia coli, Staphylococcus* spp., *Klebsiella* spp. and *Proteus* spp. [60]. In the vagina, the most abundant are *Lactobacillus* (*L. crispatus*, *L. gasseri*, *L. iners* oraz *L. jensenii*) [99].

Microbiomes differ between individuals, and are related to diet, age, sex, weight, health status, antibiotic administration or even with cosmetic use [43]. However, during across a one-year observation period, the intestinal microbiome in each host is relatively stable and varies to a small extent [94].

Fungal diversity in the human gut is much lower than bacterial diversity [74]. The most abundant fungal genus in human stool is *Candida*, followed by *Malassezia* and *Saccharomyces* [74]. *Ascomycota* is the most abundant phylum among fungi, not only in the stool but also in the vagina, oral cavity and skin [74]. In the digestive tract, other sources [30, 82] additionally mention the *Cladosporium* and *Cryptococcus* genera, *Eurotiales* order and *Botrysphaeriales* as a popular family.

On the skin, the most abundant are *Malassezia restrica* and *M. furfur*, but *M. globosa*, *M. sympodialis* and *M. pachydermatis* are also frequently present [79]. *Candida* may be component of the skin mycobiome but rarely colonize human skin – usually in diabetic patients or during infections [67]. In the oral cavity, *Candida*, *Saccharomyces*, *Penicillium*, *Scopularis*, *Geotrichum* and *Aspergillus* are present [25, 26]. The bronchial and lung mycobiome is partially determined by oral and nasal fungi which spread through continuity. Therefore, in lower respiratory tract, the most abundant are: *Cladosporium*, *Aspergillus*, *Candida*, *Malassezia* and *Saccharomyces*. In the genitourinary system, the most common are: *Saccharomyces*, *Candida*, *Aspergillus*, *Cladosporium* and *Alternaria*.

3. Disease-related differences

During PMI estimation, it is important to know the medical history of the deceased person, because the microbiome in persons suffering from diseases is significantly different than a healthy human microbiome [8, 93].

Chronic alcohol abuse and cirrhosis cause a decrease of *Clostridium* presence and increase of *Proteobacteria* (*Enterobacter*) and *Bacteroides* spp. [93].

Table I
Human microbiome in regard to body areas

Skin	Oral cavity	Bronchi lungs	Nasal	Intestine
Staphylococcus *Corynebacterium* *Propionibacterium* *Brevibacterium* *Micrococcus*	*Streptococcus* *Veillonella* *Fusobacterium* *Neisseria* *Haemophilus* *Propionibacterium* *Eikenella* *Peptostreptococcus* *Eubacteria*	*Pseudomonas* *Streptococcus* *Prevotella* *Fusobacterium* *Haemophilus* *Veilonella* *Porphyromonas*	*Propionibacterium* *Corynebacterium* *Staphylococcus* *Aureobacterium* *Rhodococcus*	*Bacteroides* *Bifidobacterium* *Fusobacterium* *Eubacterium* *Ruminococcus*

Table II
Human mycobiome in regard to body areas

Skin	Oral cavity	Bronchi lungs	Genitourinary system	Intestine
Malassezia furfur *M. restrica* *M. globosa* *M. sympodialis* *M. pachydermatis* *Candida*	*Candida* *Saccharomyces* *Penicillium* *Scopularis* *Geotrichum* *Aspergillus* *Cryptococcus* *Fusarium* *Alternaria*	*Aspergillus* *Candida* *Cladosporium* *Malassezia* *Saccharomyces* *Penicillium*	*Candida* *Saccharomyces* *Aspergillus* *Alternaria* *Cladopsorium*	*Candida* *Saccharomyces* *Cladosporium* *Cryptococcus* *Malassezia* *Eurotiales* *Botrysphaeriales* *Filobasidiales*

Diabetes mellitus patients showed a higher abundance of *Bacteroidetes* and lower percentage of *Firmicutes* in the intestinal microbiota [79]. Necrotizing enterocolitis is correlated with high abundance of *Proteobacteria* [8].

Alzheimer's disease corresponds to an abundance of *Bacteroides fragilis* and *Escherichia coli* and their neurotoxin, and the presence of bacterial lipopolysaccharide (LPS) in the brain in the hippocampal area [97]. Allergies, cardiovascular diseases, cancer, psychiatric diseases and metabolic syndrome also affect the host microbiome [8].

Although there are no studies considering mistakes in PMI estimation caused by cadavers illnesses, individual abnormalities of quantity or phyla abundance can be compared with characteristic differences in particular disease. If the medical history of the cadaver is known, time since death can be confirmed more precisely, with bacterial number or presence deviation clarified by illness.

4. Thanatomicrobiome – human cadaver studies

A basic difficulty during cadaver studies is the cessation of natural barrier protection. After death, intestinal bacteria can move to the blood and tissues. Additionally other types of bacteria also begin to spread around the entire corpse. This is caused by tissue congestion, vessel enlargement and the unsealing of cell junctions. As a result, organs considered to be sterile can become settled by bacteria, and tissues where there is a specific microbiota can be contaminated by bacteria from other areas. For this reason, the longer the time since death, the lower the accuracy of the research.

Damann *et al.* [18] analyzed ribs of 12 human cadavers and divided decomposition into 3 phases – partially skeletonized, skeletonized and dry remains. It was proved that in two of the prime phases, the thanatomicrobiome was similar to a living human gut microbiome, while the dry remains phase was characterized by a thanatomicrobiome more similar to soil bacterial communities, but was not identical [18]. The partially skeletonized and skeletonized stages had a high abundance of *Firmicutes* and *Proteobacteria*. During decomposition, *Firmicutes* decreased while *Proteobacteria* and *Actinobacteria* started increasing. In contrast to soil samples, cadaver thanatomicrobiomes in the last phase had higher levels of *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* and *Firmicutes* and a smaller quantity of *Acidobacteria*.

DeBruyn *et al.* [56] divided decomposition into 2 phases, and showed that at the beginning, *Bacteroidetes* and *Firmicutes* predominated. In the late phase of decomposition, *Firmicutes* was still in abundance, while *Bacteroidetes* decreased and *Ignatzschineria* increased.

Table III
Most abundant phyla in human cadaver according to sex

Heart thanatomicrobiome differences in relation to sex		
	Male	Female
Phylum	*Firmicutes*	*Proteobacteria*
	Bacteroidetes	
Class	*Bacilli*	*Gammaproteobacteria*
Order level	*Lactobacillales* *Rhizobiales*	*Pseudomonadales*
Genus	*Streptococcus* *Lactobacillus*	
Species	*Clostridium* spp.	

Mouth thanatomicrobiomes also vary in time – to start with, the main phyla were *Firmicutes* and *Actinobacteria*, while during bloat stage there was an increased number of *Tenericutes*, and growth of *Ignatzschineria* was also remarkable. Dry remains were characterized by a increase of *Firmicutes* with an abundance of *Clostridiales* and *Bacillaceae* [77].

Although an increasing number of studies widen the knowledge about thanatomicrobiome changes, it is worth noticing that scientists discover some differences related to illnesses or sex. For instance, Bell *et al.* [5] proved differences between the thanatomicrobiomes in male and female heart samples [53], taken from 10 cadavers and analyzed 6–58 h since death [Table III]. In male hearts, most abundant phyla was *Firmicutes*, while in females *Proteobacteria* predominated, *Bacteroidetes* was in similar quantity in both sexes. *Bacilli* and *Streptococcaceae* were detected almost solely in males. *Lactobacillales*, *Rhizobiales* were found only in males, while *Pseudomonadales* and *Gammaproteobacteria* were more abundant in female hearts samples. *Clostridium* sp. was present in both sexes in a similar percentage. *Clostridium* was present in almost all cadaver samples [53]. There is rapid overgrowth after death, because *Clostridium* have the shortest doubling time. *Bacteroides* and *Lactobacillus* spp. decreased as far as decomposition progressed [39].

5. Fungi presence – thanatomycobiome

Although most thanatomicrobiome studies focus on bacteria, studies about fungal presence can be the equally as important in PMI estimation in both humans and animals [13]. Human cadaver research is less frequent due to legal reasons and smaller number of donors, therefore animal research allows to extend more general knowledge on the subject. However, animal PMI estimation is also used independently in forensic veterinary medicine [92].

Table IV
Predominant phyla in 3 decomposition stages in particular corpse parts in order to frequency of appearance

	Hair	Skin	Mucosa	Lungs	Bones	Clothes	Soil
Bloated	*A. flavus* *A. niger* *P. rugulosum*	*A. flavus* *A. niger* *P. rugulosum*	*A. flavus* *A. niger* *P. rugulosum*	*A. flavus* *A. niger* *Penicillium* spp.	–	–	–
Putrefaction	*A. flavus* *A. niger* *Penicillium* spp.	*A.flavus* *C. albicans*	*C. albicans* *C. guilliermondii* *P. piceum*	*C. albicans* –	–	–	–
Skeletonized	*A. flavus* *A. niger* *Penicillium* spp.	–	–	–	*Penicillium* spp. *A. flavus* *A. niger*	*Penicillium* spp. *A. flavus* *A. niger*	*Penicillium* spp. *A. flavus* *A. niger*

Research based on human studies showed fungi presence during three stages of decomposition (bloated, putrefaction and skeletonization) [83]. Samples were taken from the cadaver's mouth, skin, rectum, vagina, lungs and grave soil or coffin fragments. In the bloated stage, *Aspergillus flavus* was dominating, followed by *Aspergillus niger* and *Penicillium rugulosum* in all sampling locations. In the purification stage, *Candida albicans* dominated in most samples, except hair, in which the fungal pattern was the same as in bloated stage. The skeletonized stage was dominated by *Penicillium*, with presence of *Aspergillus flavus* and *Aspergillus niger* [Table IV].

Tranchida *et al.* [90] describes human cadavers in an advanced decomposition state. In soil samples beneath the remains, *Talaromyces udagawae* (*Aspergillaceae*), registered as human pathogen, was detected, while in control soil samples, there was no signs of *T. udagawae*. In soil samples from under the cadaver, *Dichotomomyces cejpii* and *Talaromyces trachyspermus* were also found. Other fungi – *Mortierella, Mucor hiemalis, Aspergillus* and *Penicillium frequentas* were detected in control soil samples taken 15 meters from the cadaver.

Xiaoliang Fu *et al.* [35] presented differences between fungal succession during decomposition inside and outside, using pigs carcasses. During the decomposition of 3 pigs indoors, *Candida xylopsoci*, *Ascomycota* spp. and *Thermoascus aurantiacus* dominated. The outdoor carcasses decomposed faster and the dominating fungi was *Yarrowia lipolytica*. In soil samples from beneath the carcasses, *Yarrowia lipolytica* and *Candida catenulate* dominated. During the initial decomposition stage, fungal succession on carcasses were similar but as decay proceeded, indoor and outdoor fungal succession started to vary.

6. Thanatomicrobiome of frozen cadavers

A distinct issue is the thanatomicrobiome after long-term freezing. Hyde *et al.* [46] described 2 donated cadavers, the first frozen for 89 days, and the second for 143 days. In both cadavers, most popular phyla in the mouth was *Firmicutes*, followed by *Actinobacteria* on the first cadaver and *Proteobacteria* on the second one. As decomposition progressed, *Actinobacteria* decreased and *Proteobacteria* increased on the first cadaver. A second case, also presented by Hyde [47], described 2 cadavers, one frozen for 22 days and the second for 14 days. *Firmicutes* and *Actinobacteria* increased in the later phases of decomposition. *Firmicutes* and *Bacteroides* predominated in fecal samples before purge, while later stages of decomposition were dominated by *Proteobacteria*. There were differences between the thanatomicrobiome genus in the two cadavers in Hyde's second research – the first cadaver was dominated by *Ignatzschineria*, *Acinetobacter* and *Pseudomonas*, while in the second cadaver, *Clostridium*, *Acinetobacter* and *Ignatzschineria* predominated. A third piece of research by Pechal *et al.* [78] was performed on 2 cadavers of children, aged 9 and 13, murdered and frozen by mother. In contrast to previous research, in this one, there were observed differences between bacterial diversity during the thawing process. While thawing, *Actinobacteria*, *Fusobacteria* and *Gammaproteobacteria* increased, and *Firmicutes* decreased. In reference to families – *Corynebacteriaceae*, *Fusobacteriaceae*, *Pasteurellaceae*, *Pseudomonaceae* and *Tissierllaceae* significantly increased, in contrast to *Prevotellaceae* and *Staphylococcaceae* which decreased.

7. Soil microbial community changes

Soil microbial communities are quite different in comparison to the human microbiome. This knowledge is crucial, because there is the possibility to prove the former presence of a buried or decomposing cadaver based on microbial changes in soil [84]. A second option is estimating PMI by defining microbial communities on remains which are different in decomposition stages and are more similar to soil communities in late stages. Comparison of microbiome changes in the soil requires the appropriate collection of samples to differentiate soil

related to thanatomicrobiome changes and a comparative soil sample, which is pure soil without contact with a cadaver and its thanatomicrobiome [90].

Proper sample collection includes taking soil samples right beneath the cadaver (0–5 cm) [17, 31, 84, 90] and control samples. A crucial assumption is receiving soil samples without contact with cadaver – most frequent distances, considered to be adequate, used in soil thanatomicrobiome studies are 1 m, 5 m and 15 m from the body [84, 90, 91].

Garriga *et al.* [2] proved that different bacterial phyla appear in particular decomposition stages, at first, *Proteobacteria*, *Acidobacteria* and *Bacteroidetes* predominate, while in later phases *Firmicutes*, *Actinobacteria* and *Proteobacteria* are more abundant,and finally *Firmicutes* and *Proteobacteria* are the most common [2, 30].

Finley *et al.* [31] described a one-year observation of soil microbiota beneath cadavers. Cadavers were divided into two groups – one was cadavers on the surface and the second group was buried bodies. In both groups, the predominant phylum was *Proteobacteria,* but in group of buried cadavers, the quantity of these bacteria was lower. *Acidobacteria* in the buried group was more abundant than in the surface group and in control samples. After 9 months, *Firmicutes* in the surface group was predominant phylum, in contrast to buried group and controls, where the amount of *Firmicutes* was low. *Acidobacteria* and *Verrucomicrobia* were more abundant in the buried group.

Singh *et al.* [84] proved that the soil microbiome beneath cadavers is significantly different than the soil microbiome 1 m and 5 m from the cadaver. Right below the cadaver, the relative abundance of *Firmicutes* and *Bacteroidetes* was greater, but the amounts of *Verrucomicrobia*, *Acidobacteria*, *Chloroflexi* and *Gemmatimonadetes* were smaller. All of the cadavers were placed on a field, and the research included 732 days of sampling.

Cobaugh *et al.* [17] in research on four cadavers compared gut bacteria communities and soil microbiota beneath cadavers. The cadavers' most abundant phyla were *Bacteroidetes* and *Firmicutes*, while in soil samples, the most common were *Proteobacteria*, *Actinobacteria* and *Firmicutes*. *Actinobacteria* and *Firmicutes* increased while decomposition progressed, in contrast to *Acidobacteria* and *Verrucomicrobia* which decreased.

There is also research on swine buried models [80], and in the soil samples, *Proteobacteria* was most abundant phylum, while the second most abundant phylum was *Bacteroidetes*, with *Firmicutes* increasing in later phases of decomposition. Control soil samples showed a predominance of *Acidobacteria*. Soil beneath the carcasses contained a reduced quantity of *Acidobacteria* but this phenomenon fluctuated in time and when pH raised, *Acidobacteria* abundance increased.

Rabbit decomposition research [92] demonstrated a predominant abundance of *Proteobacteria*, with a presence of *Bacteroidetes* and *Actinobacteria*. An interesting fact is that *Actinobacteria* was in higher abundance during early stages of decomposition, and in the group of rabbits with fur, the abundance of this phylum was definitely higher than in the bald rabbit group. In later stages of decomposition, the percentage of *Actinobacteria* in soil samples was nearly equal in both groups.

8. Season-related microbial changes

Research on rat carcasses considered different decomposition patterns and microbiota in relation to different seasons. In the spring, swabs taken from the small intestine of carcasses showed that the predominant phylum was *Proteobacteria,* followed by *Firmicutes*. In the summer, the predominant phylum was *Firmicutes*. It is noticed that *Enterococcus faecalis* had different growth patterns in the spring and summer, but ultimately in both seasons, the abundance in carcasses was similar [48].

Benbow *et al.* [6] in research on swine carcasses in a river proved that there are some differences in decomposition during different seasons. The generally predominant phylum was *Proteobacteria* followed by *Firmicutes* and *Bacteroidetes*. In the summer, the decrease of *Proteobacteria* was slower than in the winter. In the winter, while decomposition progressed, *Firmicutes* abundance was, in general, higher than in the summer. In contrast to *Firmicutes*, *Bacteroidetes* was more abundant in the summer during all decomposition stages.

Another research on swine carcasses in water in the autumn and winter [23] showed that there are some bacteria which are season-specific. *Carnobacterium*, *Marinomonas*, *Aeromonas* and *Bacteroidales* Genus 2 and 8 were present in autumn exclusively. *Polaribacter* and *Bacteroidales* Genus 4 were distinctive for winter.

9. Thanatomicrobiome and entomology correlation

After death, not only bacteria exist and graze on cadavers. Within minutes, chemical signals attract the first necrophagous flies [89]. *Calliphora vomitoria* and *Lucilia sericata* are the most numerous insects in Europe detected on cavers, attracted by decomposition odor [34]. Odors are chemical signals, which appear while bacteria start producing postmortem compounds. Bacteria are capable of producing or decomposing substances like indole, ammonia, putrescine, and benzoic acid during cadaver decomposition [66]. Different substances attract various necrophagous flies, but ammonia is considered to be the interkingdom signal

Table V
Correlation between bacteria and insects ocurrence on cadaver

Insect	Necrophagous flies in general	*Sacrophaga* spp.	*Lucilia cuprina*	*Sacrophaga* spp. *Cochliomyia macelluria*
Bacteria genus	*Streptococcus* *Staphylococcus* *Proteus* *Morganella* *Escherichia*	*Myroides* *Ignatzschineria*	*Streptococcus* *Staphylococcus* *Bacillus* *Micrococcus*	*Bacillus* *Providencia* *Escherichia* *Enterococcus* *Ochrobactrum*

that controls the activity of bacteria and blow flies [66]. Also, there is a correlation between insect and bacteria genus occurrence on cadavers [Table V] [89]. Relations between bacteria and blowflies work both ways – some bacteria, like *Proteus mirabilis,* occur on cadavers, transferred by the salivary glands of blowfly *Lucilia sericata. The b*urying beetle transfers its own gut microbiome onto the cadaver – *Morganella*, *Proteus, Providencia, Vagococcus, Xanthomonadaceae* and *Tissirella* [95]. On the other hand, insects, like burying beetles, struggle to obtain the carbohydrates, lipids and proteins present in the cadaver. For this purpose, insects participate in spreading oral secretions that have antibacterial activity, helping to restrain bacterial proliferation [95].

Studies in general focus on the presence of insects on cadavers or on the thanatomicrobiome, and there is only a few examples of research on how both bacteria and insect presence are related [34], [89]. It is clear that presence of some insects entails the appearance of some bacteria phyla and vice versa [89], but future studies may be able to clarify the correlation in later phases of decomposition and enable a precise definition of relations between the development of thanatomicrobiome phyla and insects in particulars decomposition stages.

10. Conclusions

Over the years, knowledge about microbiome changes decisively increased. A constantly rising number of scientific studies and research leads to the possibility of PMI estimation using the thanatomicrobiome. Nowadays, we can distinguish three to five decomposition stages basing on cadaver microbiomes and bacterial community shifts during decay. Moreover, distinctive microbiome changes in the soil beneath the remains, either on the surface or soil beside a buried cadaver, can equally precisely determine the time since death. Distinctive differences occur in thanatomicrobiome changes in water or during the thawing process too. In addition, the microbiome on the cadaver is dependent on the season. Some bacteria can be transferred onto the cadaver by necrophagous insects. Furthermore, the microbiome is not the only indicator we can use in PMI estimations, as fungal changes after death (thanatomycobiome) are also characteristic and specific to different decomposition stages and body parts.

Some research focuses on differences between human decomposition and animal models. The final conclusion is that there is a sufficient similarity in different mammal decomposition stages, and process to expand animal model's records to believable conclusions for the human cadaver decomposition model.

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