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PAPER

PATHOLOGY/BIOLOGY

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The Effect of Body Coverings on the Formation of Adipocere in an Aqueous Environment

ABSTRACT: Adipocere is a postmortem decomposition product that consists of a mixture of fatty acids. The rate of formation of adipocere from pig adipose tissue in an aqueous environment has been monitored. The effect of various clothing and carpet material types on the process was investigated. The fatty acid composition of the adipocere was determined at regular intervals using gas chromatography–mass spectrometry. Examination of the changes to fatty acid concentrations allowed the degree of adipocere formation in the different environments to be estimated. The study demonstrated that the rate at which adipocere forms is particularly accelerated by the presence of coverings produced from natural materials. Elemental analysis by inductively coupled plasma–mass spectrometry revealed, for the most part, little change to the cations present in the adipocere formed. However, an increase in Ca concentration was observed for tissue wrapped in acrylic carpet, which was associated with a $CaCO_3$ additive used in the carpet manufacture.

KEYWORDS: forensic science, adipocere, carpet, clothing, fatty acids, gas chromatography–mass spectrometry, inductively coupled plasma–mass spectrometry

Adipocere is a gray-white waxy decomposition product of adipose tissue that may be observed postmortem. The appearance of adipocere is of interest to forensic practitioners, as it may slow the decomposition process or even preserve human remains, thus providing valuable information regarding the nature of a death. Adipocere may be observed in bodies found in aqueous environments, and it has been reported in a number of studies that a wet environment is conducive to the formation of adipocere (1–5). The conditions recognized as most conducive to adipocere formation in water include the presence of fatty tissue with skin and putrefactive bacteria in a moist, warm, and anaerobic environment (3). Comparatively little work has been reported about the characterization of the adipocere formed in aqueous environments, with most reports describing case studies and qualitative findings. An understanding of the composition of the adipocere formed can provide an indication of the extent of formation of this decomposition product and, thus, the effect of the aqueous environment on its formation.

One of the factors that needs to be considered when investigating the rate of adipocere formation is the presence of clothing or other body coverings. Accidental death or the clandestine disposal of a body in a water environment will more often than not involve clothing. A common finding has been that the presence of clothing or other covering appears to enhance the ability of decomposing tissue to form adipocere in both soil and aqueous environments (1,2,6–9). The general consensus of opinion seems to be that in a soil burial environment, the presence of such material favors the absorption and storage of moisture.

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Adipocere is mainly composed of a mixture of fatty acids, predominantly palmitic and stearic acids, as well as hydroxy fatty acids and salts of fatty acids (4,7,10). Adipocere initially results from the conversion of adipose tissue into unsaturated fatty acids and then into saturated fatty acids. The process is characterized by the hydrolysis and hydrogenation of tissue into a mixture of predominantly saturated fatty acids, including palmitic, stearic, and myristic acids. Remaining unsaturated fatty acids, usually stearic and palmitoleic acids, salts of fatty acids, and hydroxyl fatty acids have all been identified as adipocere constituents (4,10). Gas chromatography–mass spectrometry (GC-MS) can be utilized to identify the fatty acids present in adipocere. Previous studies have involved the analysis of the composition of adipocere formed in soil environments and demonstrated that the formation may be monitored by analyzing the fatty acid composition of adipocere (8,11–15). More recently, a modified GC-MS method has been developed to analyze samples formed in aqueous environments (16). A solid-phase extraction (SPE) method in combination with GC-MS was developed and validated for the quantification of free fatty acids commonly found in adipocere.

Elemental analysis provides information about the fatty acid salts present in adipocere. Inductively coupled plasma–mass spectrometry (ICP-MS) provides a means of identifying elements at trace levels, and for this study, the ICP-MS analysis of decomposing adipose tissue in an aqueous environment is reported. ICP-MS has been successfully utilized for the analysis of adipocere formed by pig tissue in soil environments (8,12,13). These studies identified the major cations as K, Ca, Mg, and Na.

In this study, an investigation into the formation of adipocere in pig tissue covered with different clothing and carpet materials in an aqueous environment has been carried out. GC-MS was employed

to determine the fatty acid composition, and hence, the degree of formation of each adipocere sample. ICP-MS was used to identify the major elements in the adipocere.

Materials and Methods

Adipocere Formation

Model aqueous environments were established in sealed 5L polyethylene containers. The containers were washed with 5% v/v nitric acid, rinsed with distilled water, and air dried. A polyethylene basket was constructed to contain the adipose tissue and was suspended from the lid of the container to allow for complete immersion of the specimens in distilled water. Pig adipose tissue (*Sus scrofa*) was obtained from a retail butcher. A 10 cm × 10 cm piece of tissue collected from the abdominal region containing muscle and skin was used for each experiment. One gram of pig fecal material was smeared onto the skin surface of the tissue to ensure the necessary bacteria to promote putrefaction. A control environment was established with unwrapped tissue immersed in distilled water. Newly manufactured retail polyester, wool and cotton clothing materials, and wool and acrylic carpet were purchased and washed with a commercial anionic detergent in cold water before use. Each material was wrapped in a single layer around adipose tissue until the entire surface of the tissue was covered. The wrapped tissues were immersed in distilled water. Three replicates of each set-up were prepared, and all containers were maintained at a temperature of 20°C.

Samples of adipocere were collected from random sections of the tissue at 3-monthly intervals over a period of 18 months. Previous research showed that 18 months is an adequate time period for adipose tissue to complete its conversion to adipocere and for the product to be observed in an advanced state of formation under controlled conditions (17). The collected samples were placed in sealed specimen containers, homogenized, and stored at −18°C until analysis.

GC-MS Analysis

Five milligrams of each adipocere sample was placed in 5 mL of hexane, sonicated for two 10-min sessions, and centrifuged (1000 × *g*, 5 min). The supernatant was aspirated and frozen until use. The fatty acid content of the samples was analyzed using a SPE method in combination with GC-MS (16). For the SPE method, the extraction of neutral lipids was performed using Bond Elut 100 mg aminopropyl disposable cartridge columns. Mixtures of standards were dissolved in 1 mL of hexane with 200 μL of 10 μg/mL heptadecanoic acid added as an internal standard. The column was eluted with 2 mL of 2:1 v/v chloroform/2-propanol, and this fraction contained the neutral lipids (fraction 1). A volume of 2 mL of diethyl ether containing 2 v/v% acetic acid was applied to elute the free fatty acids (fraction 2). Each collected fraction was dried using a vacuum centrifuge at 50°C for 15 min. The remaining extract was taken up in 1 mL of hexane.

Fractions 1 and 2 were derivatized using bis(trimethylsilyl) trifluoroacetamide (BSTFA). Although fraction 1 contained triglycerides only, it was also derivatized as a control to ensure that no contamination or co-elution of free fatty acid was occurring. Excess BSTFA was added (200 μL) and placed in an oven for 30 min at 60°C.

The trimethylsilyl (TMS) fatty acid derivatives were analyzed using an Agilent 6890 Series GC coupled to an Agilent 5973 Network mass spectrometer using the same conditions as those described by (16). The analysis was conducted in total ion scan mode and identified those fatty acids known to comprise adipocere. The fatty acids examined were myristic, palmitoleic, palmitic, linoleic, oleic, stearic, and 10-hydroxy stearic acids. Peaks relating to the TMS esters of fatty acids were identified by comparison of their retention time and mass spectra with the NIST98 Mass Spectral Library. The relative response factor of the standards and the unknown weight of the individual fatty acids were calculated relative to the internal standard heptadecanoic acid using established equations (18).

ICP-MS Analysis

For ICP-MS analysis, adipocere samples were decomposed by acid digestion in combination with an oxidizing agent. A volume of 350 μL of each of concentrated HNO_3 and HCl was added to 5 mg of samples and heated until the evolution of brown fumes ceased. A volume of 350 μL of H_2O_2 was added, and the sample reheated until effervescence ceased. The solution was cooled and diluted using high-purity deionized water. A 250 ppb of internal standard containing ^{103}Rh and ^{45}Sc in 1 v/v% HNO_3 was added to all samples during analysis via an external source connected to the instrument. Mixed calibration standards containing Na, Mg, Al, Si, K, Ca, Mn, Fe, Zn, Sr, and Pb were prepared from a 100 ppm of stock solution via serial dilution to achieve final concentrations of 10 ppm, 1000 ppb, 500 ppb, 100 ppb, 10 ppb, and 1 ppb. Each standard was made to 10 mL using high-purity deionized water, and 350 μL of each of concentrated HCl and HNO_3 was added to achieve a similar matrix ratio to the samples. Blank samples were prepared using high-purity deionized water and 350 μL of each concentrated HCl and HNO_3. The analysis was carried out using an Agilent 7500ce ICP-MS Octapole Reaction System with an ASX-510 Autosampler attachment. The experimental conditions used were according to the method described in (19).

Results

GC-MS Analysis

Table 1 lists the relative fatty acid concentrations determined for the adipocere formed in the control environment over a period of 18 months. The main unsaturated fatty acid present throughout is oleic acid, the relative concentration of which decreases throughout the life of the experiment. There are also small amounts of the unsaturated linoleic and palmitoleic acids present up to 6 months, but these are not detected for the remainder of the study. The main saturated fatty acid present during the 18 months is palmitic acid, which significantly increases in concentration until 9 months, then the value stabilizes. Stearic acid is present in appreciable quantities,

TABLE 1—*Relative fatty acid concentrations for adipocere formed in control tissue.*

	Time (months)					
	3	6	9	12	15	18
Unsaturated fatty acids						
Oleic (18:1)	13	12	5	6	4	3
Linoleic (18:2)	<1	<1	0	0	0	0
Palmitoleic (16:1)	<1	<1	0	0	0	0
Saturated fatty acids						
Myristic (14:0)	1	1	1	1	1	1
Palmitic (16:0)	49	44	69	66	68	72
Stearic (18:0)	24	24	20	20	18	18
10-hydroxystearic (18:0,10-OH)	12	19	5	7	9	7
% total saturated fatty acids	86	88	95	94	96	98
Stage of adipocere formation*	I	I	A	A	A	A

*I, intermediate; A, advanced.

and the relative concentration of this fatty acid decreases during the 18 months. 10-Hydroxy stearic acid is present and appears to increase until 6 months, after which the concentration declines. A small quantity of myristic acid is also present and the concentration remains constant throughout the experiment. The identification of each of these fatty acids in these relative proportions confirms the presence of adipocere (17,20).

Because of the complex nature of the reactions of the fatty acids resulting from adipose tissue, it is not a simple case of monitoring the change in concentration of a particular fatty acid throughout the process of adipocere formation. Earlier investigations divided the process of adipocere formation into three stages based on the amount of saturated fatty acids present (17,19). For the purposes of this study, an estimate of the % of saturated fatty acids at each stage is as follows: early (40–60%), intermediate (70–90%), and advanced (>90%). Table 1 shows the predicted stages of adipocere formation for the control sample based on the total % saturated fatty acids present. Based on the fatty acid composition, the adipocere appears in an intermediate state to 6 months, but appears to be at an advanced state from 9 to 18 months.

The relative fatty acid concentrations determined for the adipocere formed in the tissue enclosed in polyester clothing are listed in Table 2. The data reveal that the same fatty acids as those found in the control samples are detected in the adipocere collected from this environment, but there is variation in the fatty acid concentrations at different time intervals. Samples collected at 9 months were excluded from this environment because of a loss of sensitivity, even after repeated GC-MS analysis. Notably, the palmitic and 10-hydroxystearic acids form in higher concentrations at earlier sampling times when the tissue is enclosed in polyester clothing compared with the control. An examination of the total relative % saturated fatty acids obtained for the adipocere formed while enclosed in polyester clothing reveals that adipocere is in a more advanced state earlier than the control. Adipocere formed in polyester clothing appears to be in an advanced stage by 6 months.

Table 3 lists the results of the analysis of GC-MS adipocere formed within the wool clothing. The same fatty acids are detected with similar trends observed, but the changes associated with a transformation of adipocere into an advanced state are observed to occur at an earlier sampling time compared with both the control and the polyester environment. The presence of wool clothing appears to have an ability to accelerate the formation of adipocere in an aqueous environment. Greater amounts of saturated fatty acids are observed in the first 6 months of the experiment, corresponding to an advanced state of formation.

TABLE 2—*Relative fatty acid concentrations for adipocere formed in tissue wrapped in polyester clothing.*

	Time (months)				
	3	6	12	15	18
Unsaturated fatty acids					
Oleic (18:1)	10	8	2	2	1
Linoleic (18:2)	<1	0	0	0	0
Palmitoleic (16:1)	<1	<1	0	0	0
Saturated fatty acids					
Myristic (14:0)	1	2	1	1	1
Palmitic (16:0)	48	48	78	71	81
Stearic (18:0)	21	19	17	16	14
10-hydroxystearic (18:0,10-OH)	20	23	2	10	3
% total saturated fatty acids	90	92	98	98	99
Stage of adipocere formation*	I	A	A	A	A

*I, intermediate; A, advanced.

TABLE 3—*Relative fatty acid concentrations for adipocere formed in tissue wrapped in wool clothing.*

	Time (months)					
	3	6	9	12	15	18
Unsaturated fatty acids						
Oleic (18:1)	9	5	2	2	4	2
Linoleic (18:2)	<1	0	0	0	0	0
Palmitoleic (16:1)	<1	<1	0	0	0	0
Saturated fatty acids						
Myristic (14:0)	2	2	1	1	2	1
Palmitic (16:0)	56	57	68	77	66	74
Stearic (18:0)	19	16	17	16	18	16
10-hydroxystearic (18:0,10-OH)	14	20	12	4	10	7
% total saturated fatty acids	91	95	98	97	96	98
Stage of adipocere formation*	A	A	A	A	A	A

*I, intermediate; A, advanced.

The presence of cotton clothing appears to have an even more extensive effect on adipocere formation in an aqueous environment. Table 4 lists the GC-MS results for this environment and illustrates the very high concentrations of saturated fatty acids throughout the experiment. Adipocere appears to be in an advanced state even by 3 months, with 94% of the fatty acids detected being saturated by that time. Again, the same trends are observed for the specific acids, but the time at which they occur is earlier in this environment compared with the control and the other clothing environments.

The GC-MS results obtained for the synthetic and natural fiber carpets are listed in Tables 5 and 6, which show data obtained for acrylic and wool carpets, respectively. The acrylic carpet results indicate that the presence of this material enhances the formation of adipocere compared with the control environment, given that the adipocere formed appears to be in an advanced state by 6 months. At 3 months, the adipocere still appears to be in an intermediate stage within the acrylic carpet environment. The results obtained for the wool environment demonstrate that wool carpet has the ability to accelerate the formation of adipocere, given the advanced state of formation observed even at 3 months, and there is very little unsaturated fatty acid remaining by 9 months.

ICP-MS Analysis

The abundances of each cation present in the adipocere samples were calculated as a concentration in ppm. The values were converted to a relative concentration value as a proportion of the total cations of interest. Figure 1 illustrates the relative concentrations of the major elements (K, Mg, Ca, and Na) for the control and the clothing and carpet environments as a function of the sampling time. The average values for the entire sampling period are also shown for each environment. The most abundant element present in each experiment is K, followed by Mg, Na, and Ca. For most elements, the concentration data do not appear to be following a significant trend with increasing immersion time. There does appear to be an increasing trend in the Na concentration with longer time in all environments. A comparison of the average concentrations for tissue exposed to different environments reveals no significant changes in most cases. There does, however, appear to be an overall increase in the Ca concentration in the tissue formed in the acrylic carpet environment. This appears to be at the expense of the K concentration in this environment.

TABLE 4—*Relative fatty acid concentrations for adipocere formed in tissue wrapped in cotton clothing.*

	Time (months)					
	3	6	9	12	15	18
Unsaturated fatty acids						
Oleic (18:1)	6	5	2	1	2	1
Linoleic (18:2)	0	0	0	0	0	0
Palmitoleic (16:1)	<1	<1	0	0	0	0
Saturated fatty acids						
Myristic (14:0)	2	2	1	1	1	1
Palmitic (16:0)	57	49	82	81	79	84
Stearic (18:0)	17	13	12	11	12	11
10-hydroxystearic (18:0,10-OH)	18	31	3	6	6	3
% total saturated fatty acids	94	95	98	97	98	99
Stage of adipocere formation*	A	A	A	A	A	A

*I, intermediate; A, advanced.

TABLE 5—*Relative fatty acid concentrations for adipocere formed in tissue wrapped in acrylic carpet.*

	Time (months)					
	3	6	9	12	15	18
Unsaturated fatty acids						
Oleic (18:1)	8	5	2	1	1	<1
Linoleic (18:2)	<1	<1	0	0	0	0
Palmitoleic (16:1)	1	<1	0	0	0	0
Saturated fatty acids						
Myristic (14:0)	2	2	<1	<1	1	1
Palmitic (16:0)	60	50	71	83	83	79
Stearic (18:0)	14	14	16	13	12	12
10-hydroxystearic (18:0,10-OH)	14	29	11	3	3	7
% total saturated fatty acids	90	95	98	99	99	99
Stage of adipocere formation*	I	A	A	A	A	A

*I, intermediate; A, advanced.

TABLE 6—*Relative fatty acid concentrations for adipocere formed in tissue wrapped in wool carpet.*

	Time (months)					
	3	6	9	12	15	18
Unsaturated fatty acids						
Oleic (18:1)	5	5	1	<1	<1	<1
Linoleic (18:2)	<1	0	0	0	0	0
Palmitoleic (16:1)	<1	<1	0	0	0	0
Saturated fatty acids						
Myristic (14:0)	2	2	1	<1	<1	<1
Palmitic (16:0)	67	62	81	88	80	78
Stearic (18:0)	15	13	10	11	12	11
10-hydroxystearic (18:0,10-OH)	10	18	7	<1	7	11
% total saturated fatty acids	95	95	99	100	100	100
Stage of adipocere formation*	A	A	A	A	A	A

*I, intermediate; A, advanced.

Discussion

The GC-MS study of the formation of adipocere in the control environment revealed that this product is indeed formed in an aqueous environment. The adipocere appears in an intermediate stage of formation until 6 months and in advanced stage thereafter. A comparison with the quantitative analysis of adipocere formed in a controlled soil environment reported at a 12-month burial time reveals a similar composition at this time (8). There is some difference observed in the relative amounts in the palmitic and stearic acids, but the overall percentage of saturated fatty acids is the same after 1 year in both environments.

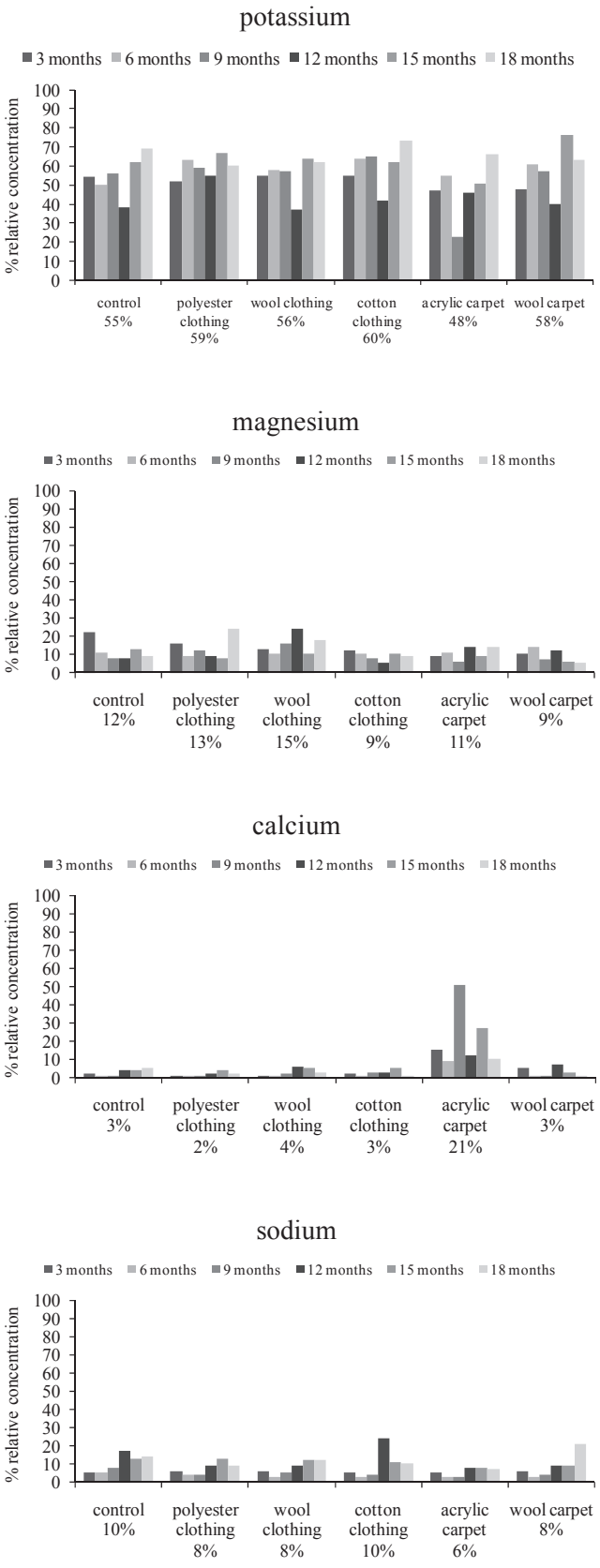

FIG. 1—*Elemental analysis of adipocere using ICP-MS.*

A comparison of the stages of adipocere formation at the different sampling times based on the % total fatty acids determined reveals that the materials produced from natural fibers allowed the adipocere to proceed to a more advanced stage earlier in the process. Wool carpet and cotton clothing appear to be the most effective at inducing adipocere formation, followed by wool clothing. The materials produced from synthetic fibers allow the adipocere formation process to proceed sooner, when compared to a control without any covering, but showed adipocere in a less advanced state compared with all the natural fiber coverings. Acrylic carpet appears to be more effective at producing the decomposition product than polyester clothing and the control.

The observed differences based on fiber type are most likely due to the manner in which water interacts with the fiber structures. Natural fibers tend to be more water absorbent. Cotton is comprised of cellulose, which attracts water via hydrogen bonding and absorbs a large quantity of water, making it one of the most absorbent textiles. Wool is also known to be water absorbent. Synthetic fibers tend to have fewer sites that attract water molecules and so do not have the same capacity to absorb water. Polyester and acrylic fibers are known to be poor absorbers of water. This difference in water absorbency means that there is a difference in the movement of the liquid adjacent to the tissue surface, which is likely to contain decomposition products. The absorbency ability may be responsible for the removal of the decomposition products, and this action could feasibly disrupt decomposition processes and allow the formation of adipocere to more readily occur. Some caution should be exercised when extending the findings of this study to other fiber types. If absorbency is a significant property, then one cannot simply generalize about the properties of natural versus synthetic fibers. Certain cellulose-based ''synthetics,'' such as rayon and acetate, have good absorbance characteristics, indicating that the ability to absorb adjacent decomposition products could be potentially similar to that observed for fibers such as wool and cotton.

The thickness of the material used also appears to be a factor affecting the rate of adipocere formation. The wool carpet environment produced adipocere in a more advanced state than that produced by the tissue wrapped in clothing made of wool. This may be due to the increased absorbency of the carpet material or the thermal insulating effect of the thicker material.

Given the observation of adipocere in an advanced or intermediate stage of formation at the 3-month stage, as a result of coverage of clothing or carpet materials, it will be of interest to investigate the effect of body coverings at earlier sampling time intervals. A previous study indicated that adipocere was not formed in the first month of immersion of pig adipose tissue in an aqueous environment (19), but the results of the current study indicate that there is certainly interest in investigating sampling times of <3 months for tissue enclosed in body coverings to determine the early onset of adipocere in these environments.

Based on the ICP-MS analysis of all samples, the major element present throughout is K. This finding agrees with the findings of previous studies, where pig adipose tissue was used as the source material (8,12,13,19). This higher K concentration differs from studies involving human tissue, where Na is found to be the major element present, even at early postmortem times (19). Unlike the studies of adipocere formed in soils, the adipocere formed in the model aqueous environment does not demonstrate significant changes in the cation composition. There is an indication that the Na concentration may increase with time in all environments, and this is probably associated with the release of Na ions from the interstitial fluid. There also appears to be an increase in the Ca ion and a decrease in K ion concentrations in adipocere formed in the acrylic carpet environment. A source of Ca leading to this cation exchange may be contained in the acrylic carpet. Calcium carbonate is used as an additive in coatings on the reverse side of carpet employed to hold the fibers in place.

Conclusions

A study has been made of the rate of formation of adipocere for pig adipose tissue covered in various clothing and carpet types in an aqueous environment. It has been demonstrated that adipocere forms faster when covered with all clothing and carpet materials when compared to an uncovered control tissue in distilled water. Coverings made of natural fibers were shown to accelerate the formation of adipocere compared with the coverings produced from synthetic fibers examined. The thicker carpet coverings were also shown to have an accelerating effect, but this factor does appear to be as significant as the effect of fiber type. As the findings are believed to be associated with the water absorbency of the fibers and the removal of decomposition products adjacent to the tissue, there is interest in extending this type of study to one in which a flowing aqueous environment can be investigated. The elemental analysis of the adipocere formed reveals limited change because of the environmental conditions. A difference in the Ca concentration observed for the tissue enclosed in acrylic carpet is believed to be associated with a $CaCO_3$ additive used in the manufacturing process. The formation of adipocere in water is clearly dependent on immediate environmental factors. The other significant factors that affect the formation of this decomposition product, including temperature, pH, and water chemistry, are also being investigated to ascertain their relevant significance to the process.

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