The Microbiology of Death

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By

Lorraine White

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Lorraine White

University of Sheffield

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Abstract

The main aim of this research was an attempt to clarify whether the protagonists of bacterial bone destruction were of a bodily origin as opposed to environmental contamination by soil bacteria and furthermore to demonstrate a time frame for such attack. It is hypothesised that bacteria from the gut commensal flora are responsible for micro-focal destruction (MFD) of bone postmortem that leaves distinctive tunnels. Microorganisms live with a person throughout their life and somewhat ironically after death persist to exploit this now non-operational substrate. They continue to thrive and without a working immune system are capable of crossing mucosal barriers and invading both soft and hard body tissues. Experimental protocol using pigs as human analogues were combined with archaeological sections of both humans and animals. The experimental research was almost absolute in the conclusion that only the fetal material was free of MFD one year post-mortem; these were entirely skeletonised and open to contamination by soil bacteria. All of the other pigs had suffered some form of attack, including those that had not skeletonised and were not therefore subjected to soil bacteria. The archaeological material tended to support the hypothesis that endogenous gut bacteria were the cause of MFD as both fetal material and animal bones were much less likely to be affected. It is suggested that soil bacteria are not normally accountable for MFD although their involvement cannot be ruled out entirely and they may be involved at a later stage. It is therefore likely that endogenous gut bacteria having access to a dead body immediately are most often the cause of MFD and that this occurs well within the early postmortem period. This has negative implications for biomolecular studies and positive implications for *in-situ* preservation.

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Table of Contents

Abstract i
Acknowledgements ii
Table of Contents iii
List of Figures vi
List of Tables ix

Chapter 1 Introduction

1.1 Introduction 1
1.2 Bibliography 7

Chapter 2 Taphonomy to the Point of Skeletonization 9
2.1 General Introduction 9
2.2 Necrology: Microbes and Death 9
2.3 Pre-Burial Modifications and Differential Burial Practices 12
2.4 Experimental Research: Decomposition Studies 17
2.4.1 Experimental Research: Entomology 20
2.4.2 Experimental Research: Children 21
2.5 Diagenetic Change 24
2.6 Summary 25
2.7 Bibliography 26

Chapter 3 Microbiology of the Body 33
3.1 General Introduction 33
3.2 Introduction to Microbiology 33
3.3 Adult Human, Newborn Human & Sus Scrofa Microbiology 35
3.4 Intestinal Bacteria and Timescale of Transmigration Postmortem 39
3.5 Summary 43
3.6 Appendix: Collagenase Producing Gut Bacteria 44
3.7 Bibliography 45

Chapter 4 Environmental Microbiology 48
4.1 Introduction 48
4.2 Notes on Bacterial Diversity and Culture Methods 48
4.3 Soil Bacteria 49
4.4 Soil Depth versus Bacterial Counts 50
4.5 Bacteria, Laboratory Culture Methods and Temperature 51
4.6 Experimental Research Microbes 53
4.7 Maggots, Microbes and Antibiotic Secretions 54
4.8 Maggots and Collagenase 57
4.9 Summary 58
4.10 Bibliography 60

Chapter 5 Diagenesis: Nature of Bone and Bone Tunnelling 64
5.1 Introduction 64
5.2 Gross Anatomy of Bone 64
5.3 Sus Bone: Comparison to Human 67
5.4 Diagenetic Pathways 70
5.5 The Nature of Bone Tunnelling 72

5.6 Types of Tunnelling and Location 73
5.7 Summary 78
5.8 Bibliography 80

Chapter 6 Case Studies of Diagenesis 83
6.1 Introduction 83
6.2 Laboratory Based Studies 83
6.2.1 Bones Exposed to Seawater (Marine Settings) 83
6.2.2 Modern Terrestrial Real-Time Experiments 84
6.2.3 Modern Forensic Cases 85
6.2.4 Predator Scat 86
6.3 Archaeological Cases 86
6.3.1 Marine Type Tunnelling in Archaeology 87
6.3.2 Terrestrial Tunnelling 88
6.3.3 Ossuary (Archaeological) 89
6.3.4 Pompeii 90
6.3.5 Cist Burials where the Remains are Disarticulated 91
6.3.6 Cladh Hallan Mummies 93
6.3.7 Infant Remains 94
6.3.8 Animal Bone 98
6.4 Summary 100
6.5 Bibliography 101

Chapter 7 Materials and Methods 104
7.1 Site Location and General Conditions 104
7.2 *Sus* Experiments 105
7.3 Site Temperature 107
7.4 Boxed *Sus* Experiments 108
7.5 Soft Tissue Decomposition 112
7.6 Further Experimentation 114
7.7 Winter Burials 114
7.8 Spring Burials 115
7.9 Microscopy 119
7.9.1 Anaerobic Growth of Microbes 119
7.9.2 Staining 120
7.9.3 Simple Stain 120
7.9.4 Gram Stain 120
7.10 Archaeological Material (Human & Sus) 121
7.11 Summary 123
7.12 Bibliography 124

Chapter 8 Results 125
8.1 Introduction 125
8.2 Results of Soft Tissue Decomposition (Boxed Pigs) 125
8.2.1 Experimental Carcasses: Pigs No's 1-4 Newborns 126
8.2.2 Experimental Carcasses: Pigs No's 5-12 Newborns & Stillborns 129
8.3 Results Soft Tissue Decomposition Real Burial Pigs 137
8.3.1 Pigs No's 15-21 Surface Depositions 138
8.3.2 Buried Pigs No's 21-30 143
8.4 Note on Description of Histological Preservation 147

8.5 Results of Bacteria Based Experiments (Boxed Pigs) 147
8.5.1 Bacterial Assays 148
8.5.2 Anaerobic Jar Results 149
8.5.3 Results of Microscopy (Boxed Pigs) 149
8.6 Results of Microscopy (Real-Time Pigs) 154
8.6.1 Surface Depositions Summary 154
8.6.2 Buried Depositions Summary 156
8.7 Results of Histology: Archaeological Sections 167
8.7.1 Results of Histology: Foetal and Newborn 172
8.8 Results of Archaeological Animal Histology 173
8.9 Summary 173
8.10 Appendix: Categories and Stages of Decomposition 175
8.11 Bibliography 176

Chapter 9 Summary/Implications for Archaeology & Forensic Science/Future Directions/Conclusion 177
9.1 Summary 177
9.2 Implications for Bone Integrity Based Studies 180
9.3 Implications for In-Situ Preservation 182
9.4 Implications for Archaeology 183
9.5 Implications for Bone Preservation in the Laboratory 184
9.6 Future Directions 185
9.6.1 Bog Bodies 185
9.6.2 Disarticulated/Excarnated Remains 185
9.6.3 Mummified Remains 186
9.6.4 Canine Bone 187
9.6.5 Juvenile Remains 187
9.6.6 Amputated Limbs 188
9.6.7 Forensic Use of Modern Human Bone 189
9.6.8 Soil versus Skeletons 189
9.7 Conclusion 189
9.8 Bibliography 191

List of Figures

Chapter 2
2. Postmortem Rigidity and Bloating (Sus) 10
2.1 Green Staining of Abdomen (Sus) 11
2.2 Purging of Fluids (Sus) 13
2.3 Maggot Activity (Sus) 20

Chapter 3
3. Comparison of Human & Sus Intestinal Tract 37
3.1 Proximal Dog Femur & Vascular Network 40

Chapter 4
4 Gross Anatomy of Blow Fly Larvae 56

Chapter 5
5 Compact Bone 66
5.1 Transverse Section of Bone 67
5.2 Sus Scrofa Bone (Microscopic) 68
5.3 Diagenetic Pathways 71
5.4 Destructive Changes in Bone 74
5.5 Amalgamation of Tunnels 75
5.6 Further Merging of Tunnels 76
5.7 Location of Tunnels 77

Chapter 6
6. Micro-Boring of Human Tooth (Modern Forensic) 87
6.1 SEM Clostridium in Bone Section 89
6.2 Well preserved Bone section from Pompeii 90
6.3 Poorly Preserved Bone Section from Pompeii 90
6.4 Cist Burial and SK5 Ingleby Barwick 92
6.5 Burials at Windmill Fields, Ingleby Barwick 93
6.6 Bone Section Cladh Hallan Male (Band of Destruction) 94
6.7 Bone Section Cladh Hallan Male (Polarized Light) 94
6.8 Well Preserved Foetal Bone from Bolsover 96
6.9 Poorly Preserved Goose Bone (Flixborough) 99
6.10 Moderately Well Preserved Goose Bone (Flixborough) 99

Chapter 7
7 The Woodland Burial Site at the Riseholme Estate 104
7.1 Average Monthly Temperatures 2005 105
7.2 Average Monthly Rainfall 2005 105
7.3 Containers Housing First Experiments 107
7.4 Maximum Daily Temperatures April-July 2006 108
7.5 Pig 1 110
7.6Pig 2 110
7.7 Pig 3 110
7.8 Pig 4 110
7.9 Pig 5 111

7.10 Pig 6 111
7.11 Pig 7 111
7.12 Pig 8 111
7.13 Pig 9 112
7.14 Pig 10 112
7.15 Pig 11 112
7.16 Pig 12 112
7.17 Winter Burial Pig 13 115
7.18 Winter Burial Pig 14 115
7.19 Cage for Pig 13 115
7.20 Cage for Pig 14 115
7.21 Surface Depositions 116
7.22 Some of the Buried Pigs 116
7.23 Mass Grave 118
7.24 Anaerobic Jar 119
7.25 Stages of Gram Staining 120
7.26 Gram Negative Stain 121
7.27 Gram Positive Stain 121

Chapter 8
8. Temperature May-July 2006 125
8.1 SK1 At Deposition 126
8.2 SK1 17 Days PM 126
8.3 SK1 One Month PM 126
8.4 SK1 Six Weeks PM 126
8.5 SK2 At Deposition 127
8.6 SK2 Eleven Days PM 127
8.7 SK2 Five Weeks PM 127
8.8 SK2 Six and a Half Months PM 127
8.9 SK3 At Deposition 128
8.10 SK3 Four Weeks PM 128
8.11 SK3 Seven Weeks PM 128
8.12 SK3 Six and a Half Months PM 128
8.13 SK4 At Deposition 129
8.14 SK4 Three Weeks PM 129
8.15 SK4 Four Weeks PM 129
8.16 SK4 Six and a Half Weeks PM 129
8.17 SK5 Four Days PM 130
8.18 SK5 Six Weeks PM 130
8.19 SK5 Six and a Half Weeks PM 130
8.20 SK5 Seven Weeks PM 130
8.21 SK6 Ten Days PM 131
8.22 SK6 Eleven Days PM 131
8.23 SK7 One Week PM Bloated 131
8.24 SK7 Three Weeks PM Mummifying 131
8.25 SK8 One Week PM Bloated 132
8.26 SK8 Two Weeks PM Mummifying 132
8.27 SK9 At Deposition 132
8.28 SK9 Two Weeks PM Skeletonizing 132
8.29 SK10 At Deposition 132

8.30 SK10 Two Weeks PM Skeletonizing 132
8.31 SK11 Five Days PM 133
8.32 SK11 Three Weeks Pm Skeletonized 133
8.33 SK12 Five Days PM 133
8.34 SK12 Two Weeks PM Almost Skeletonized 133
8.35 Average Monthly Rainfall 2006-2008 137
8.36 Maximum Monthly Temperatures 2006-2008 137
8.37 50KG Pig During Rainfall Seven Weeks PM 138
8.38 50KG Pig Beetle Larvae During Wet Period 139
8.39 50KG Pig Skin Hanging on Cranium after Rain 139
8.40 Foetal Pig Two Weeks PM 140
8.41 Foetal Pig Two Weeks PM Between Front Legs 140
8.42 Foetal Pig Four Weeks PM 141
8.43 Foetal Pig Six Weeks PM 141
8.44 25KG Pig Early Decomposition 141
8.45 25KG Pig Advanced Decomposition 142
8.46 25KG Pig Mummified Tissue 142
8.47 25KG Pig Bleaching of Skeleton 143
8.48 Buried Pig, One Year Post Burial: Adipocere 144
8.49 Methylene Blue Stain of Bacteria 150
8.50 Gram Stain of Bacteria 150
*8.51*SK15 (50kg) Surface Deposition Six Months Postmortem 157
8.52 SK15 (50kg) Surface Deposition One Year Postmortem 157
8.53 SK16 (25kg) Surface Deposition Six Months Postmortem 158
8.54 SK16 (25kg) Surface Deposition One Year Postmortem 158
8.55 SK17 (15kg) Surface Deposition Six Months Postmortem 159
8.56 SK17 (15kg) Surface Deposition One Year Postmortem 159
8.57 SK18 (10kg) Surface Deposition Six Months Postmortem 160
8.58 SK18 (10kg) Surface Deposition One Year Postmortem 160
8.59 SK10 (3-4kg) Surface Deposition Six Months Postmortem 161
8.60 SK10 (3-4kg) Surface Deposition One Year Postmortem 161
8.61 SK20 (1-2kg Fetal) Surface Deposition Six Months Postmortem 162
8.62 SK20 (1-2kg Fetal) Surface Deposition One Year Postmortem 162
8.63 SK21 (50kg) Buried One Year Postmortem 163
8.64 SK21 (25kg) Buried One Year Postmortem 163
8.65 SK23 (15kg) Buried One Year Postmortem 164
8.66 SK24 (10kg) Buried One Year Postmortem 164
8.67 SK25 (3-4kg) Buried One Year Postmortem 165
8.68 SK26 (1-2kg Fetal) Buried One Year Postmortem 165
8.69 Mass Grave Random Humerus: Buried One Year Postmortem 166
8.70 Graticule Scale 166
8.71 Archaeological Sections and % Tunnelling 167

List of Tables

Chapter 1

1 Scheduled Purpose Human Tissue Act 5

Chapter 2

2. Stages of Decomposition and Affecting Variables 12

Chapter 3

3 Environmental & Host Bacterial Requirements 34
3.1 Timescale of Bacterial Transmigration 42

Chapter 4

4 Distribution of Bacteria at Differing Depths 50
4.1 Bacteria that Produce Collagenase at Low Temperatures 53
4.2 Maggot Therapy/Disinfecting/Antibacterial Effects 55

Chapter 5

5 Bone Collagen Content 64
5.1 Key Attributes of Four Animal Species and Humans 69
5.2 Recognized Types of MFD 73

Chapter 6

6 Tunnelling Infant Remains 95
6.1 Aging of Bolsover Infants 97
6.2 Incidence of Tunnelling in Blackgate Infants 98

Chapter 7

7 Temperature Apr-Jun 2006 108
7.1 Condition of Pigs at Burial 111
7.2 Decompositional Data Scores 113
7.3 Condition at Burial 117
7.4 Weight and Age of Pigs Chosen 117
7.5 Grave Dimensions 119
7.6 Bolsover Infant Data 122

Chapter 8

8 Decomposition Data: Pigs 1-4 (Boxed) 134
8.1 Decomposition Data: Pigs 5-8 (Boxed) 135
8.2 Decomposition Data: Pigs 9-12 (Boxed) 136
8.3 Decomposition Data: Pigs 18-20 (Surface) 145
8.4 Decomposition Data: Pigs 15-17 (Surface) 146
8.5 Results of Staining: Thin Sections (Boxed) 148
8.6 Results of Collagen Destruction via Anaerobic Jar 149
8.7 Results of Histology: Boxed Pigs 153
8.8 Results of Histology: Real Burial Pigs 155
8.9 Archaeological Thin Sections 168
8.10 Foetal and Newborn Sections Histology Results 172

Chapter 1

1.1 Introduction

The main aim of this research is to investigate the determinants of microscopic bacterial degradation of bone tissue in the burial environment and to consider its implications for archaeology and forensic science. Child (1995:19), states that; "Microbial decomposition is defined as any deleterious changes to a substrate due to the action of micro-organisms, their by-products and their enzymes." Within this research theme it is anticipated that a conclusion may be drawn as to whether the attack is of bodily or environmental origin (i.e. endogenous origin/gut bacteria or exogenous origin/soil bacteria). Bacterial attack of bone is destructive and many modern investigative techniques rely on molecules retaining their original signatures. Bone integrity is critical to many biomolecular studies including DNA amplification, stable isotope studies and all other archaeometric approaches. If bacteria are affecting bone postmortem then the extent and origin must be understood before these methods are employed or there is a risk of losing primary information. Another consideration is the preservation in-situ of archaeological cemeteries. In certain cases where funds are limited or when it would be preferable to leave skeletal remains in place, until a time at which a better understanding can be gained by novel sampling or exploratory methods, it would be advantageous to have some insight in to how much further damage may occur if indeed the remains are left in the ground. Being capable of predicting which bones or sites may be affected by bacterial attack would be beneficial.

This research presents an investigation of microbiological alteration of bone in the very early post-mortem period and will employ the use of light/polarized light microscopy combined with histological techniques applied to the morphological study of bacteria contaminating human and animal bone of varying origin. In archaeological and more recent bone it has been found that patterns of microscopic tunnels of varying shape, size and distribution can often be found (Ascenzi & Silvestrini 1984, Bell 1990, Bell *et al* 1996, Bell & Elkerton, 2007, Child 1995, Collins *et al* 2002, Grupe 2001, Hackett 1981, Hedges & Millard 1995, Jans *et al* 2004, Wedl 1864, Yoshino *et al* 1991). The evidence for these tunnels can be seen directly after bone has been thin sectioned and viewed microscopically as well as indirectly from measurement of bone porosity. This is believed to be as a direct consequence of the enzymatic action of collagenase-producing bacteria that are

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either present in the grave soil or in the microflora contained within the gut of the body (Child 1995, Collins *et al* 2002). It has not been proven or shown with any certainty which if any of these is the most likely culprit. It is known that bacteria from the gut are capable of transmigrating to the normally sterile blood and surrounding soft and hard tissues within hours of somatic death once they have broken through the intestinal mucosal barrier (Canavan & Southard 1914, Janzen 1977, Jensen 1944, Kellerman *et al*, 1976, Melvin *et al*, 1984, Roberts & Mead 1986). Once there, they can begin to colonize the bone and eventually destroy the collagen fraction which is believed to result in tunnelling of the bone structure. Soil bacteria may equally be responsible for this phenomena or it may perhaps be a consequence of the mutual action of both soil and gut microbes. This problem will be investigated further in this thesis in an attempt to clarify the situation somewhat.

The main objective of the research is to determine the earliest point at which bacteria can become established in bone post-mortem; to evaluate how quickly tunnels become visible and to elucidate a possible culprit; exogenous versus endogenous. A very short time span has been suggested for the advent of this type of decomposition that lies within the very early postmortem period and this is in the realm of months rather than years; however this remains unproven and sometimes unconvincing. Archaeological studies of this particular type of diagenetic change are increasingly common but there remains disagreement between authors as to when bacterial tunnelling happens and also which microbes are responsible (Bell *et al*, 1996, Child 1995, Hackett 1981, Hedges & Millard 1995, Jans *et al,* 2004, Yoshino *et al*, 1991). A thorough review of the literature available will be conducted.

If the process of bacterial attack in bone is to become explicit it becomes imperative to appreciate any specific corpse's decompositional history from the time of death to the point of recovery as both environmental and pre/post burial conditions will affect the prevalence of micro-focal destruction (MFD). When looking at an endogenous origin of MFD it is fundamental to investigate death assemblages of organic origin that are recovered from the ground and its surface and this particular subject area is often termed 'taphonomy' or 'forensic taphonomy' dependent upon the time period being researched. The research reported here falls in to both realms as both the results of modern forensic experiments and studies of archaeological material are combined. The term taphonomy is borrowed from palaeontology and is derived from 'tapho' (=burial) and 'nomos' (=laws). Taphonomy is the scientific study of the

processes of decomposition of remains and the accompanying events up until the period of recovery.

Human Osteoarchaeology is the study of human remains from archaeological sites, most frequently these remains exist in the form of skeletal material. The preservational state of this material is often markedly different from site to site and even between different depositional contexts in a single site and may range from a mere shadow of a body buried in corrosive sand or to a beautifully preserved complete skeleton that has been recovered from a less caustic environment. Because bones and teeth are often the only tissues we are left with it becomes imperative that they are studied to the best effect. Yet in the past, bones have been dealt with mainly at the macroscopic level with a view to establishing life history properties such as age, sex, height, pathology and demographic reconstructions. These are all vital elements to an osteological assessment and form the basis of most good osteoarchaeological reports. At the microscopic level however, there is still much knowledge to be gained as to how the bones themselves degrade over time. Degradation of the hard tissues of the body is dependent upon many variables including soil type, pH and depth and duration of burial. These confounding factors are further compounded by treatment of the body prior to burial which may include factors such as disarticulation, cooking, scavenging, excarnation and trampling. Once all of the above have been considered it is then possible to view bone within its burial context and to attempt a reconstruction of its past treatment and how decomposition commences as a direct result of these agents. This research relates to one component of the process of degradation, the microbial decomposition of bone in the burial environment.

After death has occurred in humans the body becomes a substrate for the continuation of life for various vertebrate, invertebrate and microbial fauna such as carnivorous scavengers, the larvae of blow flies and especially micro-organisms. Many studies of predator scats and of entomological succession have been reported and these two areas of research are relatively well understood (VanLaerhoven & Anderson, 1999, de Carvalho & Linhares, 2001, Hobischak & Anderson, 2002). The effects of microbial degradation however are the least understood. The research presented here aims at least in part to address this deficit by examining the microbiology of death by looking at both archaeological materials and also with the application of experimental approaches to simulate the modern forensic setting.

This research project was originally intended to be a study of the decompositional processes affecting juvenile bodies and whether or not small bodies decompose differentially to those of adults. At an early stage of the research it became clear that a difference in decompositional rate may be attributable to bacterial activity in the early post-mortem period. Therefore decomposition of bone via the agonal invasion of endogenous intestinal bacteria became the main focus of the study. Bacteria require certain environmental conditions to successfully survive and reproduce, with temperature and pH playing the largest role (Alexander, 1977). In addition to this the bacteria that can use bone as an energy source must have some particular adaptations, including the ability to produce a collagenase enzyme as well as creating chemical conditions appropriate for dissolution of bone mineral (Collins *et al*, 2002).

In addition to this research a series of forensic experiments have been designed that use domestic pigs as an analogue for humans. This is entirely necessary due to the restrictions on the use of human soft tissue. The research that is presented here could be carried out using human cadavers, but both the Human Tissue Act, 2004 and ethical issues make this unlikely to happen. Of the two, ethical considerations are probably more likely to prevent this type of research and ethical permission would have to be granted from the associated university. In the United States a human research facility does exist and it is commonly known as 'The Body Farm' but religious views make it unacceptable to some people. The Human Tissue Act, 2004 replaced the Human Tissue Act of 1961, the Anatomy Act of 1984 and the Human Organs Transplant Act of 1989. It was introduced as a reaction to the retention of body parts without proper consent at some hospitals, most notably, but amongst others Liverpool's Alder Hey. The Act of 2004 is ostensibly about consent, making this the most important principle in the lawful retention of human tissue.

Although bodies after death can be donated to medical science, this is generally geared towards leaving your corpse to an Anatomy school. The scheduled purposes that require consent are listed in Table 1 (below). Part 4, obtaining scientific or medical information about a living or deceased person which may be relevant to any other person (including a future person), seems to suggest that bodies may be donated if relevance can be proved.

Using pigs as an analogue for humans presents its own problems due to the dissimilarity between them. Because pigs are being used instead of humans it will

be necessary to look at the differences in microbial loads and species diversity of pigs, human adults, children and stillborn babies as all of this will affect any eventual tunnelling found. Traditional microbiological culturing methods are still most widely used and this may lead to a biased view of the aforementioned. Intensively bred pigs will be used for the experimental work as they are freely available and it does not require the killing of any animals. Organic pigs would absolutely be preferable but these would have to be killed for this reason which entails both moral and ethical dilemmas. Pigs farmed intensively bring their own pitfalls in that their gut flora may be modified because of farming practices such as the use of antibiotics and feed additives.

Part	Scheduled Purpose Requiring Consent
1	Anatomical examination.
2	Determining the cause of death.
3	Establishing after a person's death the efficacy of any drug or other treatment administered to him.
4	Obtaining scientific or medical information about a living or deceased person which may be relevant to any other person (including a future person).
5	Public display.
6	Research in connection with disorders, or the functioning, of the human body.
7	Transplantation.
8	Clinical audit.
9	Education or training relating to human health.
10	Performance assessment.
11	Public health monitoring.
12	Quality assurance.

Table 1. Purposes requiring consent for the use of human tissue. (Data taken from Human Tissue Act, 2004).

If soil bacteria are associated with the tunnelling of bone it becomes necessary to look at how this may be possible. Environmental microbiology has a much wider spectrum of diversity and although it is known that collagenolytic bacteria are present in soils this will vary from site to site especially when looking at differences between varying soil types (Alexander, 1977, Hackl *et al*, 2004). Depth of burial (Doryland, 1909) will have a direct effect on both which microbes can colonize a body and those that can work efficiently in the low temperatures found in most English grave soils. Yet again, culture methods used to enumerate soil bacteria are not ideal (Davis *et al*, 2005). Predaceous scavengers and fly larvae will affect microbial loads but within this study the main focus will be the latter. Maggots, which work to clear up natures dead corpses, are responsible for removing and

destroying indigenous microbes as well as any that infect the corpse from the surrounding microenvironment. This aspect will have to be considered when looking at those bodies left on the grounds surface.

Diagenetic change is dependent upon various factors, one of which is the structure of the bone. This is fine when looking at archaeological samples of human bone but the experimental research uses pigs which have a rather different skeletal composition that sees bone being formed mainly of plexiform bone rather than the haversian systems seen in humans.

The experimental research is supplemented by the histological analysis of adult and juvenile archaeological samples of human bone as well as archaeological samples of pig, sheep, cattle and canine bone. These are taken from a variety of sites and differing time periods. Bacterial assay tests have also been carried out to determine at what point bacteria can be found in bone. This involves the use of specific dyes and procedures that enable visualisation of the microbes within the bone structure before any tunnelling can be identified. No attempts have been made to type the bacteria to species as this is outside the remit of this study and would be incredibly time consuming and very possibly unhelpful as any bacteria found may not be those responsible for microfocal destruction.

This research will make an important contribution to our understanding of the processes that effect the survival of skeletal remains in the archaeological record. Furthermore, the integrity of bone at the microscopic level is a major consideration when applying analytical methods such as chemical and biomolecular analysis.

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Chapter 2: Taphonomy, Necrology, Biostratinomy and Differential Burial

2.1 Introduction

Taphonomy is the study of cadaver decomposition and is a broad term that covers the entire period from the point of death to the point of recovery of the remains. Within this discipline are the processes of death (necrology), biostratinomy (stage between death and burial), burial, and diagenesis (processes affecting a corpse postdeposition). All of these factors will affect what is recovered and its final exhumation condition. Because bacterial diagenetic change is dependant upon these earlier processes, it is essential to understand how any recovered skeletal elementals have previously been treated as microbial invasion of bone will depend upon the loads available which may be higher, lower or non-existent depending on the state in which the body enters the ground. Skeletonization would be seen as a prerequisite for the commencement of MFD (micro-focal destruction) if soil bacteria were to be found responsible for this phenomenon making it necessary to understand these prior decompositional processes.

This chapter will deal with the taphonomic factors at work on any given corpse and will deal specifically with cadavers from the moment of death to the point of skeletonization or mummification/adipocere formation. It will not cover the more specific area of bone diagenesis and destruction as this will be covered in a later chapter in detail (Ch 5). There will also be a review of what has already been achieved experimentally and this will focus on studies using human cadavers and also those that use the carcasses of pigs, rabbits, rats and other animals. A short look at entomology and death will be necessary due to the close association between insects and soft tissue decomposition. This is a very brief introduction to early post-mortem changes that is far from conclusive but that offers a small insight in to the many factors that affect dead bodies.

2.2 Necrology: Microbes and Death

The very first process affecting the body is the point of death which is usually determined by the cessation of the circulation and respiration followed by a series of processes that occur at varying points in time and that include, cooling of the body, muscular rigidity (rigor mortis), postmortem staining (livor mortis/hypostasis) and putrefaction (Smith, 1945). Cooling of the body is determined by the immediate surroundings and may be affected by bed covers, clothing, climate, and refrigeration

at a morgue; it is also affected by the condition of the body and age, and by the mode of death (Kerr, 1946). Cadaveric rigidity (fig 2) is also governed by temperature and may occur earlier in cases of electric shock or by muscular exhaustion, whilst cadaveric lividity is a staining of the skin caused by blood gravitating to the lowest-lying parts of the body (Guy & Ferrier, 1888).

Fig 2. Postmortem rigidity and bloating. Newborn Pig. (Author, 2006).

Putrefaction marks the commencement of the destruction of the cadaver and commences internally almost immediately and is usually visible externally within 48-78 hours after death has occurred. This process is two-fold with enzymatic and bacterial decay working in tandem to breakdown the soft tissues (Polson & Gee, 1973). Enzymatic decay better known as autolysis (self-destruction of body cells) is denoted by cell death and then destruction by body enzymes (Dix & Graham, 2000). Intestinal bacteria play a large part in putrefaction and this can firstly be seen in the discoloration of the skin of the right flank (around the cecum) of the abdomen (fig 2.1). This greenish staining then spreads to cover the entire body and is caused by the breakdown of haemoglobin in the red blood cells by intestinal bacteria (Jackson & Jackson, 2004). Green discolouration of the skin is derived from sulphur-containing compounds as the haemoglobin undergoes chemical change (Gordon *et al*, 1975).

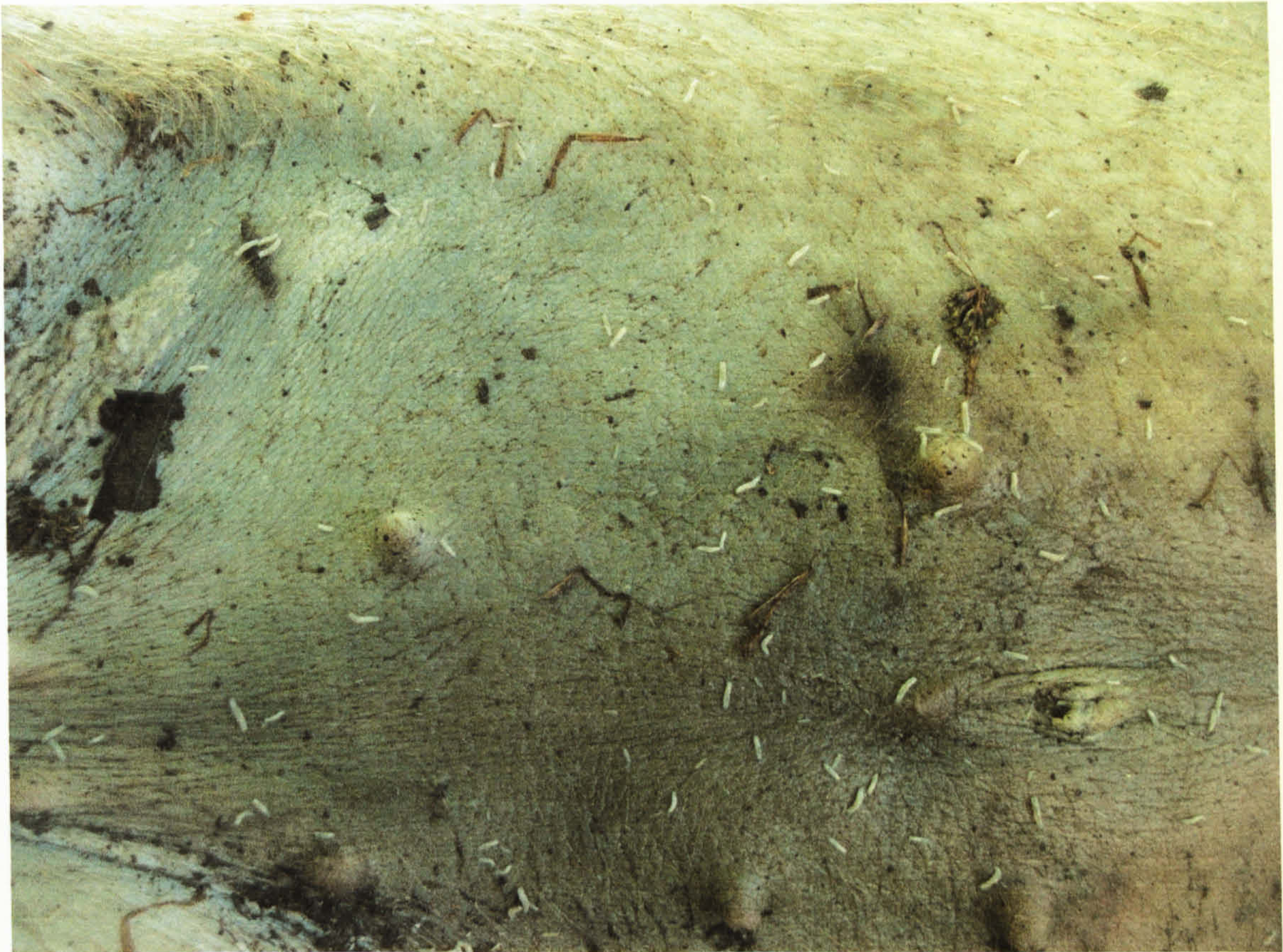

Fig. 2.1 Green staining of the abdomen. 50kg Pig. The staining occurred gradually over a number of days after prior freezing. (Author, 2007).

The bacteria involved are largely anaerobic due to a drop in oxygen levels after death. Normally, during life homeostatic mechanisms prevent bacterial overgrowth but as these cease at death the unrestrained growth of bacteria begins and is fuelled by the copious quantities of carbohydrate, protein and fat breakdown products which are released by autolysis (Clark *et al*, 1997). Bacterial transmigration will be discussed in detail in the next chapter. Once putrefaction has begun three main changes can be seen; changes in the colour of the tissues, evolution of gasses in the tissues and liquefaction of the tissues (Gordon & Shapiro, 1975). The physical state of a body at the time of death will impact on how the body decays and emaciated bodies tend to skeletonize rapidly, whilst in heavier persons there may be a trend towards the formation of adipocere in the fatty areas such as the breasts and buttocks (Mant, 1987). Septicaemia or blood poisoning (i.e. MRSA and wound sepsis) directly affects the rate at which a body decays. If a person dies with septicaemia then their blood will already be compromised by the influx of large numbers of bacteria in to the bloodstream (Perper, 1993). Such infections can lead to a rapid progression in putrefactive rate especially within the liver. Clostridium species have been indicated as contributing to this phenomenon in two sixteen year old children. The first, a boy, was found to have high-grade putrefaction of the

mucous membranes at autopsy and the authors state that bacterial sepsis should be considered a causative agent in cases of unusual advanced decomposition (Hausmann *et al*, 2004). In the second case, a 16-year-old girl was autopsied the day following her death from a clostridia infection and was found to be in an advanced state of decomposition (Totten, 1979). Breakdown of the soft tissues will persist until a point of either skeletonization or mummification.

2.3 Pre-burial Modifications and Differential Burial Practices

How bodies decompose after death is complicated by multiple factors. The early post-mortem changes have been noted above and decomposition is then further dictated by biostratinomy, how the body is dealt with prior to its interment, and then yet further by burial practice and soil conditions. So many factors contribute to the eventual end product of skeletonization or mummification that the outcome is not fully predictable. Decay may be accelerated or delayed (table 2) dependent on amongst others; the restriction or supply of oxygen, freezing and thawing, presence or absence of insects and scavengers, burial or ground deposition, soil type, flora and fauna, clothing, and obesity or anorexia (Gunn, 2006).

Stage of Decomposition	*State of Remains*	*Physical and Environmental Variables*
Initial Decay	Gases are produced internally, microorganisms are active, flesh remains intact and fresh, no discolouration or insect activity	Small versus large body size (small bodies decompose at a faster rate) Cold climate (decomposition can be slowed or halted if body is frozen, cold dry climate decay is slower than cold humid climate)
Putrefaction	Internal gasses bloat the body, microbial activity is intensified, there is a strong odour of decay, and bodily fluids are purged (fig 2.2)	Whole versus wounded/dismembered (whole bodies decompose slower than separate body parts)
Black Putrefaction	Odour of decay intensifies, gasses and fluids are purged as the body collapses, internal organs take on a creamy consistency, and exposed flesh blackens.	Hot climate (hot humid climate accelerates decay, hot dry climate decomposition slows, dry heat desiccates flesh) Nude versus clothed/wrapped (nude bodies decompose faster
Butyric Fermentation	There is a slow drying of the tissues, exposed flesh ferments.	Moist and/or acidic soils (decomposition rapid in these types of soil)
Dry Decay	Flesh is drying out and becoming leather-like.	Contained and buried versus not contained and not buried (contained and buried bodies decompose at a slower rate
Skeletonization	Bone is exposed, most soft tissue will have gone and, there may be some residual ligament and cartilage remaining, desiccated tissue or mummified tissue covers less than one half of the skeleton	Sand, clay and gravel soils with a high pH (decomposition slower) Presence of water (decompose slower unless scavenged by aquatic animals, cold to freezing water will substantially delay decomposition)
Extreme Decomposition	Skeletonization with bleaching/exfoliation/metaphyseal loss/cancellous exposure of vertebrae	Insect activity (accelerates decomposition) Carnivore and scavenger activity (promotes decomposition) Seasonal differences (winter burials require longer to decompose)

Table 2. Data modified from Galloway, 1997, Nafte, 2000, Mann *et al*, 1990.

Fig. 2.2 Purging of fluids and 'flattening' of body as it collapses during period of active decay (Author, 2007).

It is possible that the body may be disarticulated intentionally through the practices of butchery, de-fleshing or by excarnation. This will influence which bones will become buried and will also serve to sterilise the corpse by the removal of intestinal microbes from the body and hence also from the grave. Bones may be cooked or burnt which will again alter the microbial load and influence how quickly the remains will degrade.

Although in today's society it is usual for bodies in the western world to be either buried or cremated, this does not hold true in past cultures whose bodies enter the archaeological record. Many of the bodies may indeed follow these two pathways but there are others that will have been treated in a diverse manner of ways. The main protagonist of who is buried, when, where and how is man (Henderson, 1987). According to Huntington & Metcalf (1991:24): "Corpses are burned or buried, with or without human sacrifice; they are preserved by smoking, embalming or pickling; they are eaten – raw, cooked or rotten; they are ritually exposed as carrion or simply abandoned; or they are dismembered and treated in a variety of ways." Bodies may simply have been left where they died and would either become naturally buried or

remain on the grounds surface. They may have been afforded a sea burial where generally the remains will be lost, but if discovered their preservational state will be dependant upon many variables including, water and air temperature, access by scavengers, seafloor substrate and geology, water chemistry and the season of death (Sorg *et al*, 1997). Carnivores may modify remains both by removing them from the death site and also by ingestion. Pickering (2001) discovered that primate metapodials and phalanges recovered from carnivore faeces are often better preserved than larger elements. This is due in part to the fact that they are often swallowed entire unlike the larger elements which require extensive chewing prior to being eaten. Remains may be modified by amongst others; ants (Byard, 2005), pigs (Berryman, 2002), rodents (Koszyca *et al*, 2006, Tsokos *et al*, 1999), birds, reptiles and amphibians (O'Brien *et al*, 2007), canids (Haglund *et al*, 1989, Willey & Snyder, 1989), ferrets (Ferrant *et al*, 2008), hamsters (Ropohl *et al*, 1995), squirrels (Klippel & Synstelien, 2007) and bears (Carson *et al*, 2000).

It was not unusual in the past to embalm bodies, either by applying unguents known to have preservative properties such as honey, red pepper, vanilla, nuts and mushrooms all of which contain compounds that inhibit bacterial growth (Sledzik & Micozzi, 1997) or alternatively by removal of the viscera. A continuing theme amongst those of the catholic faith is the incorruptibility of the bodies of saints. There are literally hundreds of documented cases of saints who reportedly refuse to corrupt. The reasons for this are numerous, not least the effects of evisceration that successfully removes the intestinal gut flora from the remains leaving a somewhat sterile environment. Many Saints bodies were treated in this way and are often recovered in an incorrupt state. An Italian pathologist who was allowed to examine a number of bodies found that six had been preserved by both embalming and by the application of unguents (Fulcheri, 1996). This is then further aided by the application of known preservatives. Many of the saints were practising ascetics, otherwise known as 'Holy Anorexics' who deliberately starved themselves in both an explicit attempt to become closer to God and from a desire to be holy (Davies, 1985). These men and women were not averse to starving themselves even if this led to the unfortunate occurrence of their deaths and dying with an empty bowel would improve their chances of being preserved significantly.

Similar to this procedure is the act of mummifying bodies. This can either be natural, such as the bodies of newborns that are sterile, or in the case of adults that are placed in locations conducive to desiccation of the bodies. New and stillborn

babies are known to mummify and this is sometimes regarded as a natural conclusion due to their lack of gut bacteria and this is probably aided by their clandestine and generally warm hiding places that include airing cupboards and wardrobes. Often the bodies are wrapped in newspaper or cardboard which would serve to dry out the corpses further. It may also be by artificial means by either embalming or placing of the body in areas known to be preservative such as intramural locations (vaults, crypts, catacombs). Both embalming and mummification are extreme modes of modification that will seriously affect decomposition due in the case of artificial mummification to the removal of gut bacteria. In natural mummification, it is an issue of postmortem tissue dehydration overtaking the action of decomposition (Lynnerup, 2007). Basically, bacteria require water if they are to efficiently produce enzymes and if the body dries quickly then the process of bacterial putrefaction is halted.

Bodies may be preserved in peat bogs, environments that are known to have antibacterial properties. Bog moss (sphagnum) releases a substance known as sphagnan that dissolves in the bog water and converts to humic acid which results in the bacterial growth being retarded (Fischer, 1996). In this case the skin is often better preserved than the skeletal material and is presumed to be as a consequence of intestinal microbes reaching the bone structure before the viscera is infiltrated by bog water (ibid). Extensive demineralization of the skeleton is caused by the acidic environment of the peat and in a study of an Iron Age bog body from Germany, it was found that 92.7% of the bone mineral density had been lost (Schilling *et al*, 2008). The spatial structure of the bone was found to be very well preserved but this is in direct contrast to most other bog bodies where skin and hair are well preserved but where there is dissolution of the internal organs and the bones do not generally survive (Omar *et al*, 1989). Having said this, the bones of the bog bodies Zweeloo, Tollund and Meeny-braddan are quite well preserved. Brothwell and Gill-Robinson (2002) note that the bones of the hand and femur of Lindow II have something similar to microbial attack in the form of punched out lesions in the microstructure and therefore believe that microbial damage does at times occur despite the antimicrobial action of the sphagnan and this is somewhat corroborated by the work of Bell (1996) where modern forensic remains from a muskeg bog exhibit bacterial damage. Evershed & Connolly (1994) believe that two different mechanisms of decay operate differentially on muscle and skin, whilst bone was not researched in this case. However it does appear that skin is the best preserved part of the body with the collagen fibres being remarkably intact and in all cases where soft tissue is

preserved there is tanning (Painter, 1991). Adipocere that may form in bog bodies is composed mainly of fatty carboxylic acids indicating that both extensive reduction and oxidation have occurred during burial (Evershed, 1992).

Bodies are occasionally buried within sealed iron coffins and these also appear to slow down the decompositional process especially when combined with embalming as was often the case in the late 19thC (Owsley & Compton, 1997). In a study of lead coffins buried for a period of 150 years, it was found that many of the corpses had preservation of the internal structures, skin and external features, caused by the airtight nature of the coffin producing the right conditions for the formation of both adipocere and mummification (Green, 2006).

Another preservative process is the formation of adipocere which is regarded as a spontaneous inhibition of postmortem changes that makes a corpse almost completely resistant to decomposition in an unchanging environment (Fiedler & Graw, 2003). Adipocere formation is caused by a postmortem conversion of adipose tissue in to a solid material comprising fatty acids (Forbes *et al*, 2004) and both aerobic and anaerobic bacteria are involved by microbial conversion of various unsaturated fatty acids to hydroxy fatty acids (10-OHFA) (Takatori, 2001). This may occur in a variety of situations including terrestrial and marine environments. In cold sea water adipocere has been observed as early as 38 days postmortem (Kahana *et al*, 1999). It is known to occur in other animal species including pigs, cattle, sheep and rabbits and there is no evidence of any fundamental difference in composition although there remains a difference in formation with regards to time (Forbes *et al*, 2005). In research by Notter *et al* (2009), adipocere was found to form sooner in pigs than in humans and this was attributed to differences in the distribution of total fatty acids between species. O'Brien & Kuehner (2007) set up experimental research in to adipocere formation in aquatic contexts and named it the 'Goldilocks Phenomenon' because each environment must be 'just right' for adipocere to form and that both water and bacteria must be present. Although adipocere is thought to be a phenomena linked to water, such as wet burial sites or marine settings it can also be formed in dry settings. An elderly woman who had been sealed in a box and covered by many plastic bags was shown to have significant adipocere formation at the time of her discovery (Nushida *et al*, 2008). Factors such as the soil having a mildly alkaline pH, warm temperatures and anaerobic conditions promote the formation of adipocere (Forbes *et al*, 2005). Further research by Forbes *et al* (2005) in to the effect of soil type on adipocere

formation concluded that it could form in a range of soils and that bodies buried directly in soil rather than within a coffin were more likely to exhibit these fatty changes. They also speculate that soil bacteria plays no part in the process, rather the microbes originate from within the rotting corpse.

Excarnation is another postmortem act that will also affect what is buried, recovered and the amount of microbes available within the body. Redfern (2008) reports on Iron Age burials from Dorset that were excarnated, given a secondary burial and then selected bones were incorporated into structured deposits. The remains also demonstrated marks that corresponded to animal gnawing, dry fractures and perimortem blunt force trauma. Two skeletons found in a cairn at Loch Borralie had also been left above ground for some time prior to burial, as although the bodies were recovered from grave cuts, extensive scavenging had taken place (MacGregor, 2003). Similar to this (Reilly, 2003) are the remains recovered from Neolithic cairns in Orkney that had been interred articulated, allowed to decompose in-situ, then removed as disarticulated remains, whilst leaving the skulls in place or removing them to other sites of importance. All of these burial practices acutely influence what will be recovered and its condition. How you die, the diverse range of biostratinomic processes and burial practices as noted above have served to alter what we find in various ways. Diagenetic change will eventually cause more alteration and damage.

2.4 Experimental Research: Decomposition Studies

Because so many post-mortem changes take place it has become necessary to try and clarify matters by the use of experimental protocols. Most of this research has been carried out where pig or other animal cadavers (but also human) are sited within different environments (buried, left on the ground) and then recorded in respect of length of time for decomposition to be completed, insect activity, scavenging patterns, and body odour analysis (Wilson *et al* 2007, Aturaliya & Lukasewycz 1999, Prieto *et al* 2004, Morovic-Budak 1965, Weitzel 2005, Micozzi 1986, Archer 2004, Tibbett *et al* 2004, Wiltshire & Turner 1999). A large amount of this research is completed in the USA and often with Federal funding. Regrettably, much of this valuable data cannot be applied to decomposition in England where the climate differs enormously from America and is generally cooler and wetter and with less seasonal variation.

Complete skeletonization of a corpse can take many years in a burial environment and is highly dependent on geography. Bodies buried at depth in Germany may skeletonize within 8 years but this may sometimes take twice as long (Breitmeier *et al*, 2005). Bodies afforded a shallow burial in summer will probably skeletonise within 6 months; the same circumstances in winter may lead to mummification of the corpse due to cold temperatures halting the decompositional process. The timescales given vary from author to author and from place to place and Rodriguez (1997) suggests that those given a deeper burial take between two to three years to skeletonize although in some cases this will be considerably longer. Burial depth appears to be of great consequence to how fast a body decomposes; deep burials will be subjected to more constant temperatures especially in temperate climates. The deeper the burial, the slower the rate of decomposition as demonstrated by bodies buried at the research facility in Tennessee (Rodriguez & Bass, 1985). The length of time a body is buried for coupled with the time of year are both deemed significant factors whereas postmortem changes such as trauma and autopsy do not exert any influence in decomposition. Schultz (2007) carried out experimental research and also concluded that although time is highly significant to decomposition so too is burial depth. Decomposition at depth is much slower due to limited access by carrion feeding insects, cooler temperatures and protection from predaceous scavengers. When looking at soil type it was found that cadavers buried in clay decomposed slower than those buried in sand. Weitzel (2005) also looked at variables such as depth and the use of clothing. In this study the deeper burials tended to decompose faster, however it must be noted that these were still very shallow and also that some of the pigs that decomposed quickly were smaller. It was also noted that the application of clothing tended to slow down the decay process of the piglets.

Decomposition has been seen to advance differentially when animal carcasses are placed in either full sun or under the shade of woodland. A temperature based difference at the two sites resulted in the pigs from the woodland site decomposing slower under the influence of a slightly cooler setting that to some extent inhibited the destructive action of maggot larvae (Shean *et al*, 1993). If for any reason the decomposition process is slowed down or halted then mummification may occur. Many of the corpses from the 'Body Farm' have mummified soft tissue on the exterior of the bones, although the internal organs are lost and this is most likely caused by the extreme high temperatures in Tennessee. In southern Arizona

mummification is also more frequent due to the aridity and high summer temperatures (Galloway *et al*, 1989).

Komar (1998) looked at decay rates in a cold climate (Edmonton, Alberta) expecting decomposition to be retarded and it was discovered that in summer a body could skeletonize within 6 weeks and in winter by 4 months. The winter decomposition rate was faster than expected as the ground can remain frozen for long periods which should act in a similar way to cold storage/refrigeration. However, two significant factors were introduced that reflect a modified form of decay; scavengers reduced many of the remains and in other cases there may have been a freeze thaw effect. Micozzi (1986) found that previously frozen carcasses when thawed decayed slightly quicker than those that had not been frozen. Contrary to this are the findings of Stokes *et al*, (2008) who also investigated the effect of freezing prior to burial on the decomposition of soft tissue. They found the process to have no significant impact on decompositional rates but did note that microbial activity was significantly reduced. However, the soft tissue used in the study was skeletal muscle portions which should be free of enteric microbes. Both authors (ibid, Micozzi, 1997) believe that freezing will at least in part have some kind of biocidal effect against endogenous microbes.

Vass (2001) introduced a formula for soft tissue decomposition (to a time when the body has either skeletonized or mummified) for cadavers placed on the ground. The formula provides a rough estimate for decomposition by taking the figure 1285, finding the average temperature for the duration of decomposition (say 10°C) and dividing (1285/10=128.5 days to skeletonize or mummify). Using the formula in this country would probably lead to a hefty discrepancy that would be accounted for by the very high temperatures in America. The use of 'accumulated degree days' has recently been introduced that utilises a point based system to score decomposition in defined areas of the body. These scores are then added together and used with known temperature data to provide a possible postmortem interval and this research suggests that decomposition is linked to accumulated temperature and not just time (Megyesi *et al*, 2005).

All of the taphonomic studies carried out are subjected to disturbance for data collection and it was thought that this may disrupt and alter the progress of decomposition rates. However, recent research in to this problem showed that repeated physical disturbance did not alter decomposition times and no detrimental

factors were determined (Adlam & Simmons, 2007). This is most likely the case as such events tend to be very short-lived with disturbance generally lasting no more than a few minutes before a return to a controlled environment is resumed.

2.4.1 Experimental Research: Entomology

Many of the decomposition studies are heavily based on entomology and the succession of fly larvae (VanLaerhoven & Anderson, 1999, Carvalho & Linhares, 2001, Hobischak & Anderson, 2002) and also development rates to predict postmortem interval (Anderson, 2000). This is unsurprising as they have the greatest ability to affect the rate of reduction in a corpse (fig 2.3). Blowflies lay batches of eggs on corpses within a very short period after death (Smith, 1986, Anderson & VanLaerhoven, 1996) when temperature and accessibility permits. Oviposition usually occurs during daylight hours but has occasionally been observed nocturnally (Singh & Bharti, 2008). Once hatched the larvae move through three distinct larval stages that are classified as instar stages 1, 2 & 3 before leaving the corpse and pupating. Maggots have very soft mouthparts and digestive enzymes are released that help breakdown the food. Larger carcasses appear to be more attractive to the blowflies (Erzinclioglu, 1996) as presumably these are capable of sustaining many more larvae than a smaller corpse. Bodies that are buried are afforded some protection from larval activity and those species that can burrow are limited; which fauna can reach the corpse depends on the nature and depth of burial. Blowflies may be completely excluded by a covering of soil only 2.5cm deep (Smith, 1986). However, Collembola (spring tails) and coffin flies have been recovered from a corpse buried 1.8m in depth within an unsealed coffin inside an unsealed cement vault, 28yrs after death, showing that at least some flies can access deeply interred remains (Merritt *et al*, 2007).

Fig 2.3 Maggot activity exposes ribs in 10kg pig with postmortem slash wound to abdomen (Author, 2007).

Insect activity plays a crucial role in the stages of decomposition and Rodriguez & Bass (1983) relate that the four separate decay stages of fresh, bloated, decay and dry occur at timescales according to both climatic conditions and carrion insect populations. Putnam (1978) found that in reality there are two major pathways of decomposition that are characterized by the presence or absence of blowfly larvae. Seasonality and habitat did not affect decomposition in the same way (according to Johnson, 1975, differing species colonise corpses according to seasonality), although carcasses deposited in the winter or spring tended to decompose slowly and there was a propensity for these bodies to mummify. This was confirmed by Wang *et al* (2008). Payne (1965) constructed cages that either allowed insect access or prevented it when looking at decomposition in the summer months. The pig carcasses in the cages that excluded insects took many months to decompose and after 100 days 20% of the pig still remained in a mummified form.

This would suggest that those bodies that mummify may have lower microbial loads due to the drying of the corpse which then becomes unattractive to microorganisms. Joy *et al* (2006) found that pigs in shaded areas tended to decompose slower than those in full sunlight, with lower maggot mass temperatures, and smaller third instar larvae. Centeno *et al* (2002) also found differences between sheltered and unsheltered pig carcasses. Kočárek (2003) found more coleopteran species colonised corpses in forest sites than meadow sites. Destruction is also caused by beetles that are often involved in corpse reduction at a later stage, feeding on mummified tissue and hair. Larder beetles feeding on mummified tissue can reduce a body to skeletonized remains in 5 months under optimum conditions (Schroeder *et al*, 2002).

2.4.2 Experimental Research: Children

Experimental research in to decomposition rates in children are rare but it is necessary to provide a summary of what is known as much of the current research project looks at sterile versus non-sterile bodies (i.e. fetal bodies are known to be sterile and this is discussed in chapter 3). It is a known fact that very young children are often grossly underrepresented in the archaeological record. This is especially true of the Anglo-Saxon period and Buckberry (2000) suggests a number of reasons including both taphonomic and cultural factors. Lewis (2007:37) argues that the bones of children have exactly the same "potential to survive well in the same conditions that allow for the good preservation of adult bones." No experimental studies have been carried out to see if taphonomic factors rather than social or

cultural reasons could be the cause of this deficit. To gain an insight in to this discrepancy it is necessary to have some understanding of past cultural and social practices of communities that no longer exist. It is impossible to say with conviction what people in past societies believed but from modern studies carried out in developing countries it is possible to speculate (albeit with limited accuracy) about how they lived and their thoughts on life and death. Living in less blessed times before the advent of modern medicine, may have led to a belief system where children were not seen to be as important as adults, only reaching a certain level of value and autonomy at a pre-determined age; possibly at a point where endemic childhood diseases had diminished to a point where it was presumed that survival was more likely than imminent death. One scenario that is possible is that in times of great need the exposure of one mouth too many to feed may have been a necessary course of action if others were not to suffer. Any child born with a disability would have even more chance of being left to die in a field or remote location rather than a lifelong investment that may restrict the carer to a life of even worse proportions and hardship than really necessary. These abandoned children would possibly not be seen at the point of excavation in a bounded cemetery as their bodies may have been hidden or left in places where they would not be found. It is with great difficulty that we have to impose very different views from our own on to women in the past that often lived in very difficult times were altruism could prove to be an expensive luxury. Babies that were stillborn or died within the early postnatal period before the application of certain religious rites or ceremonies may be excluded from normal burial grounds due to a belief of them being polluted or impure. This again directs bodies outside the normal confines of burial areas and into unknown spaces that are not likely to be excavated on a regular basis unless they are within the confine of living quarters or the immediate surrounding vicinity.

These are of course just some of the possible reasons why children are more difficult to find from the past. Even the child that is loved and wanted but that dies in the first couple of years will in all likelihood be treated differently at the time of burial. Adults are large and bulky requiring graves that can contain their considerable size, but very young children can be buried in undersized, shallow graves; after all it would be unlikely that anyone would dig a grave six feet deep for something so small; graves are usually cut to size. Unfortunately with shallow burial comes the possibility of scavenging by carnivorous predators looking for their next easy meal. A body weighing only a few kilograms may be removed from the grave site in its entirety and this would ultimately lead to a 'missing child'. Even if the remains are

not scavenged they may be subjected to trampling and/or possibly the effects of ploughing and they are also more likely to be disturbed during subsequent grave cutting. Adult bodies are often subjected to this latter scenario and therefore children must be at even higher risk due to being buried closer to the surface. It is also possible that excavator error or unfamiliarity with foetal or infant bones may mean that children's remains are not recovered because they are not recognised as such or are entirely missed due to their size. It is well known that even excavations of adults have often in the past been inadequate as the smaller bones are not necessarily visible or diagnostic of being human to the untrained eye.

When looking at taphonomic reasons for this childless world, Guy *et al* (1997) argue that both low mineralization of the bone combined with its poorer quality may explain why their remains are not well preserved. They also note that small bones are more likely to be crushed and are attacked more easily by organic matter decomposition or by acid soils. A few studies have been conducted using child-sized remains, but these are not generally focused primarily on actual decomposition. For instance, Morton and Lord (2006) used small pigs for research in to scavenging and scattering. Whilst decomposition is noted, the emphasis on these areas detracts from decomposition without the hindrance of these secondary factors. This research was also limited by the size of the carcasses chosen. The remains ranged from 11.25-27kg and were meant to reflect the size of children aged to 2-11 years. No reason is given for the exclusion of smaller animals but it is probable that anything less than this would be taken away whole which would be counterproductive to their research. They did however discover some interesting facts in that vertebrates would avoid feeding on the carcass if invertebrate colonization had already commenced and scavengers would wait until the invertebrates had migrated away from the corpse before utilizing them as a food source. Further to this, there was found to be no apparent succession order of vertebrates feeding on the corpses. An increase in scavenging by vertebrates is seen in cooler weather and this is probably due to the fact that flies find it more difficult to locate carcasses when odours are not as apparent (DeVault *et al*, 2004), added to this is the fact that flies do not fly below 10°C. Schultz (2007) researched several decompositional variables using pigs as a proxy for humans and concluded that smaller-sized cadavers (i.e. those of child size) decomposed faster than larger cadavers. Gremillion (2005) looked at child sized remains with regards to insect colonization and found that decomposition was delayed where environmental conditions were not conducive such as times of rain, the presence of clothing and

low temperatures. The small carcasses were also subjected to scavenging by foxes even when covered by cages. Although Gremillion found rain to be counter productive to decomposition, in another study by Archer (2004) high temperature combined with rainfall actually reduced the time taken to decompose. The study focused exclusively on neonatal remains (using *Sus scrofa* as a proxy) and therefore this result cannot be directly extrapolated to adults. However, Archer states that rainfall is likely to have the same effect on larger bodies. This is mainly because the influx of water mechanically breaks up flesh, keeps the surrounding soil moist and most significantly rehydrates soft tissue, which in turn allows maggot ingestion and permits re-colonization by maggots.

Taphonomic factors are much less likely to be the cause of the shortage of juvenile remains than cultural reasons. Most likely is that the babies are not there in the first place and in cases where children's remains are recovered from archaeological sites they appear to be just as well preserved as the adults. Having no direct correlates with data already gathered makes it difficult to presuppose what will happen to smaller or sterile corpses due both to their size and the English climate. Add to this the current difficulty of experimental research in this country because of the 'Human Tissue Act, 2004' (http://www.hta.gov.uk/about_hta/human_tissue_act.cfm) it becomes increasingly tricky to find appropriate research models and methods. The methods employed here are far from ideal but go some way to explaining the decomposition process of very young children both in this country and elsewhere.

2.5. Diagenetic Change

This is a brief introduction to diagenesis as it is covered in detail in chapter 5. Diagenetic changes to bone in the form of bacterial attack in the burial environment may be reliant upon the body skeletonizing prior to any destruction. This has in fact been suggested previously as a prerequisite for the commencement of bacterial diagenetic change. Yoshino *et al* (1991) advocate that it takes 5 years for a buried body to skeletonize and this roughly correlates with bone destruction beginning after this period. They also propose that histological change will be impeded for long periods and will be less advanced in those bones left above ground. Undeniably if soil bacteria were to be the main proponents in this phenomenon then this would be of upmost importance as bacteria would not be able to enter the bones until all the soft tissue had putrefied. Conversely, if gut bacteria are instead deemed to be responsible, then the rate of putrefaction would not be of importance rather other factors such as access by insects and predaceous scavengers would be more

significant. It is therefore a fundamental necessity that the decompositional dynamics of necrology, biostratinomy, burial and diagenesis are understood.

2.6. Summary

Because cadaveric decay is so varied in the way that it advances it becomes difficult to ascertain a direct path taken by any given corpse. And although this research looks at the bare bones that are left after putrefaction of the soft tissue has ceased, these prior degrading routes are fundamental to the onset of bacterial decay of the hard skeletal tissues. Small bodies of children may be scavenged, adults may be disarticulated, bodies may be buried at depth or given a shallow burial or left on the grounds surface, adipocere may form or a body may mummify. All of these issues will have a direct bearing on the availability of the microorganisms that will inhabit a corpse and ultimately destroy collagen from the cadavers' bones. This really is where the microbiology of death is at its most pertinent as the above will be the ultimate reason why we then find bones that are either free of bacterial destruction or those that are devastated by the tunnels and degradation that is wrought by microorganisms under the right conditions.

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Chapter 3. Microbiology of the Body

3.1 General Introduction

The main focus of this research concerns microbial degradation of bone and more specifically it investigates how microbes that are capable of producing a collagenase enzyme utilise bone collagen as an energy source and whether they cause the tunnelling that is then found in both archaeological and considerably more recent bone (as early as 3 months post-mortem). This tunnelling phenomenon is poorly understood and although it is believed to be as a direct consequence of bacterial activity, this remains a hypothesis rather than a proven mechanism. The tunnelling of bone post-mortem is believed to be a) as a result of endogenous gut bacteria, or b) a phenomenon caused by soil microbes, or 3) a combination of both gut flora and soil bacteria. Within the scope of the research presented here, domestic pigs (*Sus scrofa*) have been used as an analogue for humans. For this reason it is important to note that although much of what is discussed here is relevant to humans and other animal species there is also a discussion of how pigs may differ in the amount of bacteria present, the species of bacteria found and also the specific location of any bacteria present within both the gastro intestinal and oral tract.

3.2 Introduction to Microbiology

There are three main groups of bacteria that can be determined by their ability to grow either with or without a supply of oxygen. The first group is the aerobes that must have oxygen to grow; the second are the anaerobes which cannot survive in the presence of oxygen and the third group, the facultative anaerobes that are capable of growth with or without the presence of oxygen (Alexander, 1977). In addition to this microbes require sometimes very specific, host or environmental conditions. For the bacteria to grow this must include access to the appropriate nutrients within the body and external conditions such as temperature, pH level, and reduction potential and some of these are listed in table 3 below. Temperature is very important and most bacteria are designated as Mesophiles which are adapted to intermediate temperature and are the most common of the bacteria. With all bacteria there is a range of temperature over which they can continue to thrive and this is quite large, however they also have an optimum temperature which covers a much smaller range. Usually bacteria can operate at lower temperatures over a greater range whilst an increase of just a few degrees above the optimum will

demonstrate a sharply defined maximum range. In addition to this, microbes are pH sensitive with most bacteria being designated as Neutrophiles (Table 3).

Environmental and Host Bacterial Requirements		
Type of Bacteria		Temperature Range
Most Bacteria	Range	Can grow over a range of about 30°C
	Optimal Range	Narrow
Psychrophiles	Range	0°- 20°
	Optimal Range	15°
Psychrotrophs	Range	0°- 7°
	Optimal Range	20°-30°
Thermophiles	Range	>55°
	Optimal Range	55°- 65°
Hyperthermophiles	Range	>90°
	Optimal Range	80°-113°
Mesophiles (**most bacteria**)	Range	20°- 45°C
Mesophiles in mammalian body	Optimal Range	37°- 44°C
Mesophiles in environment	Optimal Range	30°C
Type of Bacteria		**Tolerant to pH Levels/range**
Acidophiles		<4.0
Neutrophiles (**most bacteria**)		5.5 - 8
Alkalophiles		8.5 - 11.5
Growth of Bacterial Populations	**Environment**	**Doubling Time**
E. coli	Optimal	20 mins
E. coli	Human Intestine	10 hours
Mycobacterium tuberculosis	Optimal	15-16 hours
Pseudomonas aeruginosa	Soil	2-3 days

Table 3. The requirements of bacteria that are needed for them to flourish

The experimental research presented here is concerned with those species of bacteria that are capable of producing a collagenolytic enzyme.

Two types of microbial collagenolytic enzymes are: (1) collagenolytic proteases which digest collagen macromolecules, and (2) aminopeptidases which recognize and digest collagen-specific sequences. True collagenase directly hydrolyzes collagen whilst proteases and gelatinases only hydrolyze gelatine, a denatured form of collagen (Watanbe, 2004:520-521). The collagenase enzyme cleaves collagen (a scleroprotein found in hair, skin, nails tendon and bone) into small peptides. The collagen chains are tightly twisted which increases their resistance to proteolytic enzymes and Type 1 collagen (the collagen found in bone) is strengthened further

by a covalent bond that makes the molecule more resistant to enzymatic cleavage (Child, 1995). In bone and other calcified tissues the collagen is further protected from chemical attack by being surrounded by mineral. However, hydroxyapatite can be solubilized by many different bacteria that produce acids which will then make it easier to gain access to the collagen (Grupe *et al*, 1993). During life collagen degradation can occur in certain forms of pathogenic microorganism invasion such as tuberculosis and also in other diseases especially rheumatoid arthritis and periodontic inflammation (Watanabe, 2004).

Thirty percent of the body protein in mammals is in the form of collagen (Harper, 1980) and it accounts for more than 70% of the dry weight of skin and tendon (Lim *et al*, 1993). For bone to be affected the bacteria must normally penetrate the skin, mucous membranes or intestinal epithelium, surfaces that in life normally act as microbial barriers. Enzymatic catalysation is possible in a number of bacterium including but not limited to; *Clostridium, Pseudomonas, Mycobacterium tuberculosis, Bacteroides, Prevotella, Fusobacterium, Proteus, Achromobacter, Streptomyces, Staphylococcus* and *Porphyomonas.* The production of a collagenase enzyme by certain bacteria is critical for both growth and survival which is achieved by releasing amino acids from collagen and also by permitting the breakdown of defensive tissue planes that will allow the bacteria to spread and multiply. This greater nutritional diversity may confer a selective advantage over noncollagenolytic strains (Harrington, 1996). In summary, microbes are niche-filling opportunists with most being sensitive to a defined temperature, oxygenation and pH range.

3.3 Adult Human, Newborn Human & Sus scrofa Microbiology

For the experimental part of the research stillborn (foetal), newborn and older pigs were used as proxies for human cadavers at several stages of development. It is therefore relevant to discuss the microbial flora of all of the above and in addition the endogenous flora of the adult human.

The full extent of the commensal gut community is unknown, but attempts are being made to rectify this. The adult body is a hive of activity with regards to bacterial colonisation that is present virtually body-wide, from the oesophagus to the skin to the intestinal tract. It has been suggested that the colon of an adult human contains 96-99% bacterial anaerobes of the genera *Bacteroides*, anaerobic *Lactobacillus, Clostridium*, and *Streptococcus)* and only 1-4% aerobes (gram negative coliforms,

enterococcus, Proteus, Pseudomonas etc) (Gill-King 1997). The newborn infant body is a completely different matter and the microbes found here will be in much smaller quantities. The nine months spent in the womb is the only time that the human body is truly free of microbes, but this neonatal state is quickly compromised by an influx of bacteria from the mother and the surrounding environment: some of which will permanently inhabit the body at various locations such as the skin, the oral cavity and the gastrointestinal tract where they constitute an important aid to digestion (Wilson, 2005). An embryo is protected from bacteria by the maternal tissue that surrounds it. Impermeable membranes form a sac around the growing child that preserve sterility. A few hours before being born this barrier is ruptured and bacteria from the mother are free to cross over to the newborn as it travels down the vaginal canal. By the time the child is born the mouth, skin and external ear canals are all compromised and microbial colonization of the neonate will occur within 24 hours following birth (Tannock, 1995:37). By two weeks of age the resident biota will in fact be of a similar population size to that of an adult (Ingraham & Ingraham, 2000). According to Savage (1977) in the early stages after birth the biota is predominantly *Lactobaccillus* sp in those infants fed on formula milk and this is also true of the pigs. In contrast, breast fed babies will have a biota consisting mainly of *Bifidobacterium* sp. In both humans and pigs these lactic acid species will be accompanied by a range of facultative anaerobes such as *E. coli* and the strict anaerobes will not usually be detectable until solid food is introduced. The levels of the aforementioned bacteria will increase progressively within the bowel until they become the dominant microbial population and by the time of weaning an adult climax level of bacteria will be present. However, Fanaro *et al* (2003) found that *Bifidobacteria* and *Lactobacillus* are seldom found in infants and they say that the dominant species are *Enterococcus faecalis, E. coli, Enterobacter cloacae, Klebsiella pneumoniae, Staphylococcus epidermidis* and *Staphylococcus haemolyticus.* Hughes (2007) agrees that *Bifidobacteria* do not make up a significant part of the infant biota and also noted that there is a tendency towards sudden shifts in species composition in the young. This research was carried out using DNA microarray technology rather than inadequate traditional culture methods that often fail to enumerate amounts of bacteria and species present. This latter research is more plausible as previous studies relied heavily on culture methods that were inherently flawed. Species composition therefore varies between the newborn and the adult but it also differs as we age as adults, varies along the length of the gut and is intrinsically affected by the environment (Hooper & Gordon, 2001).

The microflora of animals is distinct from that of humans both in terms of composition and in its location due to anatomical and dietary differences and environmental conditions. The proximal part of the gastrointestinal tract in the pig and some other animals, (fig 3) has its own microflora that is largely absent in humans (ibid). This is also true of the stomach, which in *Sus* is heavily colonised by *Lactobacillus* sp. Pigs are also coprophagic and consume both their own stools and/or those of other pigs. There is therefore a constant influx of microbes into their guts from the ingestion of bacteria-laden faeces. In studies where pigs are experimentally inoculated with bacteria, the pigs are fitted with bags that collect faeces to prevent self infection (Swildens *et al*, 2004).

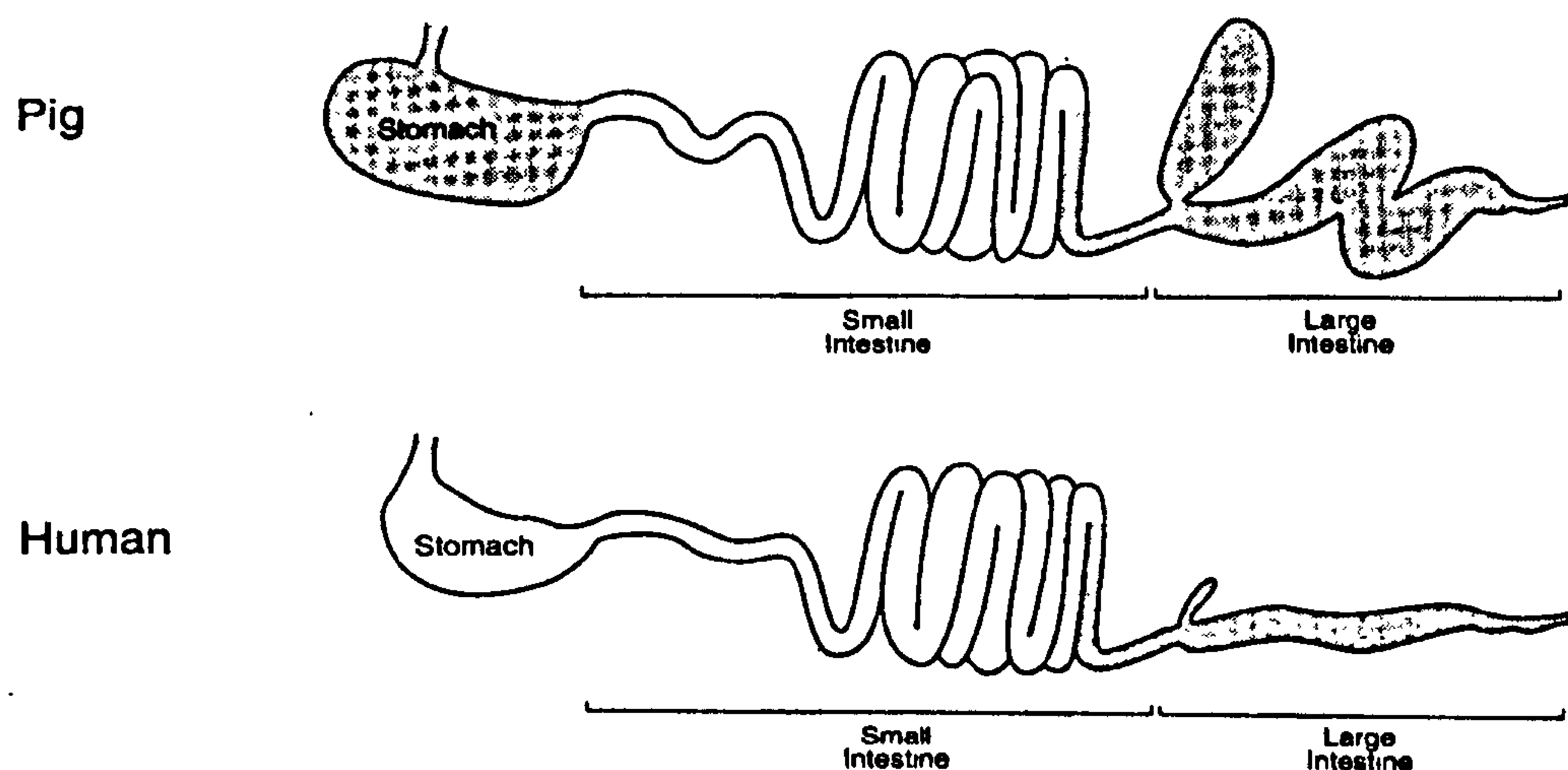

Fig 3. Areas holding the normal microflora are shaded. The pig clearly has colonization of the stomach that is absent in humans (Taken from: Tannock, 1995:40).

Numerous studies have been undertaken to estimate both the numbers and diversity of bacterial species in human and animal faecal matter. Traditionally, samples of faeces are cultured in the laboratory and bacterial counts taken. Williams Smith and Crabb (1961) found that the faecal flora of different animals were very similar during the first few weeks of life with *Bacteroides* and *Lactobacilli* predominating. In later life however, they found the bacterial counts and species identified to be grossly dissimilar. However, according to Wang *et al*, (2003) this technique was inadequate as many of the bacteria were unculturable and the methods did not reflect the true diversity. When Wang *et al* (ibid) looked at human gut flora using 16S rDNA sequence analysis they found that only 24% of the bacteria present could be identified by culture methods alone. They looked at the hindgut bacterial load across mammalian species (human, horse and pig) and found

that the phylum represented (especially *Bacteroides* and *Clostridium* which were predominant) were remarkably similar. Suau et *al*, (1999) estimate that 60-80% of gut bacteria cannot be identified through culture methods. Using 16s rDNA they also found three species to be prevalent, the *Bacteroides* and two species of *Clostridium* but noted that 76% of the clone sequences were unknown novel species. When Castillo *et al* (2006) researched total bacterial load in weaning pigs using real-time PCR, they received higher values for enterobacteria and lactobacilli, than when using traditional culture methods. This they state could simply be an overestimation due to PCR's ability to quantify dead bacteria. Eckburg *et al*, (2005) studied the microflora of humans and found significant differences between subjects (bacterial loads being host specific) and between stool and mucosa communities as previously reported by Zoetendal *et al* (2002). The bacteria eliminated in faeces may be those species that are purely transient and ephemeral rather than a true reflection of commensal gut flora, therefore, cultures taken from the intestinal wall are more likely to reproduce a true picture of microbial species present.

The pigs used in the present study are mainly intensively bred animals that are weaned at a very early age, often around several days instead of a more natural several weeks. This highly disruptive event places great stress on the digestive system of the young piglets and most of the litter will have to be treated with antibiotics on a daily basis. How this affects the natural gut flora has not been established but it must be presumed to be potentially detrimental and to modify the flora in unknown ways. According to Franklin *et al* (2002), weaning age has a significant effect on both microbial populations and concentrations of volatile fatty acids. Tests were carried out on piglets weaned at 17 days and at 24 days and in the earlier weaned pigs the concentration of faecal anaerobes declined but were maintained in the older weaned pigs.

Everything described so far deals with the living body but this research is concerned with those species of microbe that can proliferate within a corpse. At death the body is quickly deprived of oxygen and by the end stages of autolysis the environment becomes mostly anaerobic which allows the rapid growth of bacteria from the large intestine (Gill-King, 1997). A previous study by Vass (2001) attempted to explore the role of microbes in the corpse in an endeavour to utilise them as a possible post-mortem interval indicator. It was found that during the decomposition process too many microbes were involved to gain any understanding of any one particular role

carried out by different species. Vass recorded the presence of many species including; *Staphylococcus, Malasseria, Bacillus, Streptococcus, Klebsiella, Proteus, Salmonella, Agrobacterium* and *Cytophaga.*

3.4 Intestinal Bacteria and Timescale of Transmigration Post-mortem

The transmigration of bacteria from the intestine to the rest of the corpse at a point soon after death has been researched avidly with varying results. It would seem reasonable to assume this presumption to be true with a natural pathway for transmigration being present by way of the vascular system. Once within the vascular canals, highly motile bacteria would be free to migrate anywhere within the body, including the bone structure which is highly vascularized. Conversely, newborn infants are presumed to have intestinal sterility at birth which would pre-empt any transmigration.

Roberts and Mead (1986) explored the involvement of intestinal anaerobes (especially clostridia) via agonal invasion in the spoilage of meat, poultry and fish. They found that certain criteria will enhance the chances of meat spoilage including the storage of meat at elevated temperatures and stress from transportation immediately prior to slaughter. It was also found that muscle from stressed pigs contained much higher levels of contamination by the bacteria *clostridium.* It appears that translocation of bacteria out of the gut and in to the muscles and organs can occur in live animals especially where stress is a factor. In pigs, weaning is a traumatic time that can induce failure of the defence mechanisms protecting the gastrointestinal tract and stress from transportation lowers the intramucosal pH, both of these will allow bacteria to infect the internal organs after translocation out of the gut (Lieber-Tenerio *et al,* 1999 & Swildens *et al* 2004). A clinical trial by Swildens *et al* (2004) was successful in retrieving bacteria from organs (in live pigs) 72hrs after experimental intestinal inoculation in stressed animals.

If in fact, these bacteria are reaching bone via the vascular network (fig 3.1) it must be noted that during life blood has bactericidal properties and it is therefore likely

Fig 3.1 Proximal femur of a dog showing extensive vascular network. Image available @http://cal.vet.upenn.edu/projects/saortho/chapter_82/82mast.htm

that any bacteria coming in to contact with the blood should not lead to disease. However, in previous studies (Jensen, 1944) it was found that certain strains of bacteria (*Serriata, Achromobacter, Clostridium & Escherichia coli*) were resistant to the bactericidal effect of hog's blood. It is not known how long after somatic death that blood loses this unique property. However, as early as the 1850's it was noted that the blood from animals that had died of diseases such as anthrax contained thread-like structures that multiplied by division; these were actually bacteria (Lamb, 1893). This movement of bacteria out of the gut is probably one of the main reasons why many carnivores will not consume putrid corpses (Janzen, 1977.) Some research has been undertaken with blood from both the heart and lungs being sampled at the time of autopsy. Wood *et al* (1965) attempted to correlate microbes grown from heart blood with both known and unknown antemortem cultures. Where antemortem cultures were taken they were quite successful at identifying the same bacteria from blood at autopsy (less than 15 hours after death) that was incubated at 37°C for seven days. The majority of these microbes appear to be *S. aureus, Pseudomonas*, *E. coli* and *Proteus*. When they sampled blood from individuals where antemortem cultures were not made they found a similar array of bacteria

and when they tested blood from individuals where there was no anatomical evidence of infection they found the most prevalent microbes to be *S. aureus*, *Aerobacter* and *Bacteroides*. The findings are problematic as traditional culture methods were used and incubation at 37°C will allow those bacteria that thrive best at a higher temperature to proliferate. When Canavan and Southard (1914) researched the significance of bacteria cultivated from the human cadaver, it was reported that by twelve hours postmortem the majority of blood cultures were positive. Other research into postmortem bacteriology at autopsy revealed that up to 50% of samples were sterile and where bacteria were found the most common were *E. coli*, followed by *Enterococcus*. Other bacteria isolated included all of those previously mentioned with the addition of *Streptococcus* and *Klebsiella-Aerobacter* (De Jongh *et al*, 1968). More recently, as part of research in to drug levels postmortem, Elliott *et al*, (2004) sampled blood from seven human fatalities; there was clear evidence of advanced putrefaction in six of the individuals (the other' was used as a control due to the lack of putrefaction). In all cases where decomposition was advanced, *Clostridium*, *E. coli*, *Proteus vulgaris*, *Enterococcus faecalis* and *Aeromonas* were isolated from blood. In the case used as a control no microbes were found. Unfortunately, no temperature data are given and it is not known how long the postmortem interval was in any of the cases, although one would presume the interval to be short.

It is known that the mucosal membrane lining the gastro intestinal tract breaks down very soon after death; precipitating the influx of bacteria into otherwise sterile areas such as muscles etc. In a study by Melvin *et al* (1984) a piece of intestine was taken from decapitated mice. Bacterial transmigration took place within different timescales when kept at various temperatures, with transmigration apparent at only 2-3hrs when the intestine was kept at 37°C. At a lower temperature of 25°C it took 5-6hrs and at 4°C bacteria was found at 72hrs post-mortem (Table 3.1). The first microbes to be cultured were *Staphylococcus* and the last group were a variety of anaerobic bacteria. In an earlier study by Kellerman *et al* (1976) the first bacteria to be cultured from a loop of dog intestine were also *Staphylococcus*; however, this was not until 15hrs post-mortem. These bacteria then gradually declined in number, until at 48hrs they were practically absent. They were replaced by both gram-negative and gram-positive bacteria.

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Bacterial Transmigration Postmortem			
Hrs after Incubation	**Source**	**Aerobic**	**Anaerobic**
0-12 (37°C)		None	None
15 (37°C)	Dog/Intestine	*S. aureus*, Diphtheroids Non-hemolytic Streptococci	Bacteroides Peptococcus Peptostreptococcus
27 (37°C)		As above plus E. Coli	As above plus Clostridium by 19hrs
36 (37°C)		Predominant organisms: *E. coli*, Diphtheroids, Gram+ bacilli, *S. aureus*	As above
48 (37°C)		As above	As above
0-12 (37°C)		None	None
15 (37°C)		*S. aureus*, Gram+ bacilli Non-hemolytic Streptococci	Bacteroides Peptococcus
	Dog/Colon		
24 (37°C)		*S. aureus*, Gram+ bacilli Alpha-hemolytic Streptococci *Proteus*	As above plus Peptostreptococcus & Clostridium by 14 hrs?
36 (37°C)		Predominant organisms: *E. coli*, *Proteus*, Hemolytic Streptococcus, Gram+ bacilli, *S. aureus*	As above
48 (37°C)		As above	As above
0-15 (37°C)		None	None
18 (37°C)	Human/Colon	*S. aureus*, *Staphylococcus epidermidis*	
26-28 (37°C)		As above plus Streptococcus (at 26hrs)	Bacteroides Peptococcus Clostridium (at 28hrs)
31 (37°C)		As above	As above plus Peptostreptococcus
48 (37°C)		Predominant organisms: *E. coli*, *S. epidermidis*, Non-hemolytic Streptococcus, *S. aureus*	As above
2-3 (37°C)	Mice/Intestine	Staphylococcus	
4-5 (37°C)			Coliforms & fungi
6-8 (37°C)			Coliforms (dominant) & anaerobes
5-6 (25°C)		Staphylococcus	
8-10 (25°C)			Coliforms & fungi
12-16 (25°C)			Coliforms (dominant) & anaerobes
66-68 (4°)		Staphylococcus	
68-72 (4°)			Coliforms & fungi (few species)
>72 (4°)			Coliforms & anaerobes (rare)

Table 3.1. Timescale of bacterial transmigration

3.5 Summary

Once all of the above is taken into account it becomes clear that a single perpetrator of bacterial decomposition of bone would be unlikely. There are probably successions of bacteria that excrete various enzymes. Even narrowing down to a single species or even type, be it anaerobic or aerobic, is unlikely due to the difficulty of replicating this action faithfully under laboratory conditions. Not only is it difficult to culture some of the species (both of soil and intestinal origin) but also many of the flora are still unknown and what happens in the laboratory at fixed high temperatures or *in vitro* cannot satisfactorily be reconciled with a rapidly decomposing corpse. A likely perpetrator though would most likely be anaerobic due to the lack of oxygen both in the cadaver and at extreme burial depth.

Several unanswered questions need addressing. Firstly, the difference between the gut flora of humans and pigs is unclear, with some authors suggesting a dissimilar assemblage whilst others suggest that there is much similarity across all mammalian species. Thus far the entire commensal community of the adult human gut remains unknown and the gut flora may be host specific. Different authors record varied species of bacteria in the newborn child and differences do exist between breast or bottle fed babies. Much of the research is also carried out with faecal bacteria, which may be purely transient having no niche to fill within the gut or having been out-competed. It is also perceivable that intensively bred pigs will have a modified gut flora from the stress that they endure from premature weaning and from the daily dosage of antibiotics.

Secondly, the transmigration of highly motile bacteria from the gut to the surrounding tissues and blood is unambiguous. In stressed animals bacteria enter the live body (within 72hrs) and transmigration after death can occur within two hours when microbes are cultured at 37°C. Hence, at death the bacteria are already within the gut and ready to consume the nutrients that they require to thrive. However, the conditions essential for their propagation are many.

3.6 Appendix: Bacteria that Produce a Collagenase Enzyme Isolated from the Human Body (Harrington, 1996:1886)

Actinobacillus actinomycetemcomitans

Actimomadura (Streptomyces) madurae

Bacillus cereus

Bacteroides spp

Bifidobacterium sp

Brucella melitensis

Capnocytophaga ochracea

Clostridium spp

Enterococcus faecalis

Escherichia coli

Eubacterium alactolyticum

Flavobacterium meningosepticum

Fusobacterium nucleatum

Peptococcus sp

Peptostreptococcus spp

Porphyromonas (Bacteroides) spp

Prevotella (Bacteroides) sp

Proteus mirabilis

Pseudomonas aeruginosa

Serratia marcescens

Staphylococcus spp

Streptococcus agalactiae (group B Streptococci)

Streptococcus mutans

Streptococcus sobrinus (S. mutans 6715)

Treponema spp

Vibrio vulnificus

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Chapter 4. Environmental Microbiology

4.1 Introduction

The previous chapter dealt with those microbes that reside or pass through the human gut and this chapter will look at bacteria that are abundant in the environment and specifically those which are soil dwelling. It will be necessary to assess soil bacteria and their possible role in human tissue decomposition within a context of ambiguity. This chapter will look at how microbes colonise animal tissues, when they arrive and if there is a succession of bacteria similar to what would be found when dealing with entomology and also in a similar vein to the previous chapter a discussion of the inadequacies of laboratory culture methods. Soil bacteria will opportunistically use cadavers as a nutrient source if they are within range, but with burial at depth this may not be the case. A review of the literature is essential to assess at what depths microbes are active. Another point of substance that must be addressed is the role of maggots as destroyers of bacteria and their considerable role in corpse degradation.

4.2 Notes on Bacterial Diversity and Culture Methods

The current understanding of bacterial diversity is limited due to the fact that many bacteria have been termed 'unculturable' when using traditional culture-dependent microbiological methods. 'Unculturable' refers to the fact that many bacteria do not grow when using plating techniques at fixed temperatures using simple solid media in petri dishes. This is true of both endogenous animal microbes and those in the environment or more specifically the soil. Only a very small number of bacterial isolates can be cultured and any findings are flawed and confusing due to some bacteria performing better than others in the laboratory. Some headway is being made using traditional plating techniques and novel lineages of previously unknown soil isolates are being discovered (Janssen *et al*, 2002 & Stevenson *et al*, 2004). Using newly developed media combined with longer incubation times has had some success in culturing previously 'unculturable' isolates (Davis *et al*, 2005). Even so, only 4-7% of the microbial community can be recovered using this method (Cavaletti *et al*, 2006). Culture methods are still an important part of understanding bacteria but since the 1990's, new and more powerful methods that use molecular technology (16S rRNA) have been developed (Janssen 2006). 16S rRNA gene sequencing can be quite specific and has been able to prove that some bacteria (i.e. *Crenarchaeota*) that were thought to exist solely in 'extreme' environments also live

in temperate soils (Schloss & Handelsman, 2004). This method has also been useful in comparing and denoting bacterial community composition in forest soils that are determined by specific soil conditions (Hackl *et al*, 2004).

4.3 Soil Bacteria

The bacteria are the most numerous group of microbes within the soil (the others being fungi, algae, protozoa, and actinomycetes) and the size and make-up of the microfloral community will vary from site to site. Less than 1% of the soil surface area is covered by bacteria that are relatively immobile (with little ability to spread, Gray & Williams, 1971) and spend most of the time in an almost starved state (Van Veen & Kuikman (1990). When organic nutrients are added to the soil the autochthonous species will enter a period of rapid growth until the nutrients are exhausted, at which time they will decline in numbers. Both soil texture and structure control the rate at which organic matter can be decomposed, with coarse sandy soils having a higher rate than finer clay soils.

There is only a limited understanding of species diversity, spatiality and aggregation in soil. This is largely due to the old culture methods used, but new techniques such as 16S rRNA are beginning to answer questions on soil biomass. Previously, those species that only made up a minor component of the soil grew in abundance which led to a belief that these were the dominant microbiota. This is now known to be untrue and in fact those species that are truly abundant were those that appeared minimal (Joseph *et al*, 2003). Microbes are capable of surviving for many decades, but the addition of material (corpses) for consumption by such bacteria would generate an environment where microbial mass is high and of a species composition that is important to decomposition. Soil (and human gut) bacteria are either autochthonous (indigenous) or they may be allochthonous (invaders) and although the latter group may be successful for some time they will not normally become an integrated part of the soil community (Alexander, 1977). *This raises the issue of how bacteria within dead animals or humans will survive or thrive when in direct competition with the indigenous soil microflora, (they actually are the gut indigenous species of the host i.e cadaver, but are invaders in soil) and should be able to deter soil bacteria at least for some time.* Certainly at depth the gut residents may be the only microbes present. Child (1995) argued that introduced microflora in soil never become part of the soil indigenous flora and if a new substrate is introduced the incoming bacteria will be out-competed and destroyed in a matter of

a few months to a few years. Child therefore believed that soil microflora is the most significant factor in the diagenesis of bone. This can be contested. In the first instance any body buried in a deep grave (see below) will be in a relatively sterile environment due to the lack of bacteria at depth, but even at shallower depths the indigenous microbes of the corpse will have plenty of time to attack the bone, especially if bacterial tunnelling is something that occurs within the early months/years. It is possible that there may be selection pressure acting on indigenous bacteria to develop the ability to colonise bone.

4.4 Soil Depth versus Bacterial Counts

Soil depth can affect the amount of bacteria present. In a study by King and Doryland (1909) where soil samples were taken over a period of months and at varying depths, it was found that the top 5cm of soil contained an average of 14.47mpcc genomes of bacteria (millions per cubic centimetre), whilst at a depth of 30cm the average was considerably lower at just 2.4 mpcc. There are few bacteria on the surface of the soil due mainly to lack of moisture (and the possible bactericidal effect of sunlight, Alexander,1977) and the greatest number of soil bacteria exist at around 7cm in depth (table 4) with this gradually diminishing until around 200cm, where only a very limited number of microbes survive (Sewell, 1914). There exists a difference in microbial diffusion between field soils (where the bacteria are generally most profuse several centimetres below the upper crust) and shaded or forest areas (numbers are highest in the top 1-2 cm) (Alexander, 1977).

	Organisms/g of Soil x 10^3	
Depth (cm)	**Aerobic Bacteria**	**Anaerobic Bacteria**
3-8	7800	1950
20-25	1800	379
35-40	472	98
65-75	10	1
135-145	1	0.4

Table 4 Distribution of anaerobic and aerobic bacteria at differing depths in soil. (Alexander, 1977:24).

Bacteria require many different elements to thrive and of these nutrients, carbon is required in the greatest amount. However, the carbon input is relatively small being supplemented with pulses of food from the occasional rotting corpse or from dead roots (Brooks *et al*, 1985) with the result being a mainly dormant biomass. Carbon is most readily available in the form of carbohydrate but is also obtainable from other

sources such as monosaccharides, amino and fatty acids. In a study by Griffiths *et al*, (2003) it was concluded that the deficit of bacterial numbers at depth was attributable to several factors including a decrease in carbon content down the soil profile. Whilst the organic horizons higher up the profile (5cms) had a carbon content of 36.8%, the lower part of the profile (15-20cms) had only 6.5%. Other attributable factors included the lower profile being predominantly mineral which changes the soil texture, a lack of moisture and a lack of nitrogen, potassium, calcium and magnesium. Bacteria will rapidly colonize and decompose soft animal tissues, but the rate of breakdown of a solid medium is governed by the rate at which enzymes can dissolve nutrients to a soluble form (Richards 1974). For microbes to utilise bone collagen as both a source of energy and carbon they must produce collagenase. Microorganisms of soil origin that produce collagenase include; *Clostridium histolyticum,* (*C. Welchii* is also abundant and widely distributed) (Skinner, 1975) *Achromobacter iophagus, Vibrio, Pseudomonas marinoglutinosa, Pseudomonas flourescens* and *Streptomyces* sp. Streptomyces C51 can grow rapidly under aerobic conditions (Endo *et al*, 1987). According to Vraný *et al*, (1988) soil bacteria that are capable of producing a collagenolytic enzyme are widespread, especially under aerobic conditions. The authors also studied the occurrence of microorganisms that produce collagenase from various different types of soils. They found that garden soil had the highest number of isolates that were collagenolytic (36.6%) closely followed by meadow soil (30.4%) and then vegetable field soil at 29.1%. When they examined spruce growth soils they found not only less bacteria but also considerably less collagenolytic microbes (14.7%). This experimentation was carried out in a laboratory setting where temperatures were kept at 25°, no depths are given for the soil samples and reconstituted collagen was used as the substrate. Jennison (1945) notes that several species of bacteria which are able to cleave gelatine are unable to attack collagen. Gelatin is much easier to degrade as it lacks the triple-helix structure of collagen and it is probable that most serine proteases can degrade gelatine (Collins, 2009 Pers Comm).

4.5 Bacteria, Laboratory Culture Methods and Temperature

Attempts to enumerate bacteria in the laboratory may provide misleading results. *Clostridium* and *Bacillus* are genera that grow optimally at high temperatures (30°-37°C) but that are also classed as soil bacteria. Soil temperatures in the UK are nowhere near this high, (except occasionally on the surface, where lack of moisture

excludes bacterial growth) but both have been cultured in the lab from soil samples, but at the optimum temperature of 37° (Skinner, 1975). Culture methods in the laboratory are not ideal and any micro-organisms grown are a reflection of this inadequacy (Gray & Williams, 1971). Estimates tend to be low and some bacterial organisms never produce recognizable colonies on agar media (Alexander, 1977).

During research by Child *et al* (1993), it was found that only a restricted range of bacteria were capable of producing a collagenolytic enzyme at low temperatures. They discuss the fact that English grave (inhumations) temperatures at depths of 100-300cms will be around 10°C and therefore only bacteria that can grow at this temperature are relevant to microbial decomposition (table 4.1). Thirteen different strains of bacteria were found to produce collagenase at this temperature of which the majority belonged to *Pseudomonas* sp and one in particular *P. fluorescens* (which is aerobic) taken from soil was prolific in its production of collagenase at 10°C; this type is also found in human faeces.

Species	Oxygen Requirement	Origin	Species	Oxygen Requirement	Origin
Pseudomonas fluorescens (type a)	Aerobic	Faeces	*Pseudomonas maltophilia* (type b)	Aerobic	Soil
Pseudomonas fluorescens (type a)	Aerobic	Soil	*Pseudomonas putida*	Aerobic	Faeces
Pseudomonas fluorescens (type a)	Aerobic	Faeces	*Pseudomonas* sp (type a)	Aerobic	Faeces
Pseudomonas fluorescens (type b)	Aerobic	Soil	*Pseudomonas* sp (type b)	Aerobic	Soil
Pseudomonas fluorescens (type c)	Aerobic	Faeces	*Aeromonas* hydrophilia	Facultative Anaerobe	Soil
Pseudomonas fluorescens (type d)	Aerobic	Soil	*Aeromonas* caviae	Facultative Anaerobe	Soil
Pseudomonas maltophilia (type a)	Aerobic	Soil	*Aeromonas* sp	Facultative Anaerobe	Soil
Pseudomonas maltophilia (type a)	Aerobic	Soil	*Klebsiella oxytoca*	Facultative Anaerobe	Faeces
Pseudomonas maltophilia (type b)	Aerobic	Soil			

Table 4.1. Those species of bacteria that produce collagenase at low temperatures (Child *et al*, 1993).

However, no details are given as to where the bacterial samples were taken from and as already discussed it is highly probable that these bacteria probably only exist within the top few inches of the soil profile and would not consequently play a part in in decomposition of any cadavers buried deeper than this. And, although collagenase production was good, it should be noted that enzymes are most efficient at optimal temperatures and at lower temperatures they act at a much reduced rate as the catalytic action is slowed.

As previously discussed temperature clearly affects those species which can thrive in soil and it has been shown that in soils with a mesophilic microbial community (middle living with optimum growth at 30°-37°C) there will be a doubling of activity for each 10°C increase in temperature (between 0° & 30°-35° C) but there is a dramatic fall above this threshold (Kilham, 1994). And although soil researchers often store their samples at 4°C in the belief that this will impede activity this assumption is not true. Grupe *et al* (1993) agree that bacterial activity will be greatly reduced at low temperatures, but point out that in archaeological material there has been a great amount of time for bacteria to utilise the collagen and that in their laboratory experiments, it was possible to demonstrate bone decomposition by soil microbes even at 4°C. Yet again, this knowledge is based on carefully selected bacteria (which may not necessarily even be part of those bacteria which successfully colonize the corpse) that are cultured via *in vitro* experimentation rather than in a corpse based scenario.

Clearly there are is limited knowledge regarding soil bacteria in their role as decomposers of organic matter. According to Gray & Williams (1971:82):

- "The study of decomposing animal corpses in soil has never been a popular pastime so that there is little known about the succession of micro-organisms on animal tissues originating above ground or within the soil. We can presume that their body components are competed for by soil microbes and that a succession of colonisers occurs. There is also a well developed microflora in the gut of most soil invertebrates and it is possible that these microbes also play a key role in decomposition of the animal body."

4.6 Experimental Research: Microbes

Tibbett *et al* (2004) researched the rate at which pieces of sheep muscle placed in soil decomposed under laboratory settings. It was concluded that the muscle degraded quicker at higher temperatures and that soil microbes play a substantial role in the early postmortem period. The point of the research was to demonstrate that soil microbes could use muscle lacking an enteric microbial community as a nutrient source. Although the muscle did decompose there was no control sample such as muscle placed in a sterile medium, as enzymatic decay could account for some of the degradation. Neither is there sufficient information on the origin of the soil. In addition to this there is no record of how long the sheep had been dead prior to commencement of the experiments and if there was a delay of even just a few

hours then it is possible that bacteria from the gut could have transmigrated to the body tissues. There would be a definite need to sterilize the muscle to prevent contamination from enteric bacteria and prove that only soil microbes were responsible for the decay.

4.7 Maggots, Microbes and Antibiotic Secretions

Maggots play an important role in decomposition of carcasses during the summer and autumn months where the bodies are accessible to flies. During the winter and early spring, decomposition advances much more slowly when only microbes are available to breakdown the carrion (Putnam, 1978). A range of species will lay their eggs on the corpses, but especially the blow flies that are better known as green and blue bottles. A recent resurgence in their usefulness in the medical world has prompted a renewed interest in their healing and disinfecting properties. There are many published studies on the efficacy of fly larvae and their ability to heal and disinfect both skin defects, such as leg ulcers and pressure sores and also infections of bone (osteomyelitis). Many of these papers were written in the early 1930's before the advent of modern antibiotic therapy, but modern studies also exist with both eras concluding a very real antibiotic effect on microbially infected soft and hard tissues including those infected with Methicillin-Resistant *Staphylococcus aureus*/MRSA (Thomas *et al*, 1999, Courtenay *et al*, 2000, Bowling *et al*, 2007) which is the most common cause of osteomyelitis (Elliott *et al*, 1997). Septicaemia has been prevented by maggot therapy and it was effective in cases where antimicrobials, hyperbaric oxygen and disinfectants had all failed to heal the wounds (Mumcuoglu *et al*, 1999). As can be seen from the data presented below (table 4.2) maggots have been highly successful in eradicating many different strains of bacteria in hundreds of incidences involving both real life cases of infection and also in the laboratory.

Maggot Therapy/Disinfecting/Antibacterial Effects				
Location/Site	**Bacterial sp**	**Larval sp**	**Author**	**Success Rate**
Laboratory	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Streptococcus A* *Streptococcus B* *MRSA*	Not Known	Thomas *et al*, 1999	All Eliminated
Laboratory	*S. aureus* *Streptococcus pyogenes* *Streptococcus faecalis* *Streptococcus mitior* *Proteus vulgaris* *Eberthella typhi* *Clostridium Welchii*	*Lucilia sericata*	Simmons, 1935	All Eliminated
Diabetic Foot Ulcers (13 Patients)	Methicillin-Resistant *S. aureus* (MRSA)	*Lucilia sericata*	Bowling *et al*, 2007	12 out of 13 patients healed after 45 days
Pressure sores of the lower sacral area and leg ulcers (25 patients)	*Streptococcus A* (unidentified infections and septicaemia)	Phaenicia sericata	Mumcuoglu *et al*, 1999	88.4% complete 7% significant 2.3% partial 2.3% unchanged
70 wounds of mixed pathology	49 infected wounds (no sp identification given)	*Lucilia sericata*	Courtenay *et al*, 2000	30 wounds complete 20 partial 8 unchanged 1 deteriorated (90% successfully treated overall)
Laboratory	*Escherichia coli*	*Lucilia sericata*	Mumcuoglu *et al*, 2001	Eliminated
Ulcer (2)	*Pseudomonas (1)* *Staphyloccocus aureus* *Streptococci (1)*	*Lucilia sericata*	Graninger *et al*, 2002	Ulcer decreased by 80% (1) Spontaneous healing (1)
Osteomyelitis (12)	*Staphylococcus*	*Lucilia sericata* *Phormia regina*	Miller *et al*, 1932	Prompt healing
Osteomyelitis Chronic leg ulcers Compound fractures (572 cases)	Not Known	*Lucilia sericata*	Livingston, 1936	88% Improved
Osteomyelitis (2 cases)	Not Known	*Lucilia Caesar*	McLellan, 1932	Healed
Osteomyelitis (4 Children in 1928) Further 89 Individuals	Not Known *Not all infections noted but includes;* *Staphylococcus* *Non-haemolytic* *Streptococcus* *Tuberculosis* *S. aureus* *Streptococcus pyocyancus*	Blue and Green Bottle	Baer, 1931	Complete healing 79 cases healed or improved, 9 cases no improvement, 1 case death from Tetanus

Table 4.2. Lab based and Real Life Analysis of Maggot Therapy

The larvae appear to be effective for a number of reasons with most authors stating at least one of the following; there is surgical removal of the diseased tissue, the wound is actively sterilized by the maggots, which physically remove microorganisms by ingestion (Courtenay *et al*, 2000), wound disinfection via a potent bactericide that is secreted by the maggots and that has a wide spectrum of activity against many resistant pathogens (Simmons, 1935, Thomas *et al*, 1999, Sherman *et al*, 2000, Sherman 2003, Nigam *et al*, 2006 3[2]), change of the wound pH from acid to alkaline by excretion of ammonia (Mumcuoglu et al, 1999) which is then unfavourable to many bacterial species (Nigam *et al*, 2006 3[3]), most of the

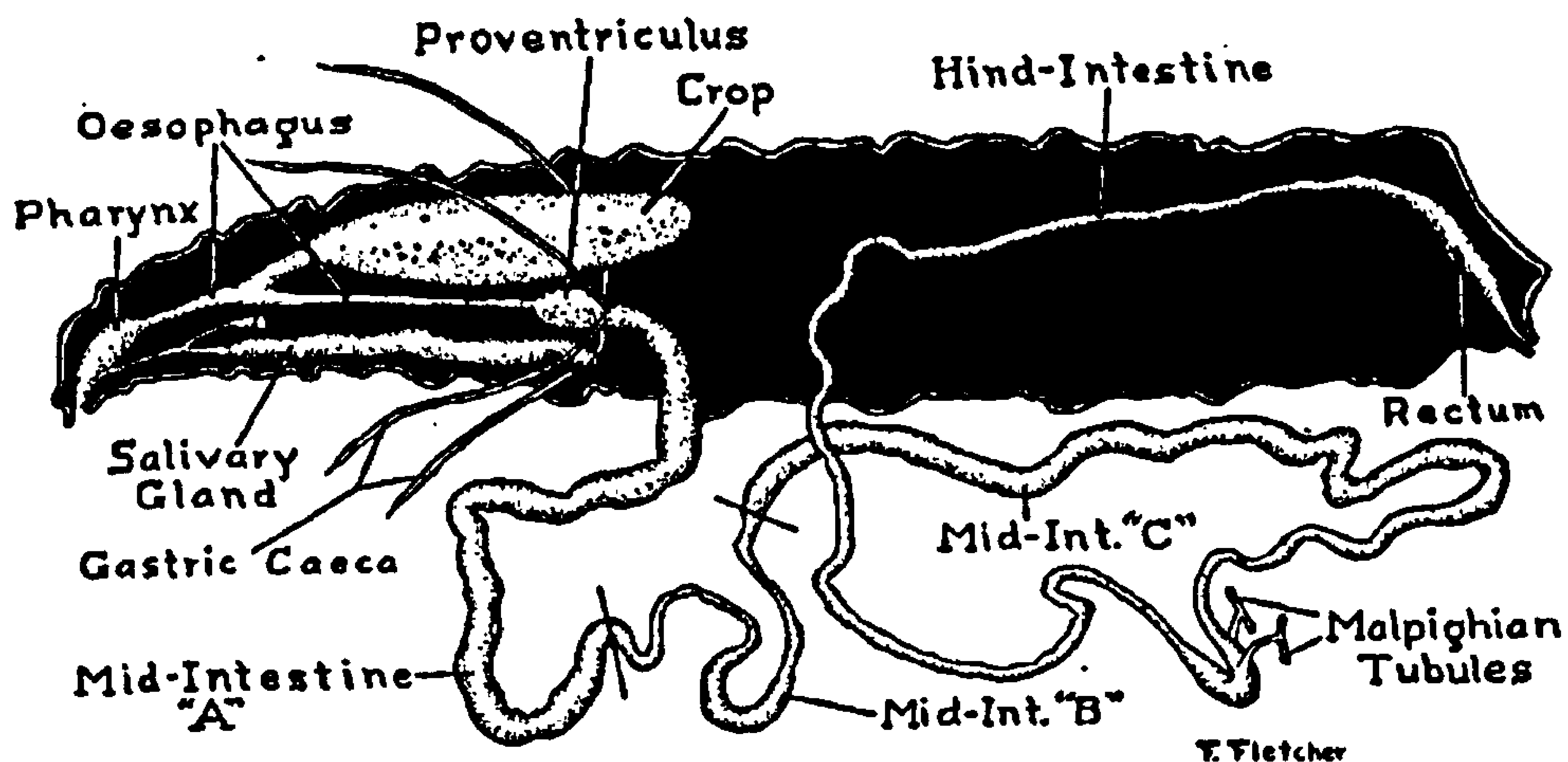

Fig 4. Gross anatomy of the Blowfly larvae (Fletcher & Haub, 1933).

bacteria are killed in the midgut of the larvae and by the time the ingested bacteria reach the hindgut (fig 4.) most are already dead and furthermore the excreta is either sterile or almost entirely free of microbes (Mumcuoglu, *et al* 2001). However, in 2007 it was decided that although maggots fulfilled the required definitions of an antiseptic (bacteria used; *Micrococcus luteus*, *E. coli* and MRSA), the maggots were excreting bacteria and as such considered to be medical waste (Daeschlein *et al*, 2007). In a study where *Lucilia sericata* larvae were placed on bacterial lawn agar of fluorescent *E coli* the larvae demonstrated fluorescence within 3 minutes (Lerch *et al*, 2003). Simmons (1935) carried out successful experiments using the excreted elimination products of maggots that included both faecal matter and cutaneous and oral secretions. He even found that desiccated excretions were potent enough to kill microbes. In a recent *in-vivo* research study in to the antimicrobial activity of maggots, it was determined that maggot therapy was more effective against those wounds that were infected with gram-positive bacteria. Alternatively, wounds infected with gram negative bacteria required a higher number of maggots over a longer period of time (Steenvoorde & Jukema, 2004). Huberman *et al* (2007) have

isolated three low molecular weight compounds from the maggot of *Lucilia sericata* (p-hydroxybenzoic acid, p-hyroxyphenylacetic acid & octahydro-dipyrrolo [1,2-a,1'2'-d]pyrazine-5,10-dione) that are effective against *Micrococcus luteus* and *Pseudomonas aeruginosa*, both when used individually and when used together. According to Nigram *et al* (2006) the disinfecting property is possibly also due to one specific type of bacteria (*Proteus mirabilis*) that forms part of the commensal flora in the maggot and that is known to produce agents such as phenylacetic and phenylacetaldehyde that both have antibacterial properties. Yet commercially used medicinal maggots are sterile and would not have any bacteria present. It is apparent that maggots are adapted to produce some kind of bactericide. The excretion of this cleansing agent possibly exists due to the fact that they live in an in an environment that is riddled with microbes of which some may be deadly. Alternatively this could equally be a mechanism to prevent bacteria from competing for the same food source.

Having studied the effect of the fly larva in a medical situation where wounds that have previously been unresponsive to conventional medical practices and antibiotics, it becomes apparent that there may also be a similar effect on the dead body. A corpse is colonized by insect larvae soon after death and many thousands of larvae will feed on the carrion. The excretions and ingestion of the tissue will quickly eliminate bacteria from the body. It has been shown that the maggots are enormously proficient at disinfecting the hard tissue of the skeleton in cases of osteomyelitis. As all of the above research is carried out entirely within a live assemblage it is difficult to extrapolate what would happen in the dead. One can only presume that a similar disinfected and largely microbe free environment will eventually be perpetuated, especially as the tiny 1st instar larvae are perfectly capable of gaining access to the interior of the bone via the nutrient foramina. If this is the case then bacterial tunnelling of bone in bodies that have been exposed to larval activity may possibly be rare or limited. This would be relevant to surface depositions and to those bodies given only a very shallow burial. Further research in to maggot secretions and their effect on carrion is desperately required if we are to advance our knowledge of this area with any note of certainty.

4.8 Maggots and Collagenase

It was found during research that maggots themselves excrete the collagenase enzyme. Two papers published on the subject which have experimental protocol

and date to the early to mid 1900's. The experiments were carried out with *Lucilia sericata* and *Phaenicia sericata* Meig. In the 1931 study, experiments were carried out using diluted excreta and collagen from the Achilles tendon of an ox and catgut (Hobson, 1931). The catgut was almost completely dissolved and it was shown that it was maggot collagenase that produced this phenomenon rather than any bacteria within the larvae due to the sterility of the maggots employed. The author suggests that:

"The collagenase enzyme plays an important part in the growth of *Lucilia sericata* on meat by digesting the fine strands of connective tissue which surround each muscle fibre." (Hobson, 1931:1462). The optimum ph for the activity was about 8.5 and increasing acidity decreases activity which will almost cease at ph 4.0. The original findings of Hobson were later confirmed in 1953 by Ziffren *et al.* They used sterile. larvae and concluded that *Phaenicia sericata* Meig maggots secrete collagenase, that its action is to break down long chain polymers to enable digestion and that the cleansing of wounds and the treatment of osteomyelitis is at least in part due to the collagenase enzyme. According to Blake (2005:80) "the decomposition of necrotic substances is primarily achieved by proteolytic enzymes like collagenase, chymotrypsin and trypsin-like substances."

4.9 Summary

Environmental corpse contamination via soil and airborne microbes is a little understood phenomena. Some of these bacteria must be capable of inhabiting a cadaver but many questions are raised by the research discussed here. Firstly, soil microbes exist in the greatest numbers towards the soil surface, yet most human bodies are buried at depths where few microbes should be active. Modern burials may not be subjected to these bacteria due to their interment at a depth of six feet, but the soil would undergo some mixing when placed back in the grave. Any burial contained within a coffin would also be protected from all soil microbes until a point at which soil infiltration or coffin disintegration had commenced; the same would apply to cist burials. In addition, little research has been carried out with animal carcasses and it remains unclear whether soil microbes can infiltrate a biomass that is already occupied by an indigenous flora.

Secondly, the decomposer ability of insects may affect the bacterial loads that survive the decomposition process. When maggots are actively feeding from the corpse any bacteria within the soft tissue will be consumed and disinfected making it

difficult to assume that the bacteria preferentially feed on the soft tissue, only moving to the hard tissues after depletion of the original food source has occurred. Is it therefore possible that a buried body with the indigenous microbiota intact would be more likely to exhibit tunnelling? Not as a consequence of soil bacteria, but as a direct effect of the gut flora being protected from predation allowing them almost exclusive access to the body if it is buried at depth.

Thirdly, is it possible that the collagenolytic activity of maggot larval secretions plays a part in bone diagenesis? The maggots are effective destroyers of bacteria, but just how efficient they are is debateable. Do any bacteria, possibly those that are gram negative, survive the ingestion/secretion/excretion process? If maggots are constantly excreting collagenase there must be some consequence for the bone that they surround in a corpse. First instar larvae being extremely minute are capable of entering bone through the foramens. One would presume that any direct destruction of the bone would be limited to the periosteal and endosteal surfaces rather than areas of attack around individual haversian systems that are so often seen. Damage to bone has never been recorded in cases of osteomyelitis being treated with larval therapy, probably due to the fact that larvae only consume necrotic tissue. The corpse however is a necrotic environment and as such may be at risk from larval activity.

Finally, having considered both all of the above and the findings from chapter 3 it is likely that there are a multitude of reasons as to why microbial attack should never occur. But it does and on a large scale. Hence, the correct circumstances and right bacteria must prevail on a regular basis and it is purely a matter of time and research in the field before the actual offenders are found.

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Chapter 5 Diagenesis: Nature of Bone and Bone Tunnelling

5.1 Introduction

Diagenesis is the study of post-mortem modification of bone in the burial environment. This chapter will firstly discuss the nature and composition of bone along with a comparison of human to porcine bone. This will be necessary as bacterial attack appears to be dependant upon the specific structure of bone and its haversian systems, whilst the experimental part of the research is carried out using pigs as human analogues as already discussed. A brief introduction to the structure of skeletal material will be presented together with an explanation of how bone forms and remodels. The action of microbes on bone is little understood but an explanation of the proposed mechanism will be provided and supplemented by a review of previous cases where it is thought that microbes have diagenetically altered bone in the postmortem period.

5.2 Gross Anatomy of Bone

Bone is a composite material that comprises both a mineral or inorganic component and a protein or organic component with hydroxyapatite accounting for the mineral phase and collagen and osteocalcin being the two most abundant proteins (Collins *et al*, 2002). The organic portion (collagen) in adults accounts for 24% of the dry weight of cortical bone but in children it has been claimed that the organic part accounts for a much larger proportion (Baker *et al* 2005, Guy *et al* 1997). In a different study (Dickerson, 1962) the opposite of this statement appears to be true with collagen accounting for less of the bone composition (whole bone) than in that of adults and a roughly similar collagen content being present in the cortical bone (table 5).

Age	*Whole Bone (femur) Collagen Content g./100g Dry Bone*	*Cortex (femur) Collagen Content g./100g Dry Bone*	*Epiphyses (femur) Collagen Content g./100g Dry Bone*
Foetus 12-14 wks	3.37	16.2	15.5
Foetus 20-24 wks	6.17	22.5	29.7
Foetus 30-34 wks	8.46	22.4	40.3
Newborn, full-term	9.28	23.3	42.2
5-9 Months	12.5	23.7	48
12-24 Months	14.1	24.3	52.9
11-12 Years	16.3	25.4	41.4
Adult 18-35 years	17.3	23	Not Available

Table 5. Collagen content of different types of bone in different age groups (Dickerson, 1962).

The initial bone that forms in children that replaces cartilage is referred to as woven or immature bone that develops rapidly but is less organized than the lamellar or mature bone that gradually replaces it (Baker *et al*, 2005). Woven bone is typical in the foetus and is the most immature type; it forms quickly, is poorly organized and is weak. Lamellar bone is more mature, forms slowly is well organized and is laid down in parallel layers (Scheuer & Black, 2004).

Bone has two basic structural components; compact (cortical) bone and spongy (trabecular) bone. Compact bone is solid and dense being found in the walls of bone shafts and on external bone surfaces, whereas trabecular bone has a honeycomb structure and is found in the ends of long bones, in short bones and vertebrae and is sandwiched between flat bones (White & Folkens, 2005). The outer surface of bone is covered by a thin tissue called periosteum during life, which is highly vascularized; blood is supplied partly by the periosteal vessels and partly by nutrient arteries, which enter bone via nutrient foramina (Gosling *et al*, 1996). At the inner surface of the bone is another osteogenic tissue called the endosteum (White & Folkens, 2000). Circumferential lamellar bone is replaced by osteons that are elongated parallel to bone long axis, circular in cross-section and that have a diameter of between 180-250µm (Ortner, 2003). These units of bone may divide, rejoin or interconnect with other osteons and are best seen in three dimensions (fig 5). The first formed matrix in bone is the osteoid which becomes calcified by the binding of calcium phosphate crystals (hydroxyapatite) to the collagen fibres. The limit of the osteon is demarcated by the reversal or cement line (Bancroft & Stevens, 1977). Bone forming cells become trapped as osteocytes within the matrix lacunae. Osteocytes are star shaped cells whose radiating processes lie within minute canaliculi. About 40% of the dry weight of bone is made up of collagen fibres that in younger individuals form randomly arranged bundles. Collagen becomes more organized as the individual matures and forms in to parallel sheets or lamellae (Walker & Liem, 1994). There are six stages to bone remodelling that revolve around 9-10 osteoclasts (cutting cones) and several hundred osteoblasts, beginning with activation. The next step is resorption where bone is resorbed at rates of up to 40-50µm per day and this is followed by reversal which centres on the transition between cutting and formation. Concentric lamellae are then laid down during the formation phase before mineralization takes place where bone mineral is grown between the layers; this phase can take up to 6 months. Finally, bone enters a period of quiescence which ends the transformation; the bone matures and becomes an active component (ibid).

Compact Bone & Spongy (Cancellous Bone)

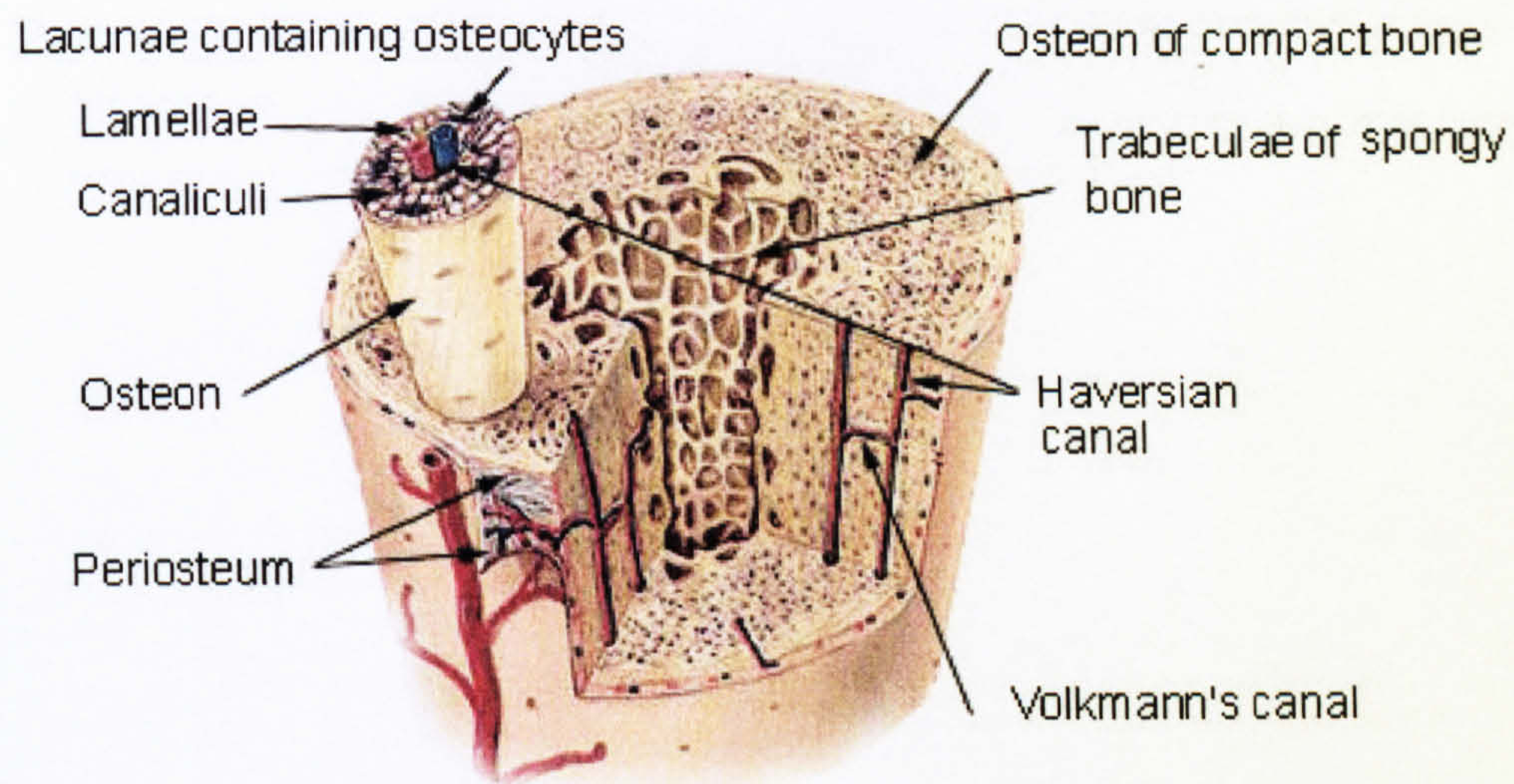

Fig 5. http://en.wikipedia.org/wiki/Image:Illu_compact_spongy_bone.jpg

For the examination of bone within the remit of this research it is necessary to observe bone at the microscopic level as the changes to the collagen structure are not visible to the naked eye. Haversian systems make up large parts of the bone and these are usually easily recognisable due to their circular nature and the central Haversian canal that in life would house a blood vessel. Fig 5.1 depicts a transverse thin section through a piece of human bone and a more diagnostic description of the structures seen are given below.

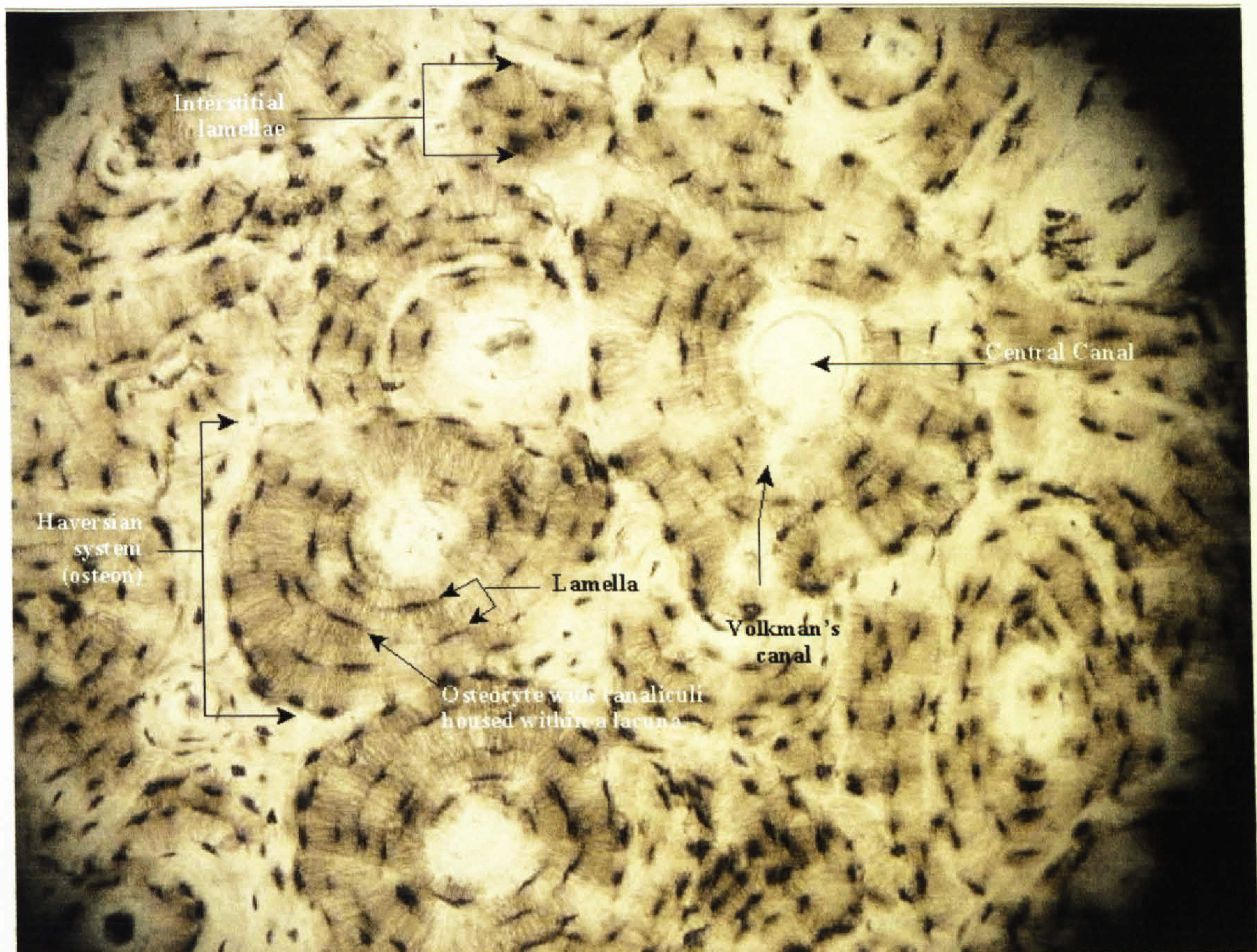

Fig 5.1 Thin section of human bone. Available @ http://biology.clc.uc.edu/fankhauser/Labs/Anatomy_&_Physiology/A&P201/Bone_Histology/Bone_Histology.htm

Haversian system = (osteon) entire complex, functional unit of bone
Haversian canal = carries blood vessel through center of osteon
lamellae = "little layer" of matrix between concentric rings of osteocytes
lacunae = "pools" which house osteocytes
osteocytes = "bone cells" which maintain bone
Volkmann's canal = feeder cross connecting vessel for blood supply
canaliculi = protoplasmic extensions from osteocytes by which maintenance of bone is performed
interstitial lamellae = layers between adjacent Haversian systems

5.3 *Sus* Bone: Comparison to Human

The main difference between human and domestic pig bone is the fact that in skeletally mature pigs the majority of the bone structure can be made up primarily of plexiform bone (Mulhern & Ubelaker, 2001). And furthermore in the immature pig, entire sections of bone will be made up of plexiform bone (fig 5.2) that has a complete absence of both haversian tissue and osteonal banding (Hillier & Bell, 2007). Plexiform bone is usual in animals that grow quickly and gain weight rapidly.

Fig 5.2 *Sus scrofa* (Periosteal) University of Sheffield

Because pigs do not reach skeletal maturity until around 6 months of age all of the pigs used in this study would be classified as sub-adult. Robinson *et al* (2003), consider that pig bones are on average more porous than other ungulates and should therefore be likely to degrade faster after burial. In immature pigs this problem is compounded even further by even more porous bone. Here however, pig bones were being compared to other domesticates although one human rib bone was included in the study. It would seem fair to say that pig bone, especially when juvenile in nature, is more porous than human bone and this may affect how quickly bacterial or chemical dissolution can occur and may have an effect on the research being carried out here. With regard to bony anatomy, morphology, healing and remodelling, pig bone is considered to be closely representative of human bone and therefore a suitable species of choice (Thorwarth *et al*, 2005). In current medical experimental research there are a range of species that are approximated to human bone including; pig, sheep, rat, canine and chicken; although in forensic anthropology, this is almost exclusively limited to porcine with the occasional use of rat bones and carcasses. The reasons for using pigs as an analogy have already been discussed, but few studies in reality quantify how close a replicate they really are. When Robinson *et al* (2003) looked at bone porosity using nitrogen porosimetry they concluded that pig bone is on average more porous than both other ungulates and humans, but this is based on a comparison between adult bone

from the other species included in the study and juvenile bone from the pigs. There is some research to suggest that though porcine bone is a satisfactory substitute, canine bone is in fact closer in (table 5.1) several ways to human bone.

Key Attributes of Similarity Between Four Animal Species and Humans				
	Canine	Sheep/Goat	Pig	Rabbit
Macrostructure	++	+++	++	+
Microstructure	++	+	++	+
Bone Composition	+++	++	++	++
Bone Remodelling	++	++	+++	+

Table 5.1 Key: Least Similar + Moderately Similar ++ Most Similar +++
(Reproduced after Pearce *et al*, 2007 p8).

During a study by Mosekilde *et al* (1987) they state that pig has a similar lamellar bone structure to that of humans, this is in direct contention with many others who state that pig bone is plexiform in nature (Mulhern & Ubelaker 2001, Hillier & Bell 2007, Martiniaková *et al* 2006) and whilst porcine bone is similar to human bone in mineral density and mineral concentration, canine bone is closer to human than is porcine, but Aerssens *et al* (1998) point out that there are large interspecies variations. However, when Laiblin & Jaeschke (1979) were looking at bone regeneration in dogs, humans and pigs, they found that the rate of regeneration in pig bone was more similar to the human rate than was the canine bone. Pearce *et al* (2007) conclude that no species (other than non-human primates) are an ideal equivalent for human bone. These studies are all reliant only on bone whereas in this research it is important to have a breed of animal that has a similar digestive system to our own. Pigs being omnivores are ideal candidates whilst dogs are carnivorous and this will affect species composition of the commensal gut bacteria. This study is heavily tied to bacteriology and strict carnivores we are not. Humans are considered to be omnivorous whilst dogs are carnivorous, but most often dogs that are kept as companions will be fed whatever is left over from the diet of the human owner and this will almost certainly be a mixture of protein and vegetable matter. Although canine bone is more similar, there are ethical implications when using animal species that are companion animals (Pearce *et al* 2007). It is perhaps easier to use those species that we already breed as food providing stock where emotive issues are less likely to be problematic. Of course the former applies mainly to often unnecessary experimentation that is carried out *in-vivo* and in the modern world, rather than examining the bones of long dead dogs. It would be

pertinent to explore archaeological canine bone as dogs were just as much pets in the past as they are today. Because of this they were often buried in a similar manner to humans, with care and emotion and were sometimes interred with their human owners. This means that they were buried in their entirety along with the microbial contents of the gut, oesophagus and skin, rather than the sterilised carcasses of domesticates that had been butchered for their meat.

5.4 Diagenetic Pathways

The ways in which a bone or skeleton can decompose are varied whilst at the same time being limited. Much will depend upon both the pre-depositional processes and then subsequently upon the environment in which the remains are finally interred. Unfortunately, pre-depositional acts are seldom clearly defined by the bones themselves. Practices such as excarnation will not generally leave a definable trace and in the same way neither will a body where an extended pre-burial period has taken place. Lyman (1994) refers to these pre-burial processes as 'Biostratinomy' and includes factors such as trampling, exposure, butchery and defleshing, cooking and burning/cremation (See Ch 2). Biostratinomic processes chiefly influence which species and which skeletal elements end up in the burial record. Therefore those remains that become buried are already compromised and to an extent 'selective'. It is clear that a human body is more likely to be buried in its entirety and even in cases of excarnation or cremation a certain amount of care may be taken to conscientiously gather most of the bones if these are then to be buried. In direct opposition to this are the remains of food providing domesticates. Here the external influences mentioned above will severely impact upon which bones survive and which are buried. In humanly created assemblages they are likely to have been differentially transported from kill sites, butchered, cooked, disarticulated, subjected to marrow extraction, fed to other carnivores or exposed to scavenging (Lyman, 1994) before possibly ending up in a midden. One study has shown that experimental cooking of bone will both modify and accelerate deterioration (Roberts *et al*, 2002). It is consequently difficult to reconcile these differences when using pigs as an analogy for humans. Any archaeological specimens that are observed will have fundamental differences in both their pre and post depositional treatment which is further compounded by the innate dissimilarity in their bony morphology.

Once the above factors are understood it then becomes pertinent to understand how skeletal remains are further diagenetically modified by their burial environment. Preliminary research in to bone diagenesis shows that degradation of the composite

material follows certain paths and is somewhat limited to deterioration of both the organic and mineral phases and by microbiological attack (Collins *et al* 2002). Further work by Jans (2005) looked at benign and corrosive environments. Recent research suggests that there are only four main pathways for change post deposition (Smith *et al*, 2007). The authors suggest that bones can either be well preserved, be subjected to accelerated collagen hydrolysis, be attacked by microbes or suffer catastrophic mineral dissolution; two of these pathways may be active at the same site. This they believe is linked to early taphonomic processes and whether or not a body is buried entire, rather than long term soil conditions (although soil type will have an effect). This research was followed up (Nielsen-Marsh *et al*, 2007) by looking at inhumed entire corpses versus disarticulated remains in what they term as either 'benign' (low pH, free draining) or 'corrosive' (neutral pH, organic rich, urban) soils (fig 5.3).

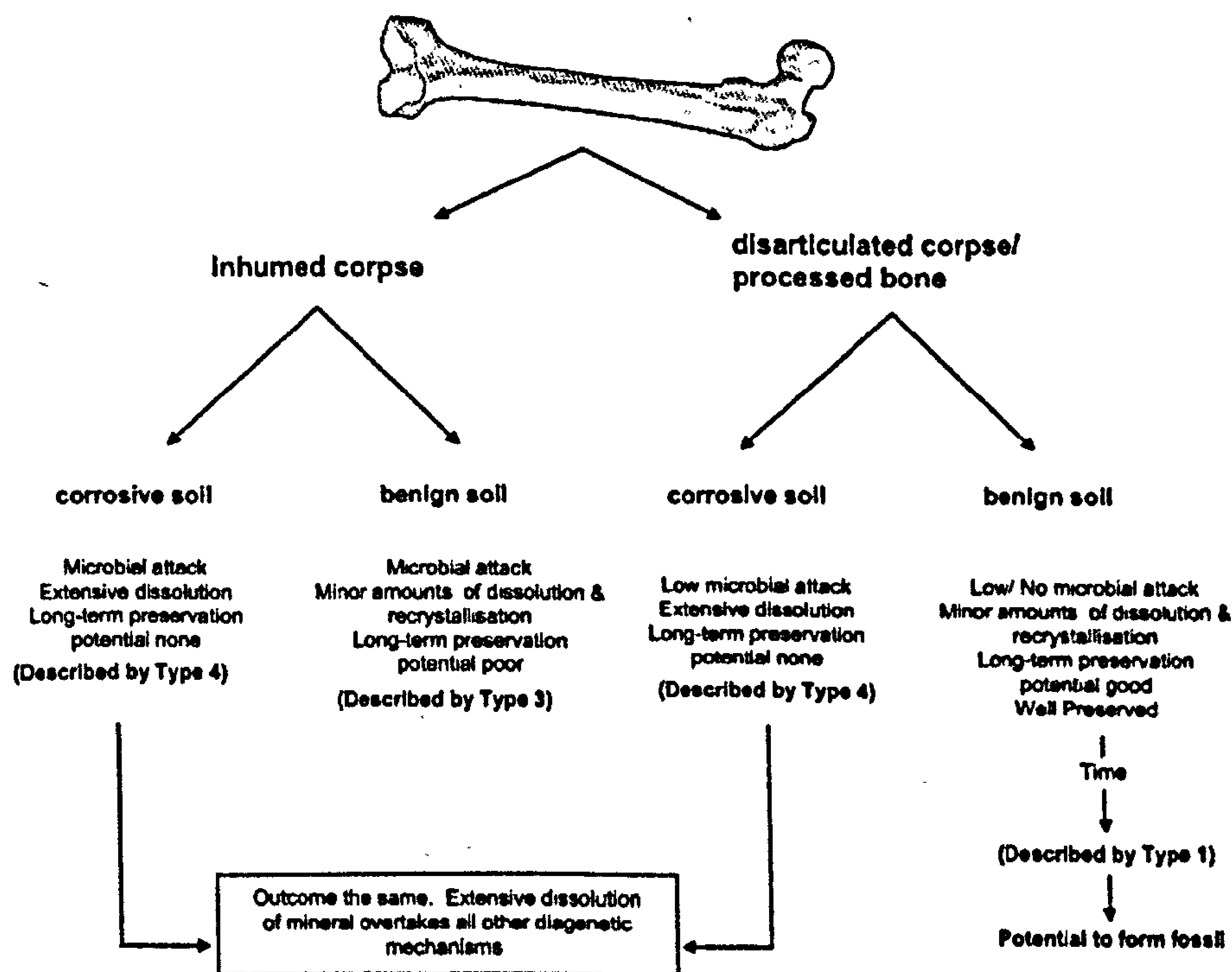

Fig. 5.3 Pathways to destruction or preservation (Nielsen-Marsh *et al*, 2007)

The information provided above suggests that the best preserved bone would originate from a disarticulated or sterile corpse that had been buried in a benign soil with neutral pH and if the hypothesis being tested here is correct then this would be highly likely as butchered remains do not have an endogenous gut flora. The other three pathways are destructive with little hope of bone remaining in the very long term. This experimental research however is looking for microbial tunnelling in the short term and the burials are based in woodland. Here the soil should in theory be

classified benign as woodland soils are generally slightly acidic with a pH of around 4-5. However, as already discussed most bacteria are Neutrophiles that work best at pH levels of 5.5 - 8. Bacterial collagenases have an optimum pH value of 7.3-7.4 (Poole & Tratman, 1978). Having measured the pH level at Riseholme it is known that the pH value for that particular site is around 7.6-7.8 which would suggest that most bacteria and those that produce the collagenase enzyme should be prolific providing an ideal environment for micro-focal destruction by bacteria. According to Gordon & Buikstra (1981) the preservation or destruction of inhumed human bone is quite strongly related to soil pH and that as the pH decreases the destruction of osseous materials increases, but this relates to acid dissolution rather than microbial activity.

5.5 The Nature of Bone Tunnelling

What happens to bone once the soft tissue has decomposed? Bone may become a substrate upon which bacteria can continue to gain their energy requirements whilst at the same time extending the organic phase available by using the hard tissue of the skeleton (more specifically the collagen) as discussed in chapter three. Previously undertaken studies have had varying success rates in the areas of; tunnelling detection, the rates at which it is found and the earliest recorded timescale for this process to occur. The very structure of the bone itself will to some extent define how and when it will be possible for bacteria to attack the bone and it has been shown that tunnelling is not random but rather reflects the lamellar nature of bone (Bell, 1990). In addition to this Hanson & Buikstra (1987) argue that post-mortem degradation proceeds independently from any extrinsic factors which only serve to hasten an intrinsically defined process. As previously discussed bone is a mixture of organic and inorganic material that comprises collagen in the form of a left-handed triple helix of polypeptide chains that forms fibrils. Collagen contains high levels of the amino acids glycine (33%), proline and hydroxyproline (20%) (Child, 1995). The spaces between the molecules are filled with the mineral component of hydroxyapatite (ibid). Hydroxyapatite is formed mainly of calcium (38%) phosphate and hydroxyl ions that form very small needle-like crystals about 20nm in length (Bancroft & Stevens, 1977). It is believed that microbial attack is initiated by localized chemical dissolution of the mineral phase followed by enzymatic attack of the exposed organic matter (Collins *et al*, 2002). Child (1995) believes that microbial proteinase (a strong bacterial acid) results in demineralization of the hydroxyapatite. Either of these two pathways is possible.

There remains considerable disagreement in the literature as to whether bacterial tunnelling is as a result of soil or intestinal microbes. Microbes of soil origin have long been suspected of having a predominant role in the breakdown of bone collagen (Grupe, 2001), but some (Jans *et al*, 2004) are confident that the endogenous intestinal bacteria are the true perpetrators. Others are so convinced that soil bacteria are responsible that they state that tunnelling will not normally commence until the point of skeletonization at a time when the bone structure becomes available to soil microorganisms around five years postmortem (Yoshino *et al*, 1991).

5.6 Types of Tunnelling and Location

There are currently four different types of tunnelling described in the literature that affect bone in terrestrial settings (Hackett, 1981). A further type affects material in marine settings and is attributed to cyanobacteria (Bell & Elkerton, 2007). Of the four that affect terrestrial remains one form is believed to be of fungal origin and is called 'Wedl'; this was first described by Wedl in 1864 and pertained to bored labyrinth-like structures in archaeological and modern bone. This research is primarily interested in 'non-Wedl' tunnels that are understood to be as a consequence of bacteria from either the gut or the environment, using bone collagen as a substrate. These types have been described and classified by Hackett (1981) and are given the terms; Lamellate, Linear Longitudinal and Budded (table 5.2 & fig 5.4). These are designated to type by shape, size, distribution and abundance, contents, demineralization, mineral redeposition and the influence of the cement line. Hackett suggests that there may be other types that do not fall into the categories above. In many microscopic thin sections bacterial tunnelling cannot easily be placed into these three types; it is obvious that the bone has undergone major decomposition and there may be a total lack of collagen, but staining or the poor state of preservation may preclude a strict diagnosis.

Recognised Types of MFD			
Wedl	**Linear Longitudinal**	**Budded**	**Lamellate**
5-10μm No cuffing (i.e. mineral redeposition) Course is never smooth straight or longitudinal They branch They are either empty or contain spherical bodies The bacteria may leave before the osteon is packed	Small round focus 5-10μm Mineral redeposition in the form of a hyper-mineralized cuff from about 5μm Restricted to osteon by the cement line May be concentrated in a single osteon	About 30μm wide Frond-like tunnels Side shoots budded at intervals of 80-90μm Slight cuffing Best seen in longitudinal section Transverse section appear rounded up to 50μm	10-20μm to 60x250μm Rounded but in transverse sections are curved following the pattern of the lamellae May appear close or away from the osteon canal Form concentric mono-lamellate patterns

Table 5.2 Categories of tunnel types and distinguishing features. (Hackett, 1981).

One of the difficulties in assessing MFD lies in the fact that bacterial invasion can be very different along the length of any given bone. Most sections are cut transversely through the mid shaft of a long bone at very thin sections. If no MFD are found it does not mean that there is not tunnelling present lower down or further up in the bone, after all only a 50µm section has been observed. Because tunnelling is 3 dimensional, sections taken longitudinally give a better chance of detection as a much larger area is covered; however, visualizing MFD in this type of section is more suggestive.

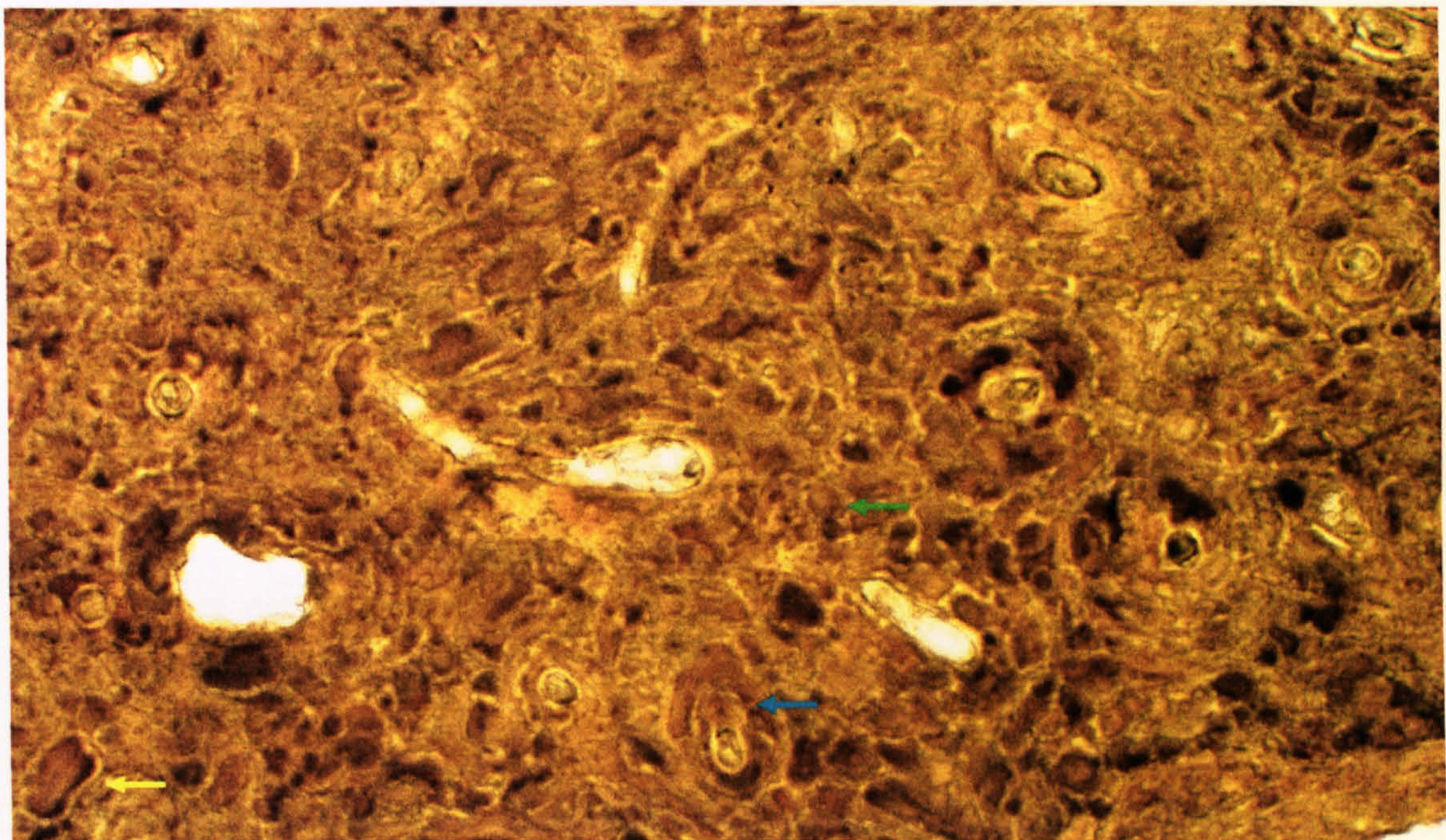

Fig. 5.4 The three recognised and different sized/shaped bacterial types of MFD. Green arrow = Linear Longitudinal (small/round), Yellow arrow = Budded (irregular, larger than LL) and Blue arrow = Lamellate (largest tunnels/follow shape of osteon). (Picture, Author, 2007 Adult from Bolsover Collection)

Further quantification of the degree of postmortem microbial degradation can be achieved by using mercury intrusion porosimetry (HgIP) that measures total pore volume and pore size distribution. Turner-Walker *et al* (2002) found that diagenetic change in archaeological bone is restricted to two discrete pore ranges. They found three broad ranges of pore size that were attributable to firstly, chemical degradation of the collagen fibrils (<0.1µm), secondly, microbial (spongiform) porosity in the range of 0.1-1µm and thirdly pores with a volume >1µm would be characteristic of the physiological properties of the bone. This correlates well with similar research by Jans *et al* (2004) in which it was found that budded and linear longitudinal types of tunnelling demonstrated pore size of approximately 0.6µm, lamellate was slightly smaller at 0.3µm, whilst Wedl tunnelling was between 0.1 and 1.2µm.

After examining many photographs of sections different types of MFD can appear very distinctive. Linear Longitudinal are very small and round, whilst Budded are much larger and Lamellate larger still and close to the osteon. In one however, this distinction has become blurred by what appears to be merging of discrete tunnels. In the lower right of the section a series of twelve or more tunnels are seen to blend into each other in a somewhat haphazard and zigzag manner (fig 5.5).

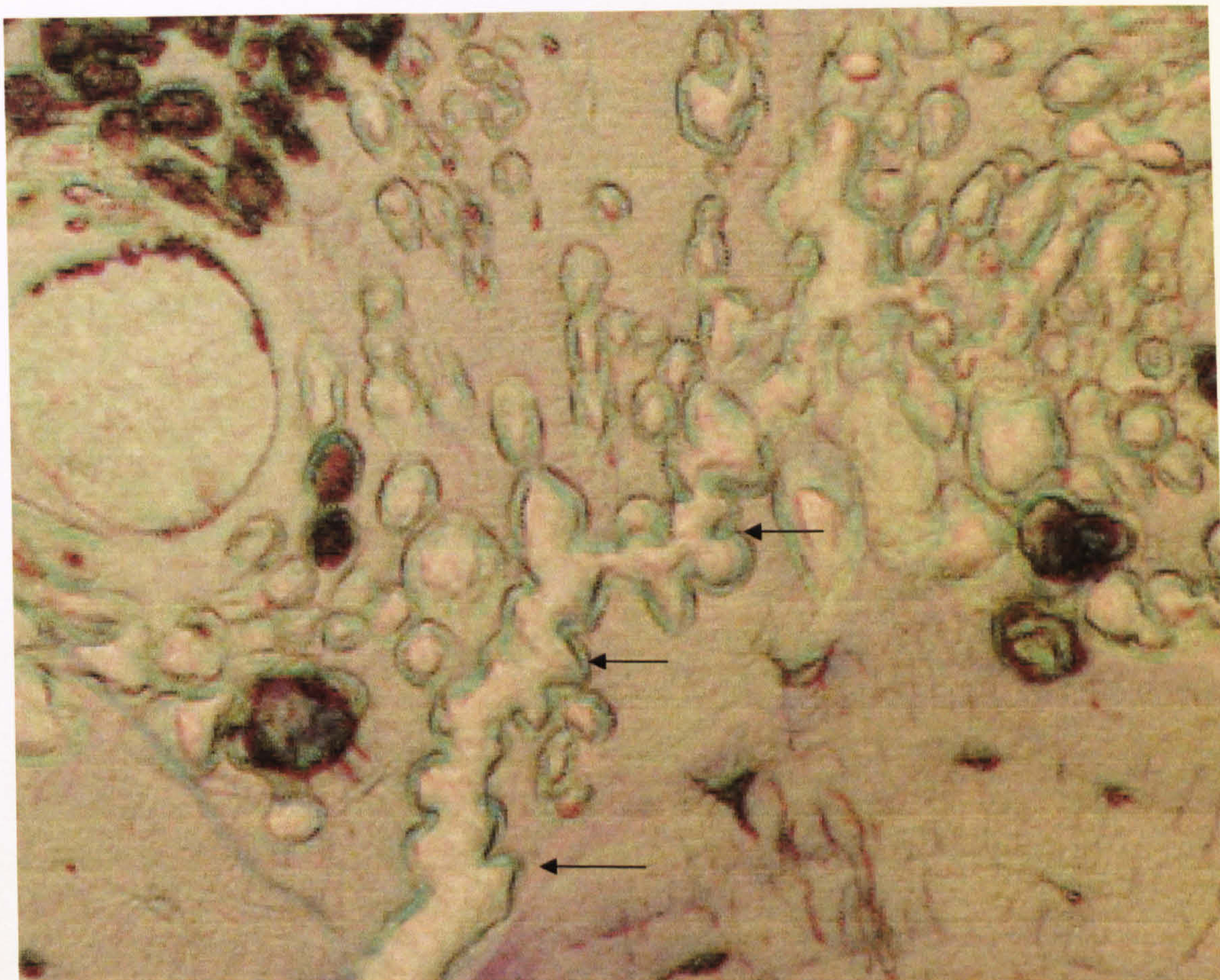

Fig 5.5 Tunnels that appear to amalgamate in to larger entities (Jans, Personal Histology Collection)

Furthermore, some of the tunnels that would be designated as lamellate appear to have roughened edges and appear to be two or more smaller tunnels that have simply merged together. If this is the case then it could be possible that what is actually being visualised is one type of tunnelling that can blend to look like something else (fig 5.6). Many questions have been raised by which bacteria are responsible for tunnelling and it has been suggested that different microbes may be responsible for individual types of MFD. Is it possible that all three types are nothing more than a series of tunnels that have joined together, at least in the cases of bacterial attack? If this is possible then one issue raised is why aren't more tunnels in very close proximity to each other joined together? It is not unusual to see an

osteon packed with linear longitudinal type tunnels. What is proposed is that some of the tunnels can merge and others cannot and this will be dictated by how many bacteria are available and where in the tunnels margin, mineral redeposition occurs. Consider two sets of bacteria adjacent to each other and both begin to cleave mineral from opposite sides. As they work around the edge if they meet in the same place at the same time then they will break through the margin, much the same as when building a large underground tunnel with workers starting at both ends and meeting in the middle. If however one group of bacteria closest to the adjacent tunnel deposited mineral without meeting the adjoining bacteria then a mineral barrier would be formed and the tunnels would remain as discrete entities. The same would be true if fewer bacteria were involved. A host of bacteria have more enzymes at their disposal than say a few solitary bacteria.

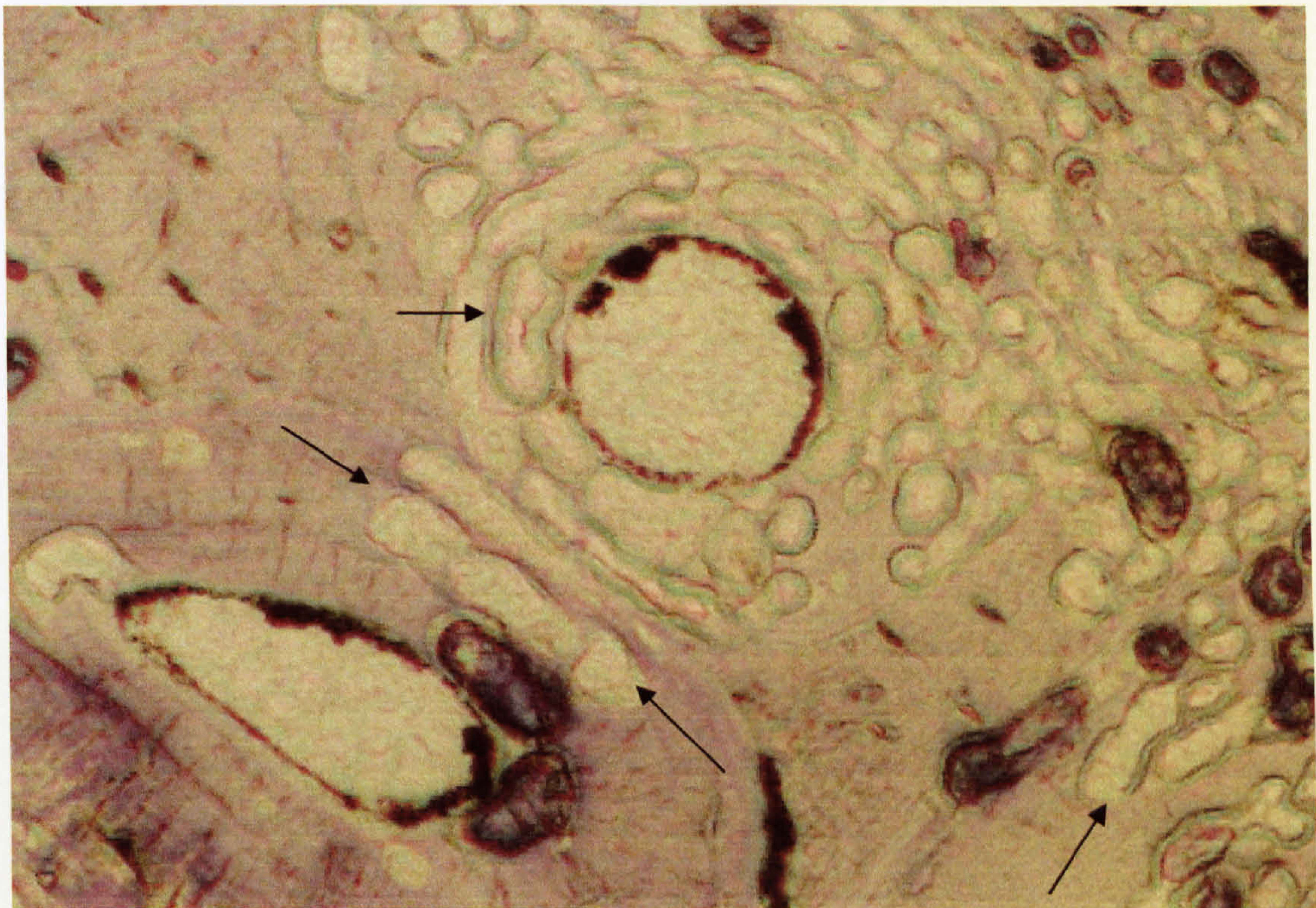

Fig 5.6 What appears to be lamellate tunnelling, but could perhaps be linear longitudinal that has merged (Jans Personal Histology Collection)

Jans *et al* (2004) assert that lamellate tunnelling is always found in association with budded foci. If the above proposition is true then this would be expected as tunnels of budded type would just be made larger by amalgamation. As an alternative explanation it is also possible that certain soil conditions such as chemical and physical factors may hydrolyse the mineral component faster prior to or after tunnelling has taken place.

In cases of known tunnelling it is evident that bacteria tend to colonise around individual canals and do not cross the cement line. Jackes *et al* (2001) consider an haversian system to be a 'closed world for bacteria' as once the colony reaches the cement line the area becomes too acid for their continued livelihood. This is demonstrated well in fig 5.7 below as the areas of tunnelling do appear to be restricted to individual osteons. Also of relevance is that in most sections observed there is a definite 'inside-out' mode of attack that affects the mesosteal region and in general the periosteal and endosteal areas will have a clear band of preserved bone (fig 5.7) where destruction has not taken place. Structural preservation of the periosteal surface has been noted by Hanson & Buikstra (1987) and Hackett (1981). This would be suggestive of endogenous bacteria entering the bone during the early putrefactive period. If soil bacteria were the cause then a more likely scenario would be a mode of attack that would start at the periosteal surface that then proceeded to the central area of the bone ('outside-in' mode of attack). Hanson & Buikstra (1987) advocate that micro-focal destruction begins with a series of isolated foci which steadily band together to form large patches of affected bone and that haversian systems are affected rather than the more highly mineralized interstitial bone. It has

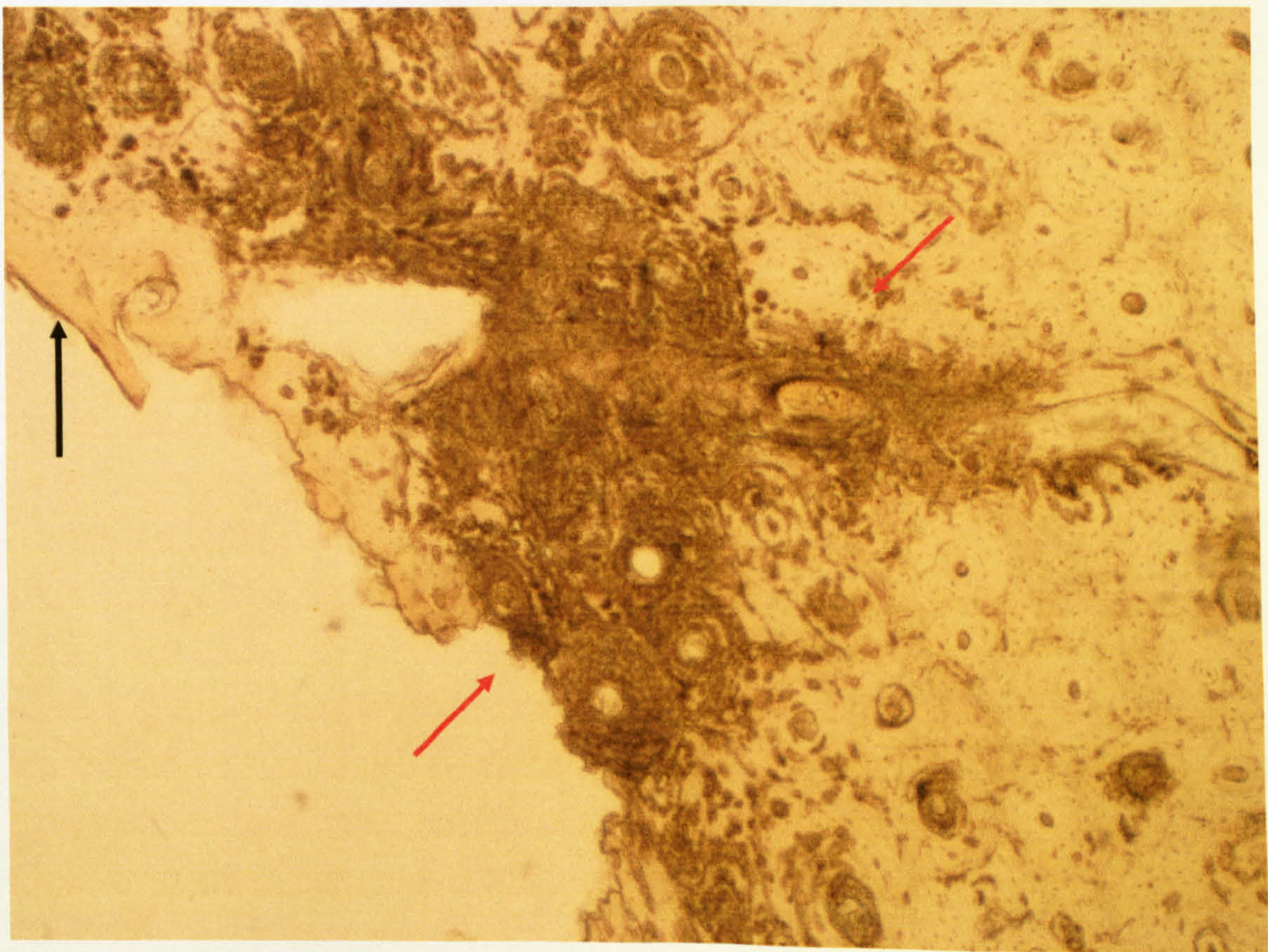

Fig 5.7 Location of tunnelling (White, 2007). Key: black arrow = preserved periosteum, Red arrows denote band of attack between the periosteum and the mesosteum.

been suggested that once microbial tunnelling has begun it will generally continue to completion (Collins *et al*, 2002) i.e. the bone will be completely destroyed. This assumption appears to be supported by recent research where a histological index was assigned to bones from archaeological sites (Hedges & Millard, 1995). One hundred and thirty nine bones were sampled and the majority had a score of either one or five; meaning that either destruction was complete or alternatively had not commenced. However the findings were site specific so most of the well preserved bones came from one site and the poorly preserved from a different site. In the case of the 27% of bones affected by microbial diagenesis most fell in to the category of being affected across the entire section and only in a few cases was the deterioration said to be intermediate. In addition to this the authors suggest that the timescale involved is much shorter than previously believed as greater age of bones did not correlate with a worse preservational quality. Hedges (2002) agrees with this but is still referring to bacterial attack happening over hundreds of years rather than in the early postmortem period. In 1995, Hedges and Millard suggested that microbiological attack is generally complete within less than five hundred years. It could be argued that this estimate is still overly long and if the forensic cases are to be believed the time periods involved are set within the first few decades. In addition Hedges (2002) adds that there is great variability in bacterial attack within sites, between sites and within a single bone. Two pertinent questions provided by Hedges are; what environmental conditions are conducive to, or inhibit, microbial attack? And also, what pre-burial conditions affect microbial attack?

5.7 Summary

The structure of bone is designed to be a strong and dense due to its supporting role during life. This makes it difficult to hydrolyse, yet not impossible. Collagen from bone can be used by bacteria to gain energy and this can be achieved by the enzymatic dissolution of the hydroxyapatite which then allows access by other microbial collagenases to the collagen fraction itself. The morphology of human adult bone differs from both human foetal bone and *Sus* bone. The main differences are that in young children and foetal material the bone will be mainly woven bone and in the immature pig the bone will mainly be plexiform in nature. This cannot be overcome by the use of other animals as these are generally difficult to obtain and quite often seen as ethically corrupt if used in this way. Although canine bone would be a preferable model the efficacy of using it would be unacceptable. It is therefore necessary to continue using porcine bone and dealing with any inadequacies that this may entail.

Four types of MFD are recognised and are often encountered in bone sections. Further analysis is required if these types are to remain distinct as the research here has demonstrated that in certain cases this may not necessarily be the case. It is possible that the same microbes are responsible for all of the tunnelling and those certain environmental conditions will allow tunnels of different sizes and shapes to form merely by amalgamation. Tunnelling is generally seen as an observable inside-out mode of attack suggesting that bacteria are gaining access to the bone via the vascular system and this in turn leads to an explanation of the microbes having been endogenous in origin, especially when it is acknowledged that butchered remains are less likely to be affected.

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Chapter 6. Case Studies of Diagenesis in the Form of Microbial and Fungal Alteration of Bone

6.1 Introduction

Diagenetic change in the form of microbial and fungal tunnelling has been well observed both in the archaeological record and in experimental studies. This chapter will look at these cases in more depth and will include terrestrial, laboratory, marine and archaeological settings. Because it is not known when bacterial destruction of bone commences it is necessary to look to prior research and experimental studies to try and ascertain both when microfocal destruction (MFD) begins and once begun how long it takes for it to progress to completion.

6.2 Laboratory Based Studies

Laboratory based studies are quite rare, but have been successful in mimicking what is found in older material recovered from ancient cemeteries and burials. However, these are often carried out under ideal conditions at optimal temperatures and with selective bacterial species. Grupe & Dreses-Werringloer (1993) used pig bones that were subjected to inoculation by either soil fungi or bacteria and then kept at temperatures optimal for bacterial growth. These were shown to be densely colonized by microbes within four months using fluorescence light microscopy. The bacteria chosen were selective, and unnaturally high temperatures were used to optimize results. It would be interesting to investigate how and if this process occurs at typical grave soil temperatures.

6.2.1 Bones Exposed to Seawater (Marine Settings)

In experimental marine based studies microbial tunnelling has been found as soon as one year after deposition. This type of tunnelling often represents an outside in mode of attack and is quite different to terrestrial tunnelling visually and looks more like fungal tunnelling; although cyanobacteria are probably responsible for the damage seen. In one experiment fresh bovine metatarsi were deposited at a depth of 60m on the sea bottom. Bored cavities were apparent after one year's submergence, and these were often filled with micro-organisms. Bacteria, algae and protozoans were all present but it appears that the protozoans of amoebic type were unambiguously involved in bone resorption (Ascenzi & Silvestrini 1984). Tunnelling was also apparent in forensic bone recovered from sea water at between 4 & 5 years postmortem by Yoshino *et al* (1991); no samples of earlier date were

included in the study. Balzer *et al* (1997) used sterilized bone inoculated with several different types of bacteria to research the implications for stable isotope analysis when bone collagen had been degraded by microbes. The bones were cultured for between 8 and 18mths at optimal temperatures and after this time bacterial invasion sites were clearly identifiable although no tunnelling phenomena were detected. It is clear from these limited studies that bacterial attack in water can commence very quickly even in disarticulated bone. This would suggest a very different process to what is seen in land based studies.

6.2.2 Modern Terrestrial Real-Time Experiments

A variety of situations have been researched using real-time experimentation. This entails depositing carcasses or disarticulated material in a variety of settings and then leaving in place for set periods before examination for diagenetic change. Although this is less likely to give false results it is reliant upon generally only one or two variables and therefore the results are limited. Using modern burials Cross (2006) examined two pig carcasses that had been buried for periods of up to 18months. In neither case was any micro-focal destruction observed although one carcass was placed in the ground as soil temperatures were beginning to get colder and both corpses were in areas where temperatures are in general quite cold. Both cadavers had soft tissue remaining which would supply any microbes present with a food source that is presumably easier to exploit than bone collagen.

Nicholson (1996) looked at buried animal remains including mammal, bird and fish that had been interred for a period of seven years. Although focal destruction is reported in a few of the cases (focal destruction in sheep metapodial from three sites) some of the animals were placed in the ground as singular bones whilst others were buried as entire corpses. Nicholson found that the most critical mode of destruction in the early postmortem period was microbiological followed by drainage and soil pH. It is also suggested that any differences can be accounted for by variance in soil microbial populations. But it should be mentioned that this paper was researching the effects of different types of soil and pH values on bone degradation rather than the effect of endogenous bacteria.

Hackett (1981) experimented with sterilized compact bone buried in three types of garden soil over a period of one year. The bones were kept at room temperature and at the end of this period several sites of tunnelling were seen. Curiously, Hackett does not give these tunnels a type (after all he was the one to categorise

them); he merely states that they were not of 'Wedl' type, but that they were more likely to be due to bacterial invasion. In forensic and experimental cases it can be difficult to assign a type as any destructive lesions may be in the very early stages and unrecognisable as to distinct groups. Most of the information on how to label a lesion comes from archaeological bone where bacteria have had many hundreds of years to complete the destructive cycle. There must be a point at which collagen has begun to be lost but its extent is narrow and without the tunnels that define MFD.

Wedl found fungal tunnelling of teeth13-17 days after being left in untreated well water (Wedl, 1864). Marchiafava *et al* (1974) carried out experimental research in to bone boring by fungi. Human vertebrae were placed in plant pots with garden soil and by the 45th day the specimens were penetrated by fungal hyphae. When using sterilized soil only one species (*Mucor*) was able to develop. These limited studies show varying degrees of destruction over very narrow time periods, but it is clear that both bacteria and fungi are capable of attacking bone in the early postmortem period. There is some optimism that the experimental research carried out for this study will add to and clarify what is already known.

6.2.3 Modern Forensic cases

Apart from real time experimentation the next best scenario is to look at medico-legal forensic cases. In these instances real bodies of humans are examined that have been exposed to the elements for possibly many years. One major drawback however, is that many of the bodies have been left on the grounds surface or are given a very shallow burial; rarely are there cases of deep interment. Yoshino *et al* (1991) looked at modern forensic bone and found that in general MFD was present in inhumed bone from around five years postmortem. They linked this to the fact that most burials would take this long to skeletonize unless in a shallow grave. In one case of shallow interment MFD was seen after 2.5 years. In bones that were found as surface exposures (no33) no MFD were seen in bones up to 15 years postmortem except for one case where bacteria were clearly responsible for a small area of bone destruction. The quickest Bell (1996) found bacterial postmortem tunnelling in forensic cases (apart from the predator scat discussed below) was fifteen months postmortem: this was recorded in a rib recovered from a surface exposure in a waterlogged muskeg bog. Further destructive changes were evidenced in a variety of settings from PMI's of 2 years for marine type alteration

and between 7 and 70 years for terrestrial types of destruction. The primary problem (apart from lack of deep interment) with all of the above is that the bodies cannot be sampled over time. They are sampled only once, at the point of discovery in the case of clandestine burials/murders and at the point of exhumation in the other. This severely limits prior knowledge and a true timescale cannot be given and any attack found could have happened much sooner.

6.2.4 Predator Scat

A very different type of case from the others so far researched is that of a predator scat. Two sites of focal demineralization were observed by Bell (1996) in a fragment of bone recovered from a predator scat at three months postmortem. This section of bone had passed through the digestive tract of a carnivore and in addition was recovered from a wet coastal environment. It must be noted that during the passage through the predator the bone would have been subjected to microbial loading from the animals' indigenous flora as well as highly acidic conditions during passage through the digestive tract. This is a scenario that is quite different from the norm and this process is known to demineralise bone and therefore is not indicative of the natural tunnelling that is being researched here. It does however prove that tunnelling may very well be a product of the early postmortem period.

6.3 Archaeological Cases

The bulk of our knowledge of MFD is as a direct result of histological sampling of ancient human and animal bone from archaeological material. This often gives the best results as the bones have been subjected to bacteria for very long time periods and in general destruction by microbes will be complete. One problem with this material is that it can be so completely destroyed that it becomes practically impossible to visualise distinct MFD. Some sections become very unclear and although bacterial attack is the causative agent nothing more than the occasional haversian canal can be detected. Further to this is the problem of reuse of cemeteries and in particular graves, which may impede any results found. This section will cover a variety of archaeological case studies from a normal demographic cemetery were all age groups are represented to those burials where singular discrete burials of infants can be found. It will also incorporate disarticulated animal remains, mummified bodies, cist burials and unique findings from both the Mary Rose Shipwreck and the bodies of Pompeii.

6.3.1 Marine Type Tunnelling in Archaeology

Bell & Elkerton (2008) published findings from the Mary Rose shipwreck and evaluated two different layers of deposition where the first formed very quickly with much silt type coverage and the second forming over a much longer time period (several decades). The bones from the first layer were all free of tunnelling whilst those from the second displayed peripheral tunnelling that is thought to have been caused by cyanobacteria. This type of destruction is visually unique (fig 6) and may be of use in forensic settings where it can be stated that bones demonstrating this type of degradation must have been in a marine environment.

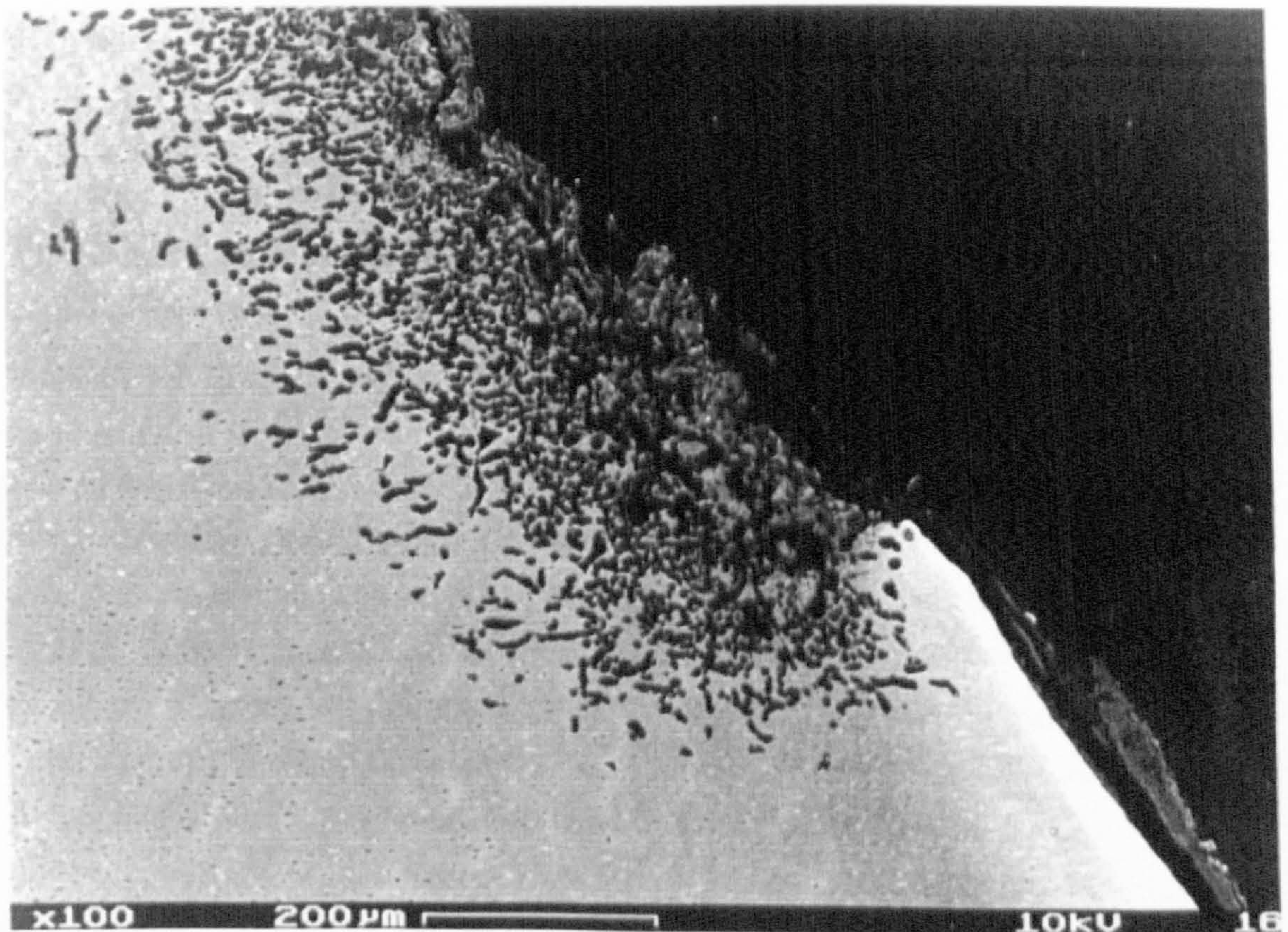


Fig 6. Micro-boring modern forensic tooth recovered seven years postmortem from a marine setting. Note the outside-in mode of attack. Periosteal surface to the upper right. (Bell & Elkerton, 2007)

Garland (1987) also found what he described as Wedl type tunnelling affecting only the cortical area of bone that had been in seawater. Ascenzi & Silvestrini (1984) found borings in archaeological bone from two medieval shipwrecks that affected the canaliculi and that were similar morphologically to tunnels produced by fungi in soil-buried bodies.

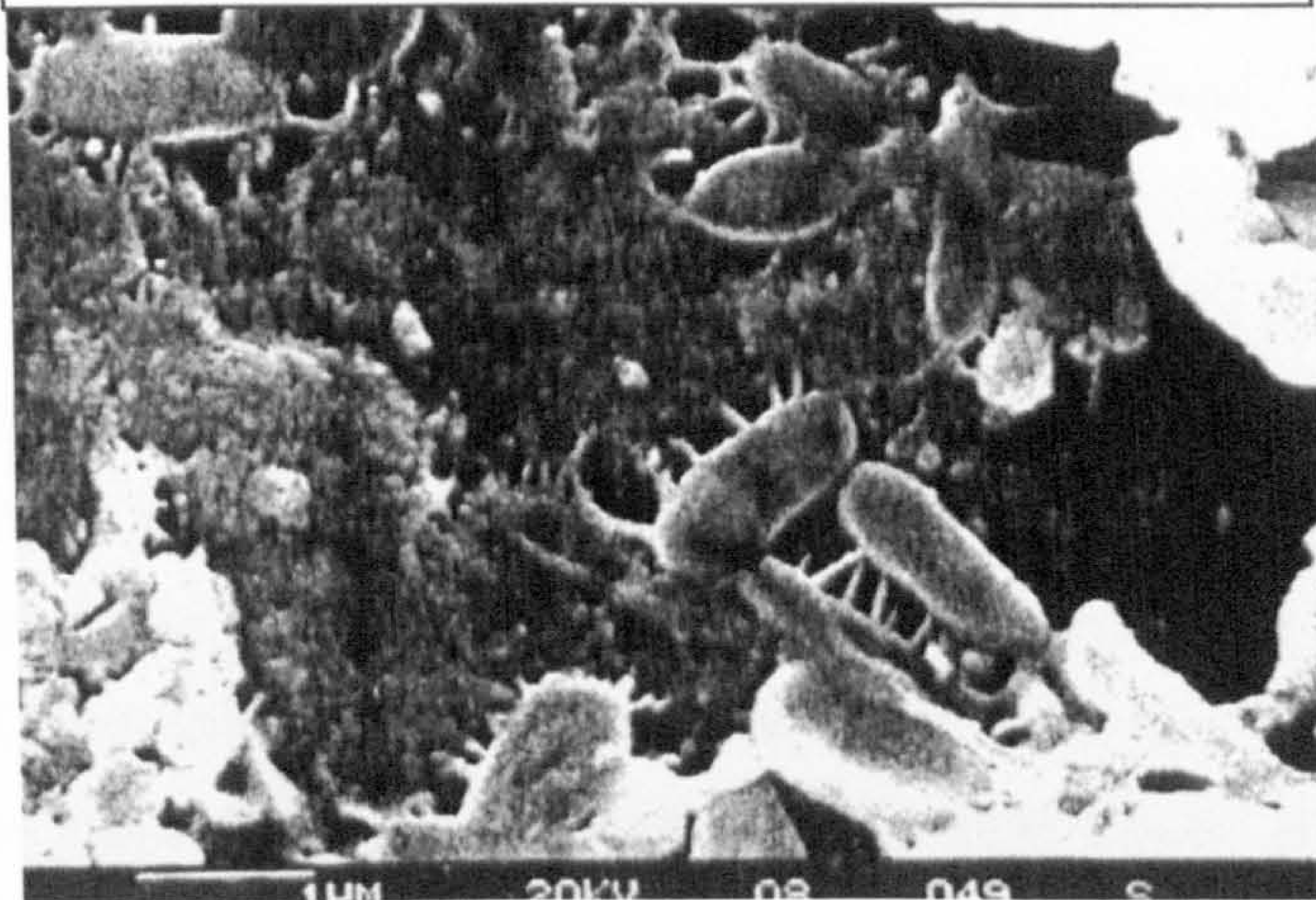
6.3.2 Terrestrial Tunnelling

Jans *et al* (2004) observed microbial changes in archaeological bone and found that out of 261 bone samples from 41 sites 68% had been altered by microbial attack and that linear-longitudinal and budded were the most prevalent types. The percentage of animal bone affected by microbes was much lower at only 34%, however bones from entire burials rather than from butchered animals were all affected by tunnelling. When Garland (1987) observed archaeological bone (n76) he found the predominant types to be Wedl, linear longitudinal and lamellate. It is interesting that it is stated that no budded foci were found as in Jans *et al* (2004) later study, lamellate foci were always found in association with budded MFD. It is possible that the distinction between budded and linear longitudinal were unclear as in the research carried out for this project budded foci are extremely prevalent in many of the sections viewed. Alternatively, this could be due to amalgamation of tunnels as previously discussed (chapter 5). Smith *et al* (2002) studied human archaeological material from the medieval site of Apigliano in Southern Italy where evidence of microbial attack is limited. Some samples do display tunnelling and several are described as being extensively affected, but the majority that are described as well preserved and which do not display microbial attack are highly cracked and the collagen fraction is depleted. It is suggested that collagen loss in the absence of microbial activity is due to the action of peptide bond hydrolysis. Hackett (1981) examined archaeological bone from several different countries and found one hundred and thirteen out of one hundred and seventy specimens to have suffered from tunnelling. Although all categories of tunnelling were recorded, no further information is provided as to types found and quantification.

One of the few studies producing visual evidence of bacteria within bone was carried out by Jackes *et al* (2001). Using SEM they were able to visualise bacterial colonies in archaeological bone (fig 6.1). On examining bone from both the Mesolithic and the Neolithic, bacterial attack was evident in both thin sections and on uncut bone. They used diagnostic criteria to identify the bacteria as *Clostridium histolyticum* (although this remains unconfirmed as it is difficult to type bacteria visually) which is known to attack bone. In thin section the bacteria could be seen either side of a central unaltered portion of bone with most of the bacterial cavities having a breadth of around 1µ, and other cavities were closer to 0.5µ in diameter. Evidence of tunnelling was apparent and these are described as being straight, tortuous or dog-legged. Colonies of bacteria were seen around the Haversian canals which were then reduced in size due to a coating of calcite around the canal

margins and the destructive foci are orientated around the circumferential lamellae rather than appearing randomly. Due to their findings experiments were carried out on modern human bone using three species of *clostridium* (*C. sporogens, C. perfingens and C. septicum*).

Fig 6.1 Clostridium Bacteria in Bone (Jackes *et al*, 2001)



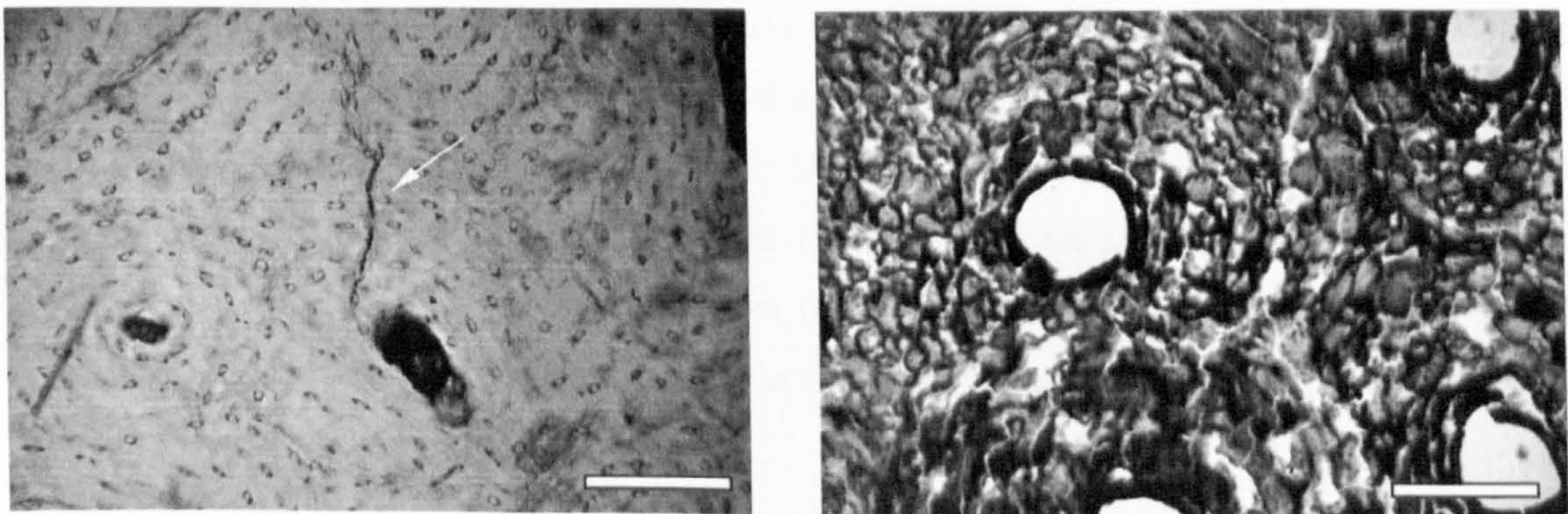
The bones were examined after seven months at which time the specimens incubated with *C. perfingens* and *C. septicum* showed no alteration, however the surface of the specimen incubated with *C. sporogens* had an altered bone surface. In no case were tunnels seen in the bone. It is worth stating that a succession of colonisers would be presumed to be the norm (as with insect colonization of cadavers that occurs in distinct waves) and therefore even if bacteria are isolated from destructed archaeological bone, it is unlikely that these will be the pioneering decomposers (Grupe, 2001) and may instead be more modern environmental contaminants.

6.3.3 Ossuary (Archaeological)

Piepenbrink (1986) examined bones from the medieval ossuary at Göttingen and found that all of the samples had been subjected to extensive penetration (n20). This presents an interesting theory as the bones are from an ossuary. There is no recorded information as to where the bodies were originally buried and the duration of interment is not stated, although, it is usual for bones in ossuaries to have been deposited there in the early post-mortem period. It would be likely that any tunnelling would have taken place during interment as once within an ossuary the microclimate would probably not be conducive to tunnelling phenomenon.

6.3.4 Pompeii

Guarino *et al* (2006) looked at unique bones that originated from the site of Pompeii and stored at Terme del Sarno. Twenty-seven femurs of unknown provenance were sectioned that had for a period of around 2000 years been buried beneath 5-6m of pyroclastic material. Causes of death in the city were separated in-to two distinct phases. Firstly, when Vesuvius erupted there was immediate fallout of pumice lapilli that reached depths of up to 2.8m (Luongo *et al,* 2003). This material began to build up on the roofs of buildings and eventually these collapsed under the weight, causing death by the falling tiles and other debris. Many of the remains found at his level have fractured skulls. Within six hours of the eruption, a further and more fatal stage began that involved pyroclastic density currents (PDCs). The PDCs were responsible for overwhelming flows that destroyed everything in their path and formed a compact and very cohesive layer approx 1-3m thick on top of the previous pumice lapilli layer. The research by Guarino *et al* showed that the majority of the bones were very well preserved (fig 6.2) and visibly very similar to fresh modern



A B

Figs 6.2 & 6.3 Two very different bone sections from Pompeii. A= Very well preserved with evidence of microcracking (arrow) B= One of the few sections that is entirely destroyed by microbial tunnels (Guarino *et al*, 2006). Scale A= 90µ Scale B = 180µ

bone and only 33.4% were affected by MFD (fig 6.3). One explanation for this is that the dense material above the corpses prevented air and moisture from reaching the corpses. Microbial attack would then be unexpected due to an unfavourable microenvironment. However, a few of the bones (at least 2) did exhibit signs of extensive diagenetic change in the form of lamellate tunnelling. Because provenance is unknown it is difficult to say why these few bones were subjected to diagenetic change in the form of MFD. One possibility is that the remains are from a group of people who were huddled together at the time of the eruption and some of them were better protected by the overlaying of bodies or by trapping of oxygen in a larger void. This is not improbable as many large groups of dead have been

recovered; this includes groups of 11, 12, 13, 14, 17, 18 and 20. It is also interesting to note that the influence of soil bacteria would not be an issue here as the bodies were not buried, merely covered or more generally layered/sandwiched between the two separate deposits. In addition to this the pyroclastic material would have been sterile due to the high temperatures under which it was produced (although this would mainly have been deposited cold due to the small size of the particles) and therefore very little exogenous bacteria would have been introduced during the period of volcanic activity. The authors (Guarino *et al*, 2006) themselves exclude bacterial infiltration from soil as a mechanism for the diagenetic change in the bones and offer instead the explanation that this was as a direct result of the invasion of intestinal bacteria. Another study of Pompeian remains (Cipollaro *et al*, 1998) produced similar results. In this research the provenance of the remains is well documented and consists of thirteen individuals from the house of Caius Iulius Polybius. In this instance 53.8% of the bones analysed histologically are categorized as Good. This was the highest score possible and of the other categories one was intermediate, four were poor and one was very poor. It appears that the unique environment of the remains prevents bone diagenesis in some bodies better than in others. Turban-Just (1997) declares that soil bacteria are "an always present component of all burial conditions" and that "they substantially contribute to the decomposition of bone collagen". This is a slight inaccuracy; as demonstrated above this is not always the case and in many other scenarios this is also untrue. For instance lead coffins prevent the influx of soil and even wooden coffins will delay the soil bacteria reaching a corpse.

6.3.5 Cist Burials where the Remains are Disarticulated

Early Bronze Age burials from Windmill Fields, Ingleby Barwick, Stockton on Tees, have provided an interesting insight into disarticulated remains from a cist burial (Annis *et al*, 1999). The skeletal remains form part of a rescue excavation with eight sets of remains being identified with a MNI of eleven individuals. Of these diagenetic analysis by light microscopy was carried out on four of the burials (Booth, 2008 SK2, 3, 5, & 6) and it was found that two of the skeletons displayed immaculate bone preservation (SK2 & 3) whilst the remaining two were almost completely destroyed by advanced bacterial attack. SK5 was articulated and crouched as was SK6 (fig 6.4 & 6.5) Excarnation has been implicated in one of the burials (SK3) that exhibits near perfect preservation as only the skull, long bones and a few finger bones were recovered from the grave that was highly suggestive of disarticulation prior to burial. In addition, the remains were also interred in a cist that

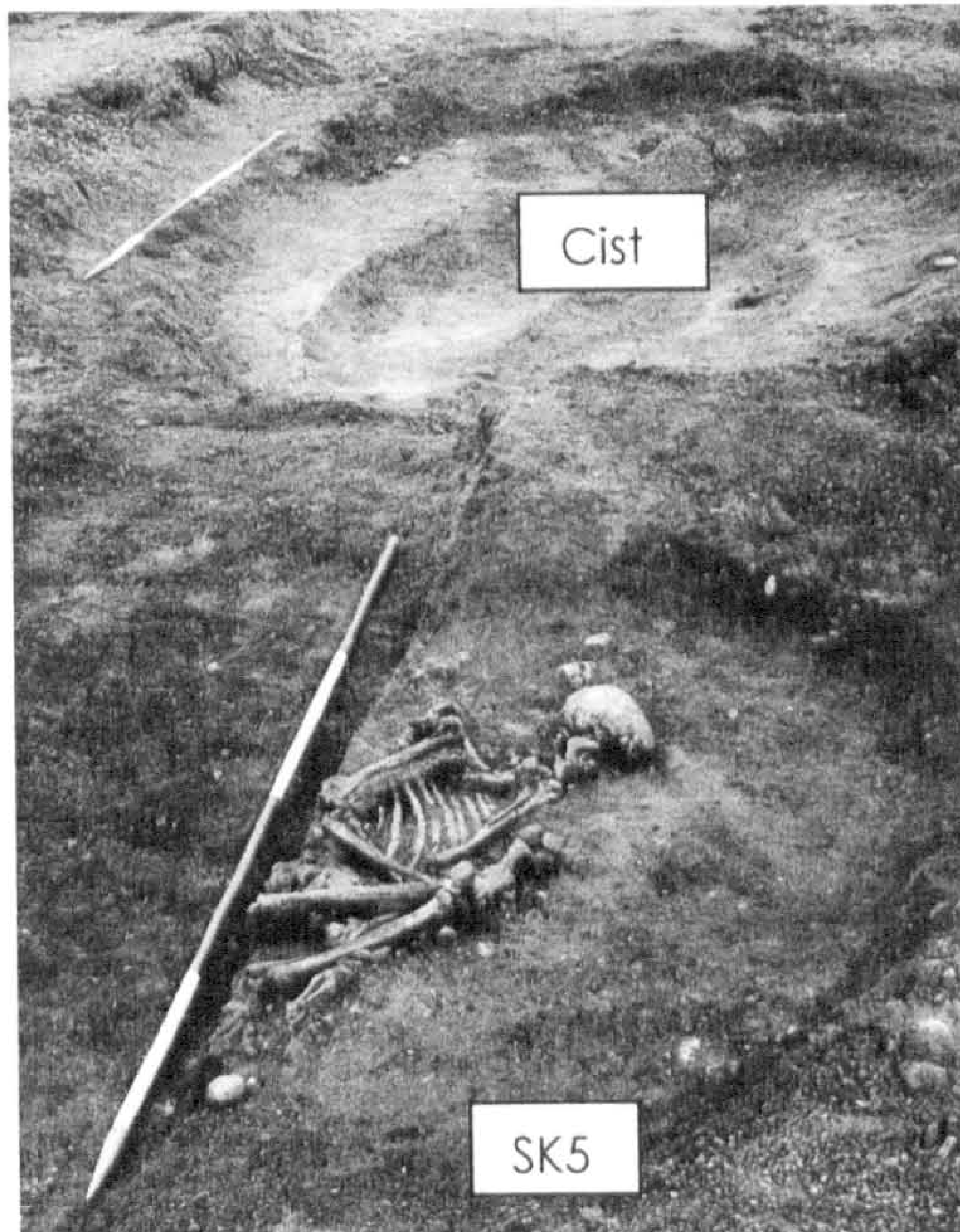


Fig 6.4 Photograph of SK5 in-situ with Cist in the background (Annis *et al*, 1999)

was originally constructed from wooden planks. It is difficult to ascertain the provenance of the further well preserved specimen (SK2) as these were disturbed during construction work and the bones were mostly recovered from the spoil heap. In addition to this it is thought that a number of the bones belong to SK1 and a female pubis belonging to an older woman was also found with the remains. If soil bacteria were the protagonist of bacterial postmortem tunnelling of bone then one would expect all of the bone from the site to be similarly affected. The fact that these two sets of bones are not, may be primarily influenced by the lack of gut bacteria entering the inhumation environment by the removal of the gut at a point soon after death. It must also be noted that a cist burial is often a sealed environment, with planks or stones lining the sides and also covering the top. From prior research (Taylor, 1959), it is known that any fill within this type of burial is often as a consequence of soil slowly leaching in to the cavity over many years, either as the wood degrades or the stone breaks down. Therefore soil bacteria would not be available to colonise the body in the early postmortem period.

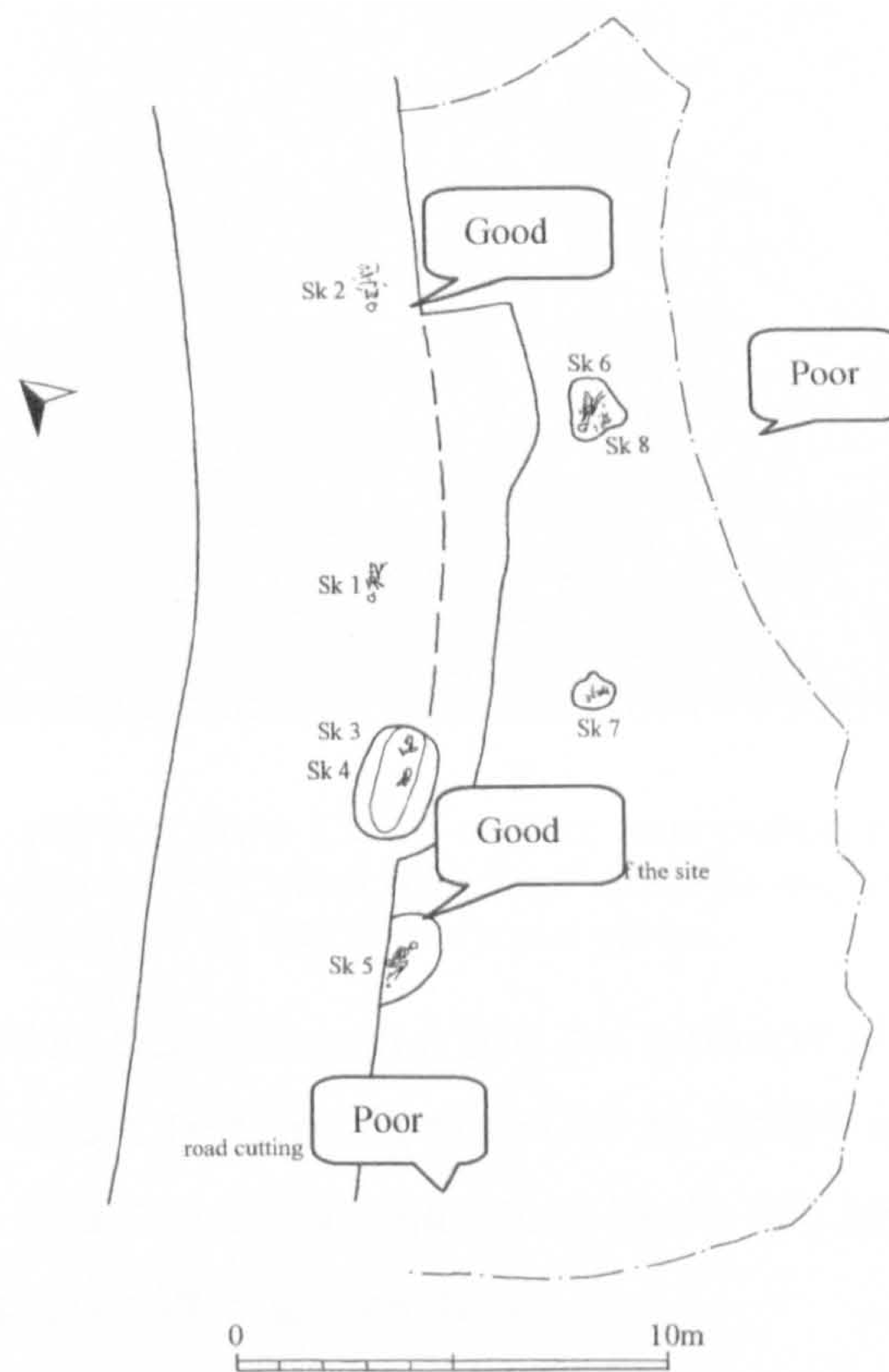
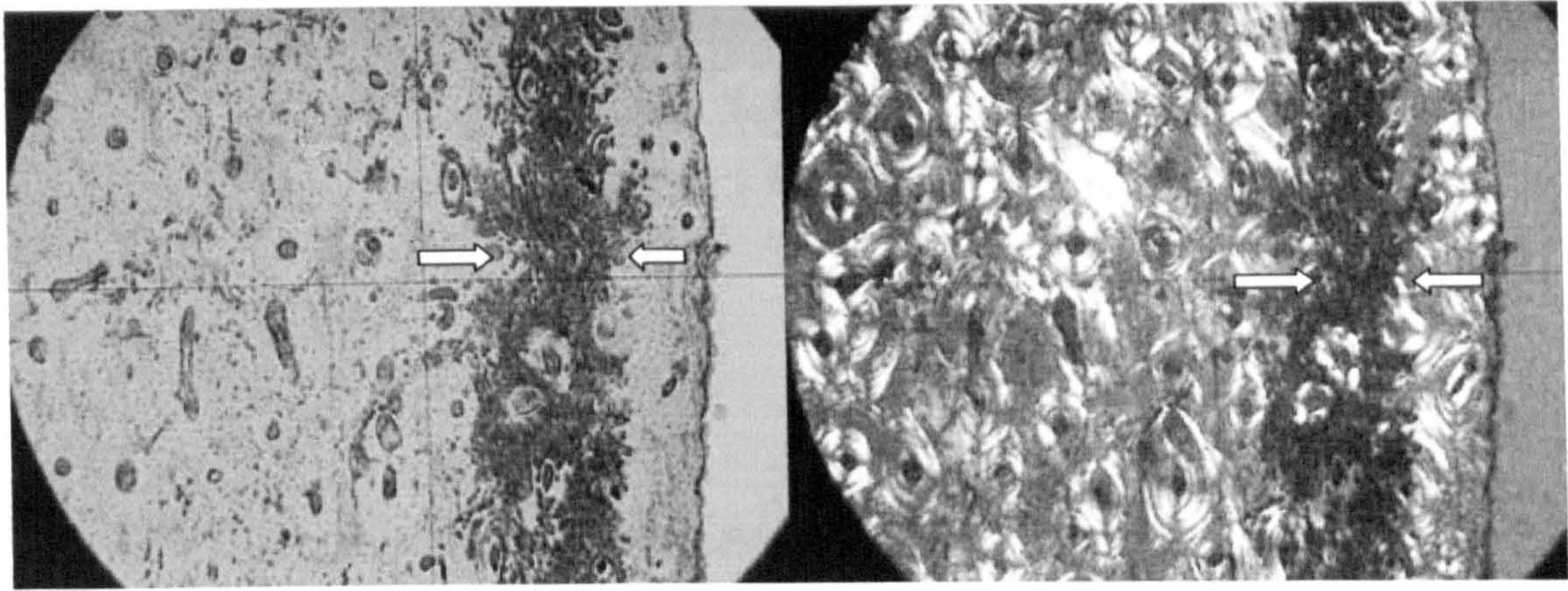


Fig 6.5 Layout of Burials at Windmill Fields (Annis *et al*, 1999).

6.3.6 Cladh Hallan Mummies

The Cladh Hallan mummies are an interesting enigma (Parker Pearson *et al* 2005). Research points towards their being mummified soon after death. This is supported by the arrangement of the bones and by multiple people in one set of remains (i.e. the skull and jaw and postcranial skeleton belong to three different people, yet the remains are articulated), carbon dating and demineralization of the bone. Summerfield (2003) originally looked at the remains microscopically and found an unusual pattern of diagenetic change in the remains of the adult male (SK2638). There is a thin band of destruction slightly beneath the periosteum that encircles most of the section (figs 6.6 & 6.7). This appears slightly unusual as often bacterial attack will affect the entire section but in this case for some reason the degradation has been halted. According to Parker-Pearson *et al* (2005) this is as a consequence of mummification in the early postmortem period and it was proposed that the



A B

Figs 6.6 & 6.7 A= Bone section from Cladh Hallan mummified male showing a band of destruction of inside-out origin B= Polarized light view of same section showing corresponding loss of collagen (Parker-Pearson *et al*, 2005). No scale given.

evisceration had preceded deposition in a bog for a period of time after death. After further research the authors (Parker Pearson *et al*, 2007) revised this speculative opinion and accepted that the body was most likely not eviscerated and that the diagenetic change was a result of gut bacteria.

6.3.7 Infant Remains

The initial and most profound purpose of this research was to gain an understanding of microbial attack and to decipher whether this was a phenomenon limited to intestinal bacteria or alternatively as a result of soil bacteria or a combination of both. To this end both parts of the experimental research are heavily biased towards children. The primary burials were very much an attempt to prove or disprove the theory of endogenous bacteria being responsible for bone tunnelling. The previous chapter discusses in depth the issue of presumed sterility in foetuses and with this in mind it is then possible to surmise that if a foetus is buried sterile then any resulting postmortem modification by bacteria must surely be as a result of soil bacteria, unless other contamination can be ruled out. Children's remains are not researched as avidly as those of their adult counterparts and there is a deficit in the literature regarding their histological integrity. Two sites where infant remains have been studied are Wijnaldum in the Netherlands and Bolsover, England. The remains from the Netherlands (Colson *et al*, 1997) comprise of seven skeletons that date from the 2nd-9th C AD of which six are newborn infants and the other is a young female adult of 18-19yrs. Five of the infants have excellent preservation status without any MFD visible and scored 4-5 using the OHI (Oxford Histological Index). The remaining infant has some damage to the bone with destructive foci present that was given a score of 3. However, this child is not given an age and comes from

scattered infant bones that are associated with other finds. This presents the difficulty of suggested reasoning for the attack without full provenance and age status. Human remains analysed from a cemetery in Bolsover, dating to Norman times have given interesting results. According to the author (Economou, 2003), the remains of foetuses that were buried as discrete singular burials showed no signs of tunnelling in the form of Linear Longitudinal MFD (table 6). The presumed sterility of pre-term infants would suggest that no bacterial attack should be found in their bones unless they are contaminated by either the mother (those children who died as a consequence of the mother dying) or another source whilst in the ground if endogenous bacteria are culpable of the tunnels that are found. However, apart from one exception, a foetus of 28 weeks that has a bone structure very similar to fresh bone (fig 6.8), all of the other infants suffer from tunnelling of the budded type. In the other remains (non-infants) both LL and budded occur regularly but Wedl and Lamellate were not recorded by the author. Most of the remains that were sectioned from this cemetery scored 1 on the OHI, meaning that they were all very badly preserved. Having reanalysed these skeletal remains, it appears that LL type tunnelling is present in at least four (n=7) of the foetuses. In two of the seven, destruction is so advanced that visualising any type of MFD is impossible. It is also possible that three of the foetuses are actually full term as one is aged at 39 weeks and the remaining two at 40 weeks. Aging of the skeletons was carried out during this research (table 6.1) and by using Fazekas and Kosa (1978) and prior probability (Gowland & Chamberlain, 2002) it is probable that some of the foetuses are older than the original ages given. Here at least, presumed sterility cannot be proved

Skeleton (BOL)	AGE	*OHI (Economou)*	*OHI (This research)*	*MFD (Economou)*	*MFD (This research)*
007	Neonate	5	5	None	None
008a	Foetus	5	5	None	None
008b	Foetus	5	No Section	None	-
009	Neonate	5	No Section	None	-
010	Foetus	0	No Section	Budded	-
011	Neonate	0	0	Budded LL	Budded LL
012	Post-neonate	3	2	Budded LL	Budded LL Wedl
014	Foetus	1	0	Budded	Budded LL L
017	Neonate	0	0	Budded LL	Budded LL
028	Neonate	1	1	Budded LL	Generalised Destruction
056	Foetus	1	0	Budded	Generalised Destruction

Table 6 Results of Bolsover thin sections.

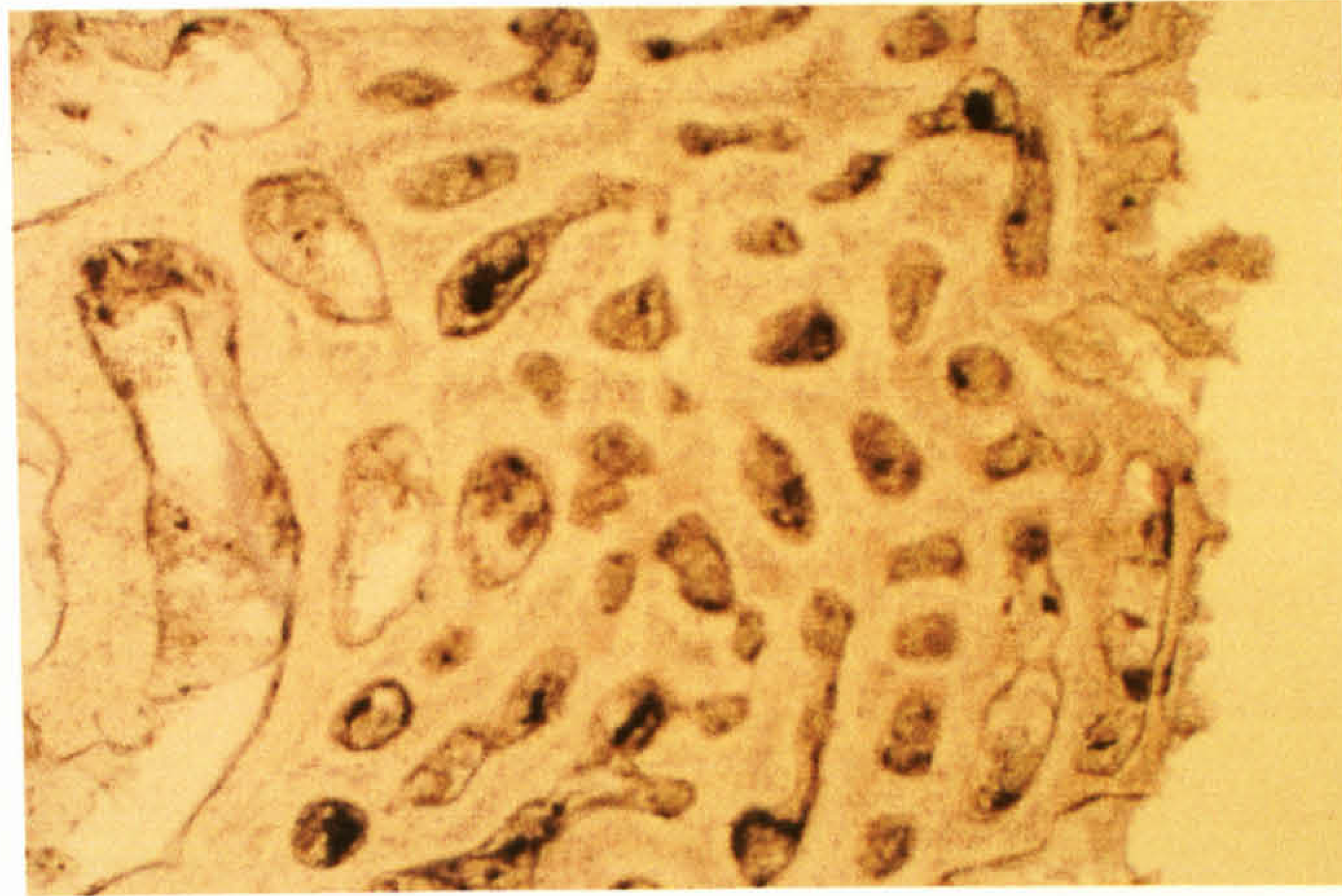


Fig 6.8. Exceptionally well preserved bone section. (Author, 2008).

as childbirth is not always easily definable in terms of when a child is actually born. Premature babies are a daily occurrence with modern day infants surviving, admittedly with medical assistance, from as early as 22 weeks gestation. This would surely not have been the norm in the past but both 39 and 40 weeks gestation are certainly classed as viable. Additionally, not all of the remains were discrete singular burials. The North side of the church was almost exclusively used for infant remains, with just a few modern intrusions from cremations. So, in theory the infants found in this section of the graveyard, if found to have tunnelling, should have MFD only from intestinal bacteria if buried at depth and if indeed this is how microbial attack commences. It is interesting to note however, that in at least one case (BOL007) the infant remains have been interred in a grave cut that is adult in size. Is this a case of reuse of an old grave or simply a grave used in an unusual way. It is therefore entirely possible that these three infants survived at least long enough for bacteria to begin to colonise their bodies. This leaves 4 foetuses, one completely preserved and the other three being very poorly preserved. Of course, it must be remembered that their remains have come from a long used cemetery that has an infiltration of endogenous human intestinal flora every time a corpse is interred. Recent research (Wilson *et al*, 2006) in to microbial loads in soils containing decomposing animals, have found that the amount of anaerobic organisms (that can be cultured in the lab) triples in twelve months from the time of deposition. Additionally, basal respiration measurements, a more accurate method of counting bacteria looked at three deposition scenarios. The control grave, a pit dug but no burial, had the least amount of microbes, whilst a grave with a burial, (but that was subsequently scavenged) had double the amount of bacteria, and a third burial of a pig that was un-scavenged had double the amount as the scavenged pig.

Skeleton (BOL)	Original Status	Discrete Burial	Femoral Length	Humeral Length	PP	F&K	Aged	Original Age
North Side of church								
007	Neonate	Yes But in grave cut of adult	89.2	73.7	46-48wks			42-44wks
008a	Foetus	Yes but with twin		38.2				
008b	Foetus	Yes but with twin	46.3	42.2	28wks	26wks	26-28wks	26-28wks
009	Neonate	Yes	73.7	64.3	40-42wks	40-42wks	40-42wks	38wks
010	Foetus	No Intercuts grave of child	35.9	34.7	26wks	22wks	22-26wks	24wks
011	Neonate	Yes	75	67	40-42wks	40-42wks	40-42wks	39wks
012	Juvenile	Yes	93.1	76	46-48wks	Over 10 lunar mths	1-2mths postnatal	1-3mths
014	Foetus	Yes	59.7	53.9	34-38wks	34wks	34-38wks	33wks
017	Neonate	Yes	78.7	68.4	42-44wks	Over 10 lunar mths	42-44wks	40wks
General Cemetery								
028	Neonate	No	75.4	66.8	40-42wks	Over 10 lunar mths	40-42wks	39wks
056	Foetus	No	50.8	45.5	28-30wks	29-30wks	28-30wks	30wks

Table 6.1 Aging methods used in this research PP= Prior Probability (Gowland) F & K= Forensic Fetal Osteology (Fazekas & Kosa, 1978)

Whatever the cause of MFD clearly the two youngest foetuses were not affected yet others merely weeks older were and in fact almost all of the skeletal material recovered from the site is very poorly preserved. Being unaware of past cultural practices makes any real suggestion untenable. In modern cases of stillbirth it is not unusual for mothers to keep the child at home until the time of burial. Visitors will call, most of whom will hold and kiss the child and whilst doing so pass on bacteria, the baby will be washed further contaminating the child. It is impossible then for the child to be presumed sterile although it is presumable that the intestines will not have been colonized, merely the skin and possibly the respiratory tract.

The Blackgate collection has a number of very young infants and foetuses. Twenty-four sections of all ages were taken of which nine are aged between 27 weeks to less than one year (table 6.2). Of these, four are free of MFD, one has generalised destruction where it is difficult to ascertain the cause, one has possible budded tunnelling and three have positive tunnelling. The three with definite tunnelling are aged between thirty-nine and forty weeks and this could mean that they were live born only to die within a few days of birth. All of the sections from the adults have visible MFD.

Skeleton No	Age	MFD
BG3277	27 wks	Generalised Destruction
BG90 3285	32 wks	Possible Budded
BG90 3191	36 wks	None
BG90 3166	39 wks (Tibia)	Generalised Bacterial Attack
BG90 3166	39 wks (Humerus)	None
BG3215	39 wks	Budded LL
BG90 3204	40 wks	Budded LL
BG3191	46 wks	None
BG90 3207	<1yr	None
BG90 3830	18mths	Generalised Bacterial Attack
BG90 3184	4-5yrs	Generalised Bacterial Attack
BG92 3664	5-6yrs	Generalised Bacterial Attack
BG81?	Adult	Generalised Bacterial Attack
BG81?	Adult	Generalised Bacterial Attack
BG464	Adult	All 3 types
BG91	Adult	All 3 types
BG8	Adult	All 3 types
BG481	Adult	All 3 types
BG548	Adult	All 3 types
BG127	Adult	All 3 types
BG614	Adult	All 3 types

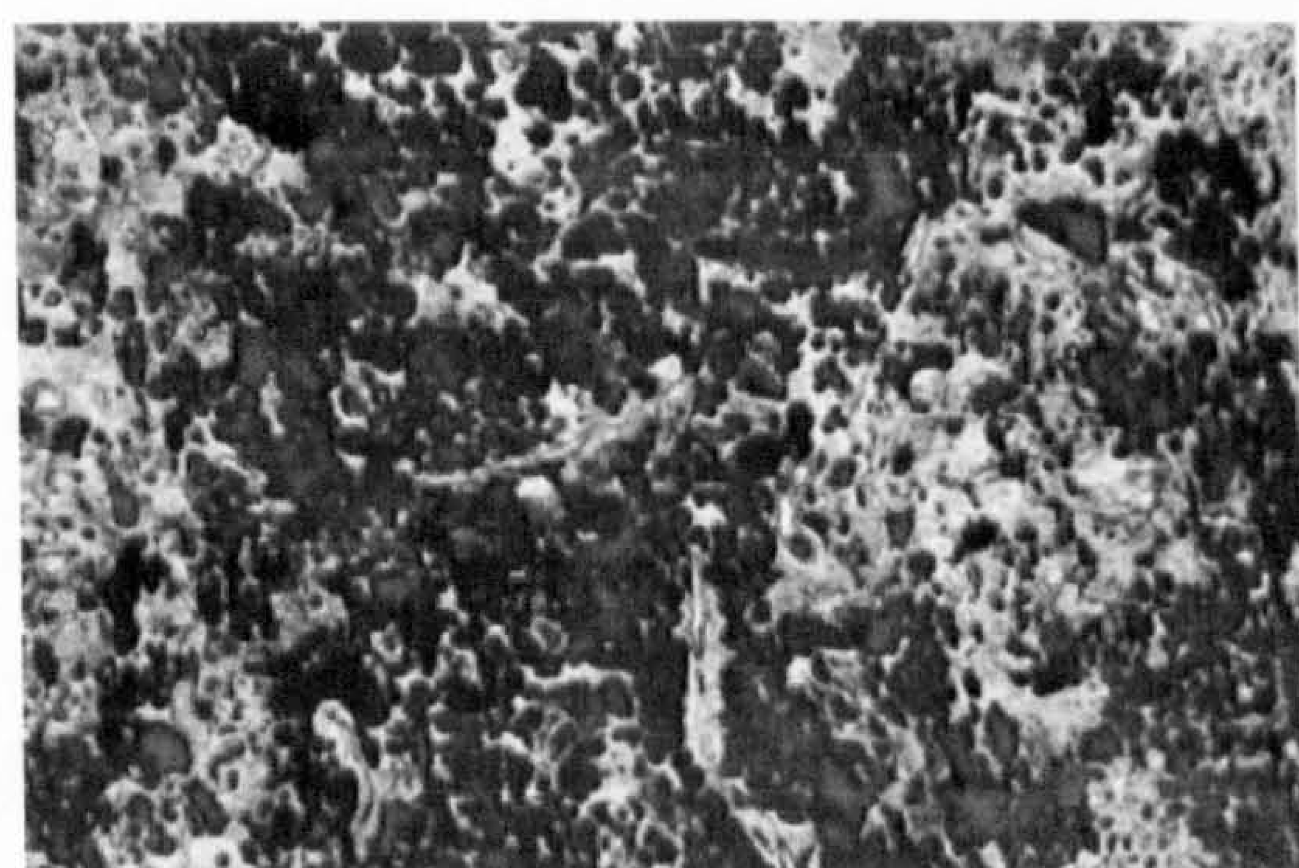
Table 6.2 Blackgate collection showing differential preservation between juveniles and adults. Data from studies by the author.

6.3.8 Animal bone

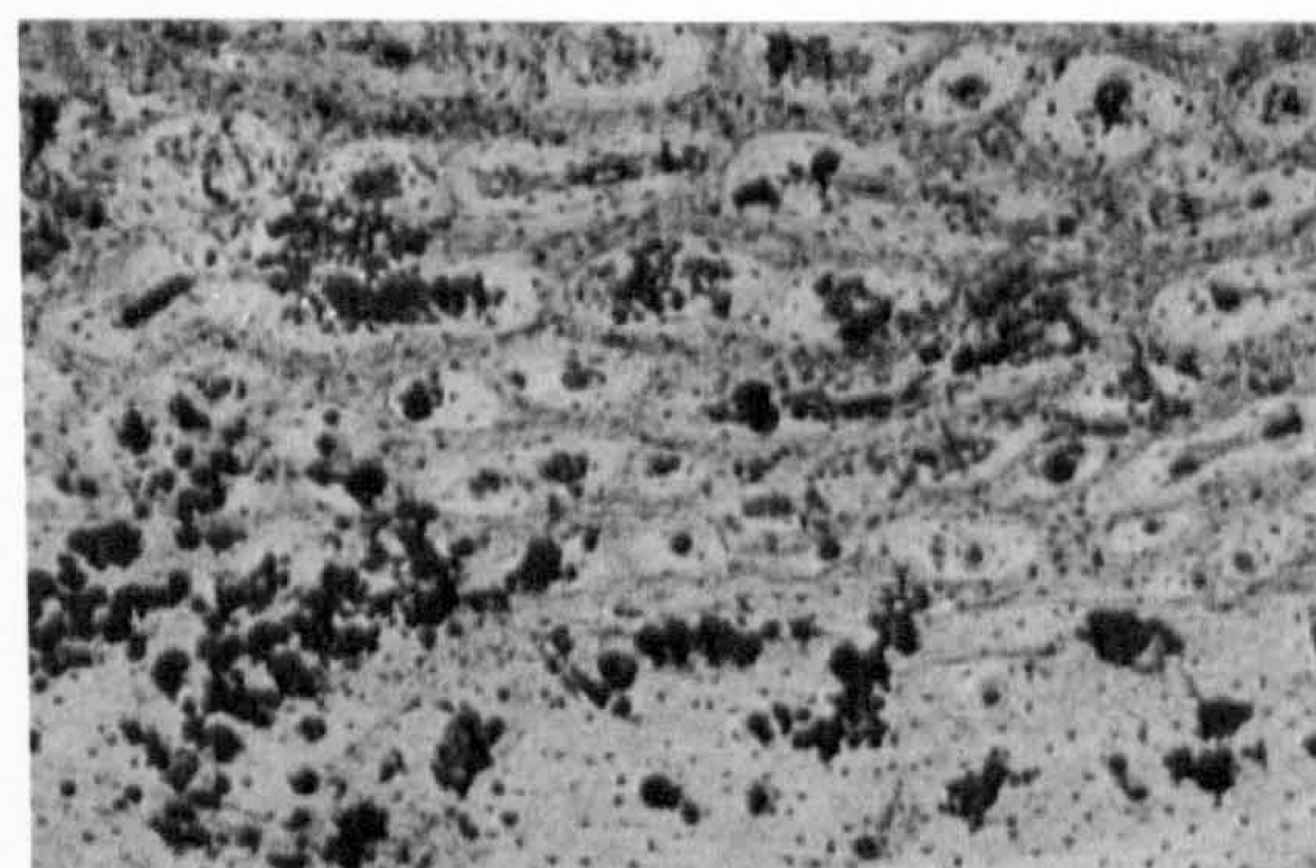
It has been suggested that microbial attack in animal bone is less likely due to the fact that butchery practices result in sterile carcasses and that if any diagenetic change is present this will be in the form of fungal invasion. However, butchering can take different forms and this is evident at the butchery site of Paso Otero 1 in Argentina where bone samples are regularly found to have been diagenetically altered by bacteria (Gutierrez, 2001). Two distinct usage phases have been identified and excavated from which Guanaco (camelid type animal) bones have been recovered and analysed. The site is classified for these two time periods as stable landscapes dominated by buried A horizon soils that developed within a poorly drained and very moist setting with a typical vegetation of grasses and reeds. Two different processing methods were employed and in the earlier time period the whole carcass was deposited at the site and only the edible meat was taken back to the campsite. In the later period select animal parts in the form of fore and hind limbs were left in piles at the site. Both assemblages suffered from bacterial MFD although the later assemblage was slightly less affected. The group with the entire carcass being left behind is easier to interpret as the intestines would also have been left allowing bacterial attack to commence immediately. However, the stage where only limbs were deposited requires more thought. Presumably if a carcass is being processed in this way then the main objective is to leave inedible/bulky/unproductive parts at the site to ease the burden of carrying an already large and ungainly animal back to camp. This being the case it would not be presumptuous to suggest that the hunter/gatherers would also have gutted the

carcass. The intestines would not be identifiable later as their decomposition would be complete. It would make sense that these parts would also be deposited at the very least in the same locality as the limbs and very possibly deposited at the exact same location i.e. within the pile of limbs. The two strategies of procuring meat result in different assemblages with much the same outcome. Although in the second phase the intestines would not be within each and every bone and this may explain why these bones from this usage period are slightly better preserved. In addition to this any carcass being taken back to camp would be sterile and it then becomes feasible that these bones if later discovered will not suffer from bacterial MFD. Both assemblages (mni=36) demonstrate higher frequencies at stages 3 & 4 of the histological index (where 1= worst preservation and 5= best preservation). In this case at least where microbial destruction has begun it has not generally continued to completion.

Archaeological goose bones (n=320) from the Middle-Late Saxon site of Flixborough in Lincolnshire show MFD that demonstrates an inside out mode of attack (Haynes *et al*, 2002). The majority of the bones scored 3 using the HPI (figs 6.9 & 6.10), but overall the distribution of categories is quite evenly spread.



A



B

Fig 6.9 & 6.10 Archaeological Goose Bone from Flixborough. A= HPI 1 B=HPI 3. No scale given. (Haynes *et al* 2002).

Again this is in direct conflict with the findings of Hedges *et al* (1995) who recorded a bi-modal distribution where the bones were either very well preserved or almost completely altered. The authors (Haynes *et al*, 2002) suggest that rather than there being a difference between mammalian bone and faunal bone, the difference lays in the burial environment. Bi-modal versus even distribution may be both a question of numbers. The larger the sample size, the more likely it will be that the distribution will be even or more possibly it relates to how the animals were buried. Intact corpses are more likely to display MFD than those that are disarticulated/processed before interment. When Reiche *et al* (2003) studied animal bones from the Neolithic

site of Bercy, France, they found that bone buried in the waterlogged zone had a high organic content and good preservational state, but during dry periods tunnelling by microorganisms was evident. This they attribute to an oxygen-rich environment.

6.4 Summary

Reported cases of MFD suggest that the time scale involved is most likely within the early postmortem period. Archaeological cases although useful are not definable in terms of when the attack took place. They are however beneficial in terms of which remains exhibit tunnelling and those that do not. It has been shown that disarticulated remains may be very well preserved when articulated remains from the same cemetery are subject to MFD possibly pointing to an endogenous reason for microfocal destruction. The Cladh Hallan mummies appear to have a limited form of MFD perhaps due to their early postmortem treatment and some of the remains from Pompeii that are not subjected to soil bacteria are also affected. Fetal remains are also less likely to be affected. All of these instances point towards a point of origin for MFD firmly within the body itself. Experimental and forensic cases are however providing some answers, even if these are not immediately answering very clearly defined questions. Three months is the earliest recorded timescale for MFD to occur but this cannot be equated to what is seen in the archaeological record. It is however clear that true cases of tunnelling may well be evident in terrestrially located bone within less than two years and possibly even earlier.

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Chapter 7 Materials and Methods

7.1 Site Location and General Conditions

By kind permission of the Department of Forensic and Biomedical Sciences at the University of Lincoln, we were allowed to use land belonging to them for the experimental part of the research. Their assistance is gratefully acknowledged. The site is located at Riseholme, Lincolnshire. This is a 240 hectare estate that encompasses agricultural, animal related and biological science. The Riseholme estate is mainly arable, with mixed woodland making up 20 hectares of the total. Riseholme Hall, which used to be the Palace of the Bishop of Lincoln, is still situated at the site. The actual location of the experimental material is a small wooded area within this (fig 7) that has vehicular access. The site is to the rear of the Rural Science Centre and adjacent to paddocks, a public footpath/bridleway and a golf course. It is a quiet location that is not used by either students or members of the public making it an ideal location for privacy and to reduce the risk of accidental discovery. The woodland is comprised of Scots Pine, Norway Spruce and Beech, with very little under storey and the burials are central within the wooded area. The site is located at 53.27°N latitude and 0.52°W longitude with an elevation of 50 meters above sea level. Average climatic data for the site as recorded at RAF Waddington (Jan-Dec 2005) is depicted below (figs 7.1 & 7.2). Waddington is 10km south of Riseholme and at the same altitude.

Fig 7. The woodland site at Riseholme. The graves are within a small clearing in the wood. (Author 2007)

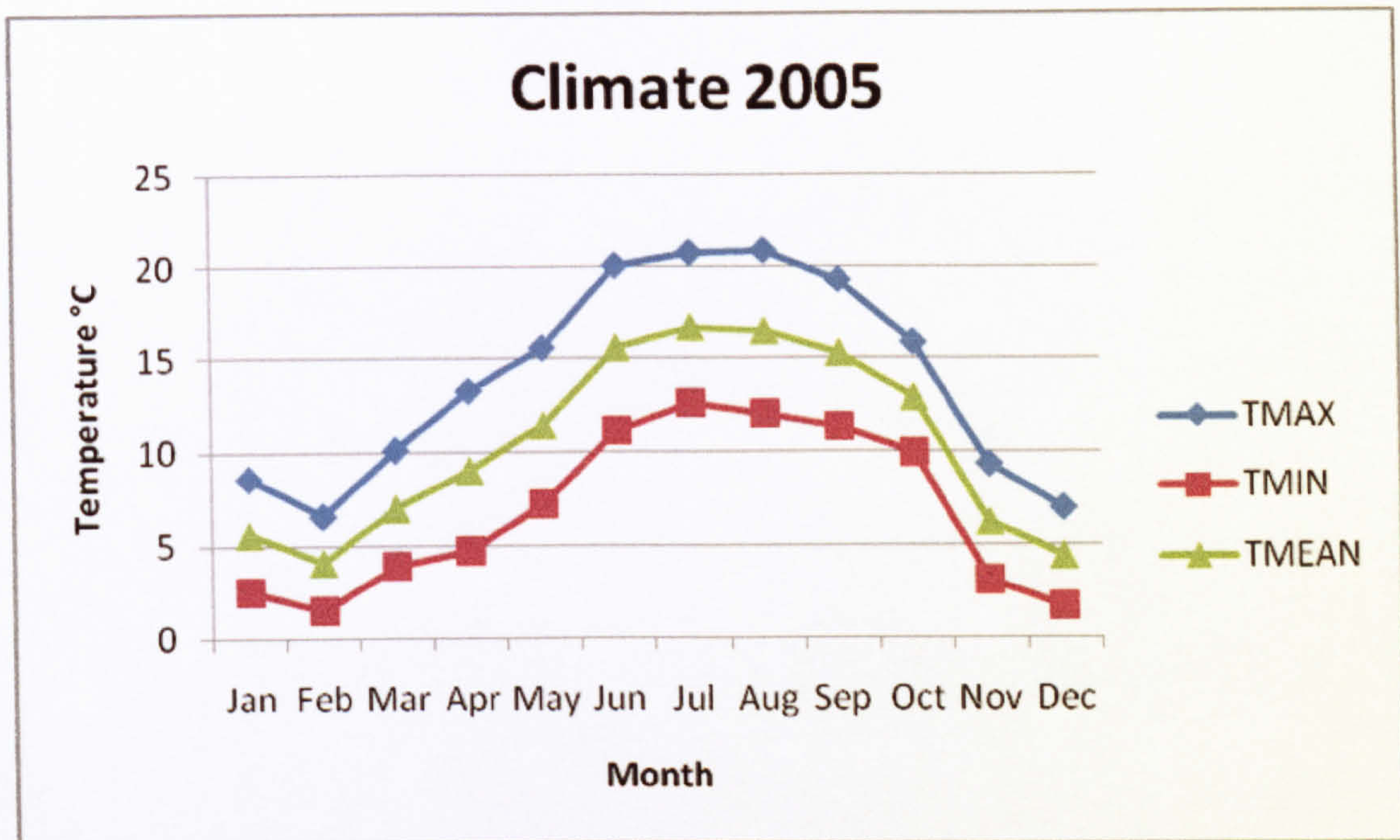


Fig 7.1 Average temperatures relating to the Riseholme site as collected from RAF Waddington 10km from site. KEY: TMAX = Maximum Air Temperature, TMIN = Minimum Air Temperature, TMEAN = Mean of TMAX and TMIN

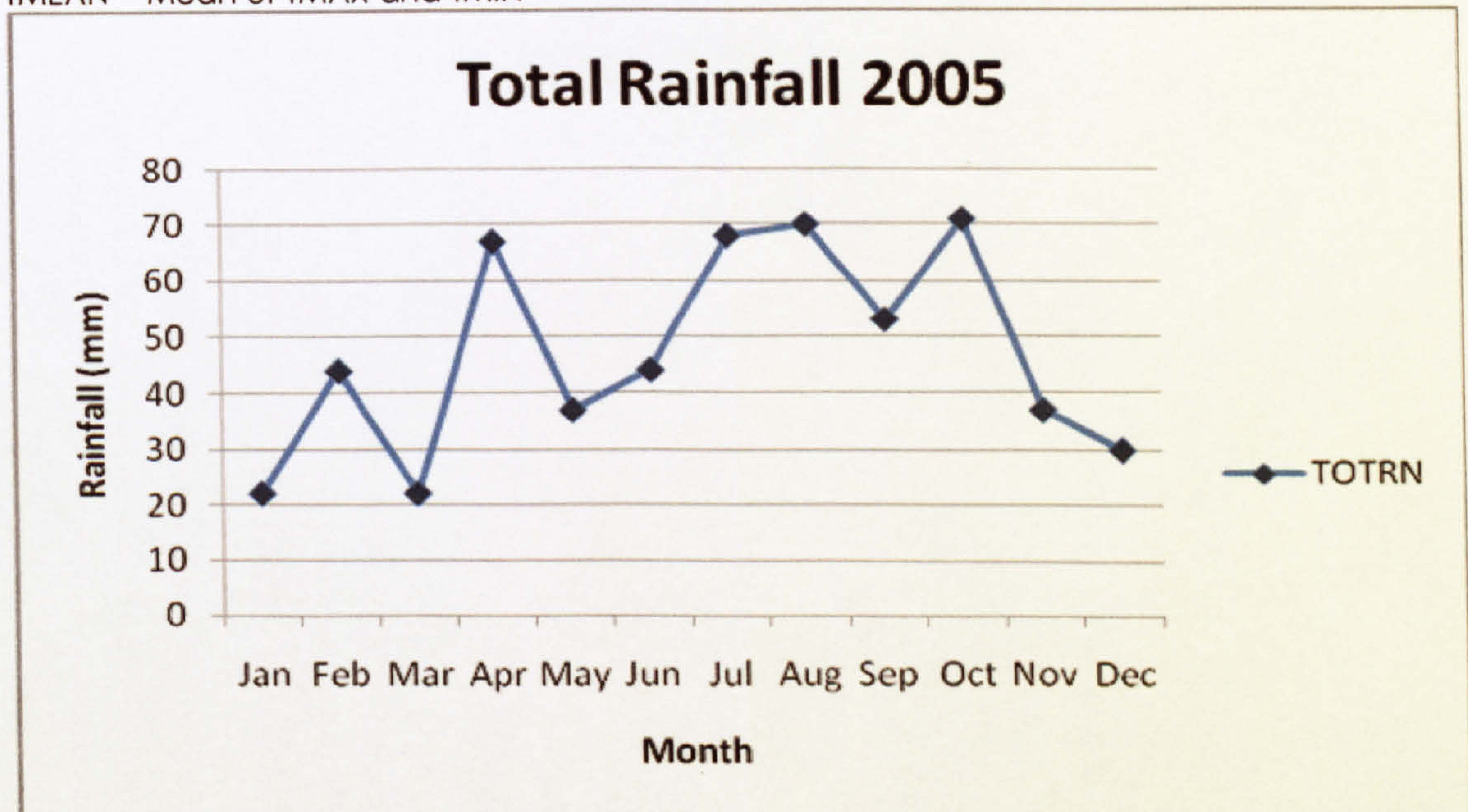


Fig 7.2_ Total monthly rainfall throughout the period Jan-Dec 2005. KEY: TOTRN = Total Rainfall

7.2 *Sus* Experiments

For the first part of the experiment 12 newborn domestic pigs (*Sus scrofa)* were 'buried/placed' inside water tanks. The pigs were supplied by the local knackerman, who delivered them to the site and all had died within 24 hours of collection and were therefore considered fresh on arrival. Seven of the pigs had been refrigerated for a period of 4 days. They had all died of natural causes and were either stillborn or rolled on by their mother. Because in the UK it is difficult to gain ethical permission to use human cadavers (adult or juvenile) for this type of experimental research it was

determined that juvenile pigs would be used as a proxy or substitute for experimentation purposes. These were considered to be an appropriate model for newborn children, due to their similar size, lack of body hair and broadly comparable postcranial skeletal anatomy. The pigs used in this study ranged from 1.4-3.1kg (3.08-6.8lbs). The lowest weight is comparable to stillborn children whilst the higher weight roughly equates to newborn children. Many other animals have a thick coat of fur or hair, but pigs tend to have a much lighter covering that is similar to the amount of human body hair. Pig carcasses are often used in anatomical studies of both the soft and hard tissues of the human cadaver and although there are physiological and biochemical differences, there are also considerable similarities. However, the ratio of body fat to total body mass in a newborn human is relatively greater than in the neonatal pig; 16.1gm per 100gm, versus 1.1gm respectively (Widdowson, 1950). It should also be noted that piglets can weigh anywhere between .5kg-1.5kg whereas newborn humans usually weigh around 3.5kg. Many other previous studies in human taphonomy and decomposition have relied on this source also (Wilson *et al* 2006, Morton & Lord 2006, Weitzel 2005, Micozzi, 1986, Turner & Wiltshire 1999, Schultz *et al* 2006, Haefner *et al* 2004, Hobischak *et al* 2002, Joy *et al* 2006,) due to ease of appropriation, ample availability, ethical considerations and the fact that animal by-products are permissible for diagnostic, educational and research purposes.

Due to strict regulations and guidelines (Animal By-Products Regulations, 2005) enforced by the Department for Environment, Food and Rural Affairs, it is illegal to bury or contaminate land and soil with 'fallen livestock' and this regulation extends to cover stillborn animals.

- *"Fallen stock can no longer be buried or burnt in the open because of the risk of disease spread through groundwater or air pollution. Instead, animals must be taken to/collected by an approved knacker, hunt kennel, incinerator or renderer, either by private arrangement, or under the National Fallen Stock Scheme." (ibid).*

This is largely to prevent microbes from notifiable diseases such as BSE and scrapies entering the soil and/or public water supply. A need to prevent scavenging by other necrophagus wildlife is also obligatory. Personal health whilst handling the pig carcasses was addressed by the use of latex gloves and the application of antibacterial hand wash after each visit to site. University held personal safety guidelines were followed and the site was only ever visited when two or more people could be present at the same time. This was achieved by inviting University of Lincoln Forensic Science students to help out with data collection. It was necessary to propose an experimental protocol that met these guidelines whilst retaining the general purpose of the

experiments. As no putrefactive liquids were allowed to penetrate the soil, the pigs had to be housed within boxes that were leak-proof. Small domestic water tanks were found to be an ideal size for young pigs. These were then filled to a depth of 3-4 inches with small pebbles that were subsequently covered by a layer of fine grade mesh (to prevent loss of small bones and epiphyses). The pigs could then be laid on top of the mesh for putrefaction to take place. Any liquids draining downwards are then contained within the pebbles which prevent the body from becoming waterlogged. The water tanks were then securely fitted with clear corrugated PVC lids attached to small sections of wood fitted internally to the top of two sides of the tank (fig 7.3). The lid was held in place by cross head screws and served the purpose of both preventing scavenging by carnivorous animals or carrion feeding birds whilst also keeping out any heavy rainfall which may have flooded the boxes. They also discouraged human interference. The lids were spray painted white to reflect sunlight, reduce the temperature of the box internally and to avoid public scrutiny if accidentally stumbled across. Access holes for flying/crawling insects were drilled along two sides of the boxes. The standardized design of the boxes reflected the need to provide identical environments for all of the individual pigs.

Fig 7.3. The containers for the pigs with lids in place (Author, 2006).

7.3 Site Temperature

A HOBO temperature data logger was placed inside the tank containing pig no. 2 to log the internal temperature of the boxes on a two hourly basis throughout the day. It was set to launch at noon on the 26th April 2006 and Onset software was used to retrieve temperature data via use of Boxcar 3.7. External temperature was provided by the Meteorological Office at monthly (provides daily data including a minimum and maximum daily temperature) and yearly (provides monthly data including maximum and minimum temperatures for the month) intervals from RAF Waddington. Data comprised

of maximum/minimum temperature, amount of rainfall and hours of sunlight for the four most relevant months can be seen below (table 7 & fig 7.4). Relevant decompositional data were collected at set intervals (this ranged from every day to every other day or weekly depending on stage of decomposition). July 2006 appears to have been unusually hot and sunny with a max average temperature of 25.8°C compared to 20.8° C for the previous year.

MONTH	AVERAGE MAXIMUM DAILY TEMPERATURE (° C)	AVERAGE MINIMUM DAILY TEMPERATURE (° C)	RAINFALL (mm)	SUN (Hours)
APRIL 2006	12	4.7	31.6	164.1
MAY 2006	16.4	8.3	86.2	187.3
JUNE 2006	21	11.3	11.6	207.4
JULY 2006	25.8	14.2	50.4	294.5

Table 7. Climate data April-July 2006, from RAF Waddington.

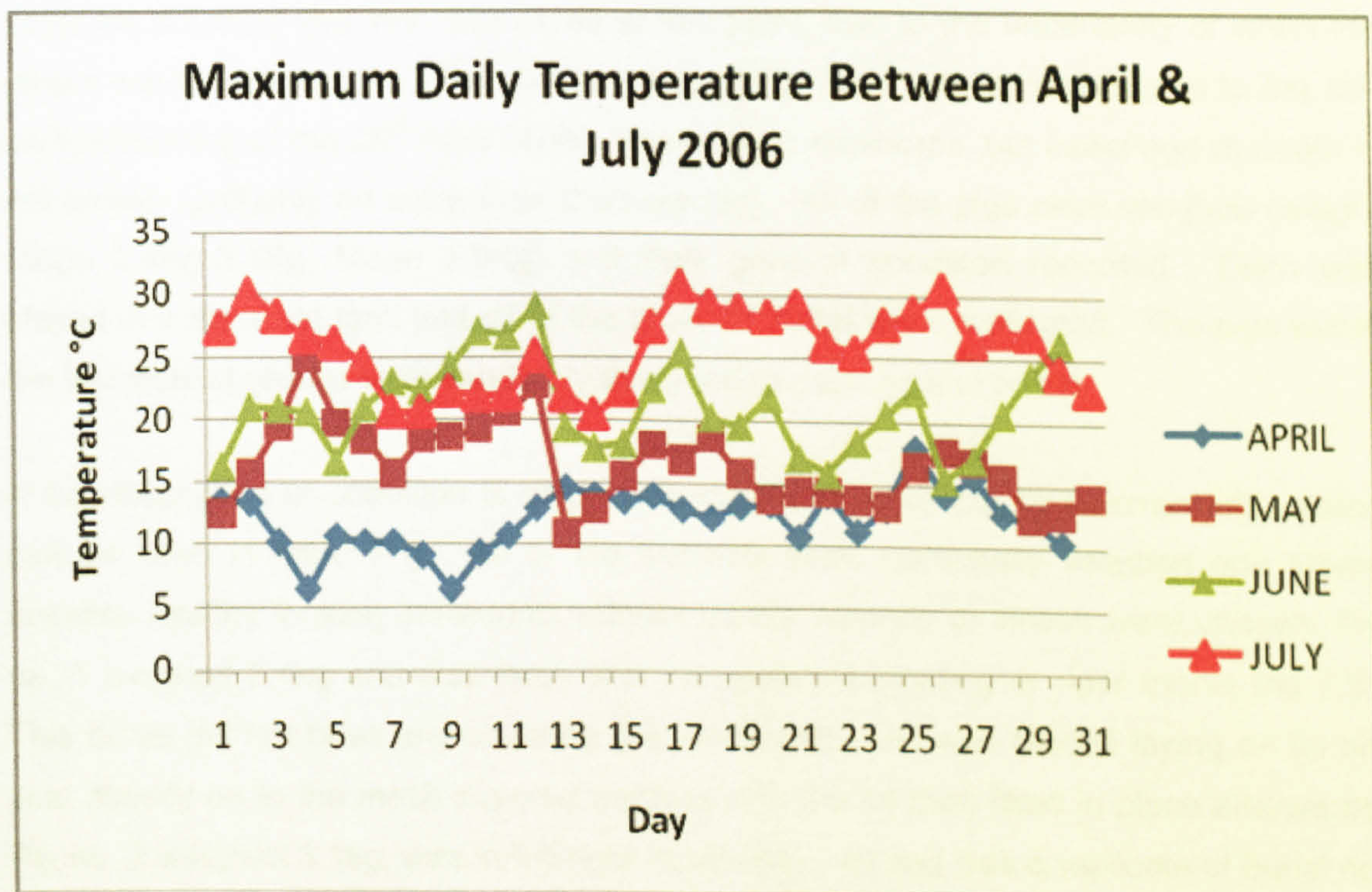


Fig 7.4 Comparison of maximum daily temperatures at Riseholme.

(Data from Waddington compares favourably to Riseholme, although the site temperature is usually around 2°C lower due to tree coverage).

7.4 Boxed *Sus* Experiments

The boxed pigs should provide a clear definition between bacterial sources with bacterial origin being tracked effectively due to the controlled environment of each pig. If a new born pig is buried without any covering and is subsequently found to be subject to rapid internal microbial attack then these bacteria are likely to be of internal origin.

Conversely, if a still born pig covered with soil demonstrates bacterial attack then this is more likely to indicate a soil origin for the bacteria. Stillborn pigs were included in the process due to their theoretical intestinal and respiratory tract sterility at birth. Soil (to detect soil microbes) was added to a third of the burials, builders sand to a further third and the remaining pigs were left uncovered. The soil was taken, with permission, from a local graveyard (from mole hills) as there is some suggestion that such areas would be highly laden with bacteria (either dormant or active) due to the steady influx of decaying material on to which bacteria can readily colonise.

Six stillborn and six newborn pigs were used and were all single burials (i.e. one pig per box). From the six newborn, two were placed in the boxes with no covering (to reflect above ground burials), two had a shallow covering of soil (to reflect true burial) and two had a covering of sand as a control. The same protocol was used for the stillborn animals. The experiments were set up in two phases due to difficulty in obtaining twelve pigs of the right size and age all on the same day. Freezing of the pigs until all of them could be obtained was not considered at this point, due to the uncertainty of when the others would be available. The first four pigs (pigs nos. 1-4) were delivered to the site on the morning of the 26th April 2006. These were newborns, but exact age at death is not known (probably no more than 2 weeks old). All of the pigs were weighed (weight range 2.4Kg-3.1Kg, Mean 2.9Kg) and their general condition recorded. Each was placed in a separate tank and all of the types of burial were replicated. The pigs within the two distinct groups were randomly assigned to each type of burial.

A full description of condition is given individually for each pig (a summarized version can be seen in table 7.1). All of the subjects were personally selected and where possible healthy looking individuals without visible wounds or illness were chosen. Pig no. 1 weighed 2.4kg and was fresh with no apparent bloating or rigor mortis (fig 7.5). This burial did not have any covering (i.e. soil/sand) and was placed laying on its left side directly on to the mesh covered pebbles with the lid then fixed in place afterwards. Pig no. 2 weighed 3.1kg, was in full rigor mortis (fig 7.6) and was a replicate of burial no. 1. Pig no. 3 weighed 3.1kg and was actively decomposing internally (fig7.7) as evidenced by the abdominal area which was markedly discoloured green. This pig was again placed on its left side and then given a shallow covering of soil. Pig no. 4 weighed 3.1kg, was considered fresh (fig 7.8) as no bloating, rigor mortis or decomposition was evident. This burial was covered with sand.

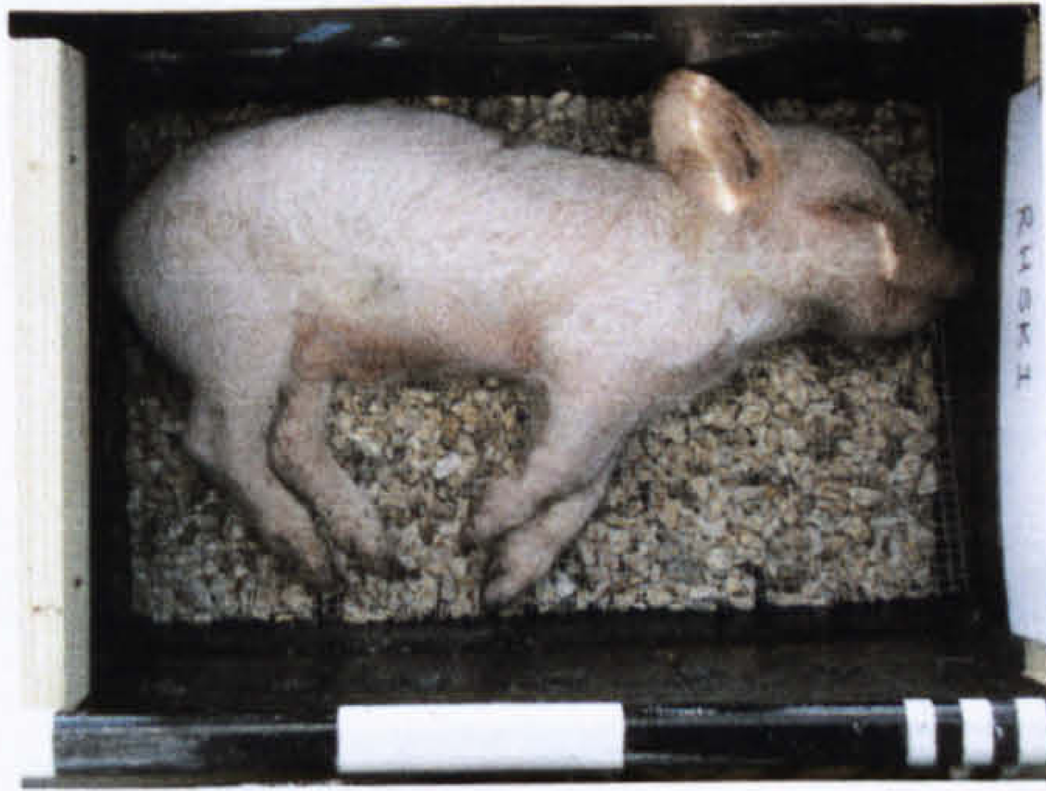


Fig. 7.5 Pig no 1 Fig 7.6 Pig no 2.
Both Newborn, No 2 being in full rigor mortis. Neither was given any covering. (Author, 2006)

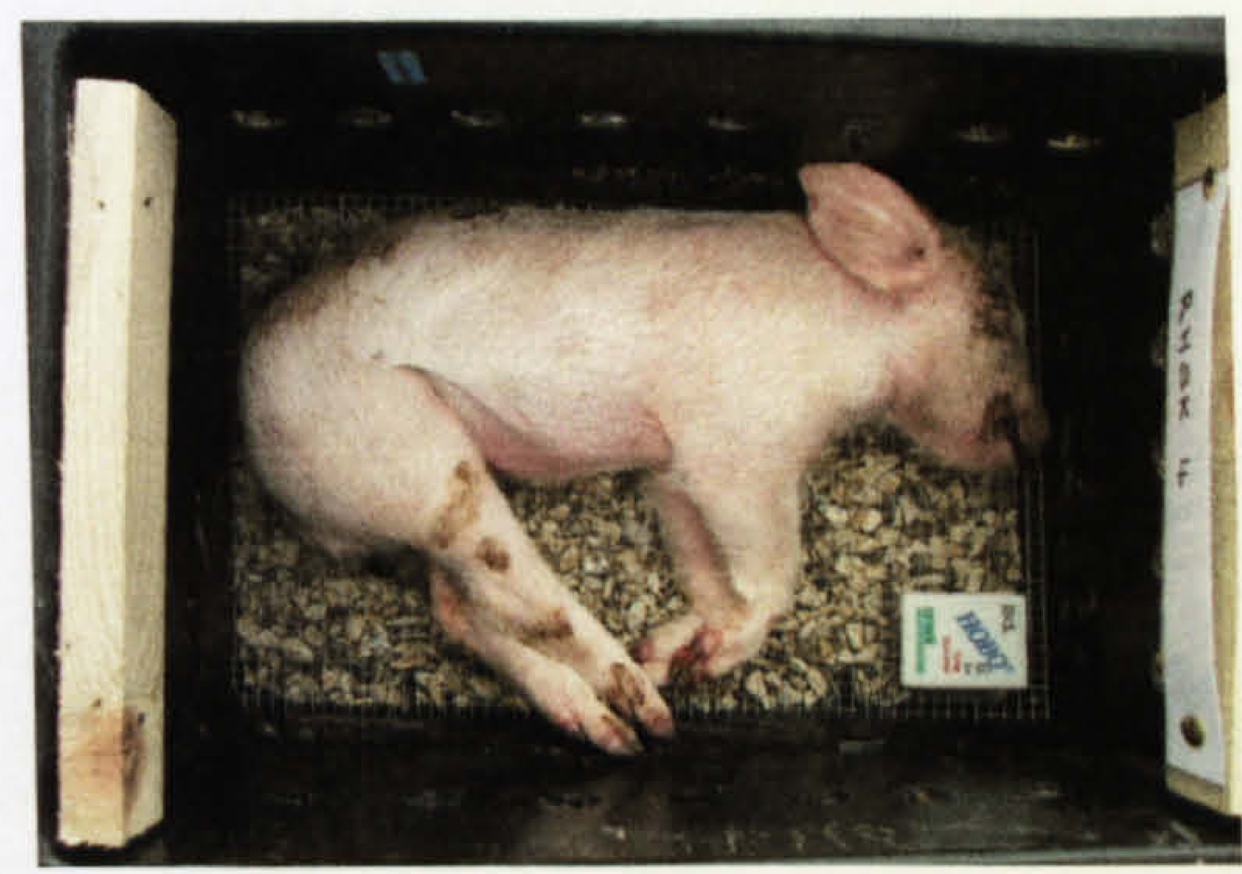


Fig 7.7 Pig no 3 Fig. 7.8 Pig no 4
Both newborn. No 3 has discolouration of the abdominal area. No 3 was covered with soil, No 4 with sand. (Author, 2006)

The eight remaining pigs (pigs' nos. 5-12) arrived at the Riseholme site on the 30th May 2006 (roughly five weeks after the 1st experiments were set up). These were again recorded as to bodily condition and weighed (table 7.1). Pig no. 5 (fig 7.9) weighed 2.9kg and was a replicate of burial no. 3, whilst pig no. 6 (fig 7.10) weighed 2.8kg and was a replicate of burial no. 4. Both of these pigs were fresh with no obvious signs of decomposition. It was decided at this point to reduce the amount of soil/sand covering the remaining pigs in an attempt to reduce the possibility of mummification due to the very dry nature of the tanks and to allow insects access to the carcass.

The remaining six pigs placed at that time were theoretically stillborn and most still had the umbilical cord attached. However, the basis of being stillborn was mainly taken from their weight (this ranged from 1.4Kg-2.1Kg with a mean of 1.9Kg). Normal birth weight in pigs is 1.6kg ± 0.4kg (1.2kg-2kg). Four of the pigs were at the top end of this range (1.9-2kg) and two were slightly higher at 2.1kg and it is therefore possible that some of these juvenile pigs were not stillborn.

Burials nos. 7 & 8 (figs 7.11 & 7.12) were both considered fresh and were firstly a stillborn weighing 1.9kg that had no sand or soil covering and secondly another stillborn

weighing 2.1kg that was a duplicate burial of Pig no. 7. Burials nos. 9 & 10 (figs 7.13 & 7.14) were two stillborns weighing 2kg and 1.4kg respectively. Both of these animals had fly eggs already present around the facial and abdominal areas, suggesting that they had lain outside for a period of time prior to collection and refrigeration. Both burials were covered with soil. Burials 11 & 12 (figs 7.15 & 7.16) weighed 2kg and 2.1kg in that order, were considered fresh and both were covered with sand.

PIG	WEIGHT	CONDITION	COVERING	REFRIGERATED	DATE	REPLICATES
No 1	2.4kg	Fresh	No Covering	No	26.04.06	No 2
No 2	3.1kg	Full Rigor Mortis	No Covering	No	26.04.06	No 1
No 3	3.1kg	Abdominal area discoloured green	Soil	No	26.04.06	No 5
No 4	3.1kg	Fresh	Sand	No	26.04.06	No 6
No 5	2.9kg	Fresh	Soil	Yes	30.05 06	No 3
No 6	2.8kg	Fresh	Sand	Yes	30.05 06	No 4
No 7	1.9kg	Fresh	No Covering	Yes	30.05 06	No 8
No 8	2.1kg	Fresh	No Covering	Yes	30.05 06	No 7
No 9	2kg	Blowfly eggs present	Soil	Yes	30.05 06	No 10
No 10	1.4kg	Blowfly eggs present	Soil	Yes	30.05 06	No 9
No 11	2kg	Fresh	Sand	Yes	30.05 06	No 12
No 12	2.1kg	Fresh	Sand	Yes	30.05 06	No 11

Table 7.1 Condition of Pigs at Time of Burial

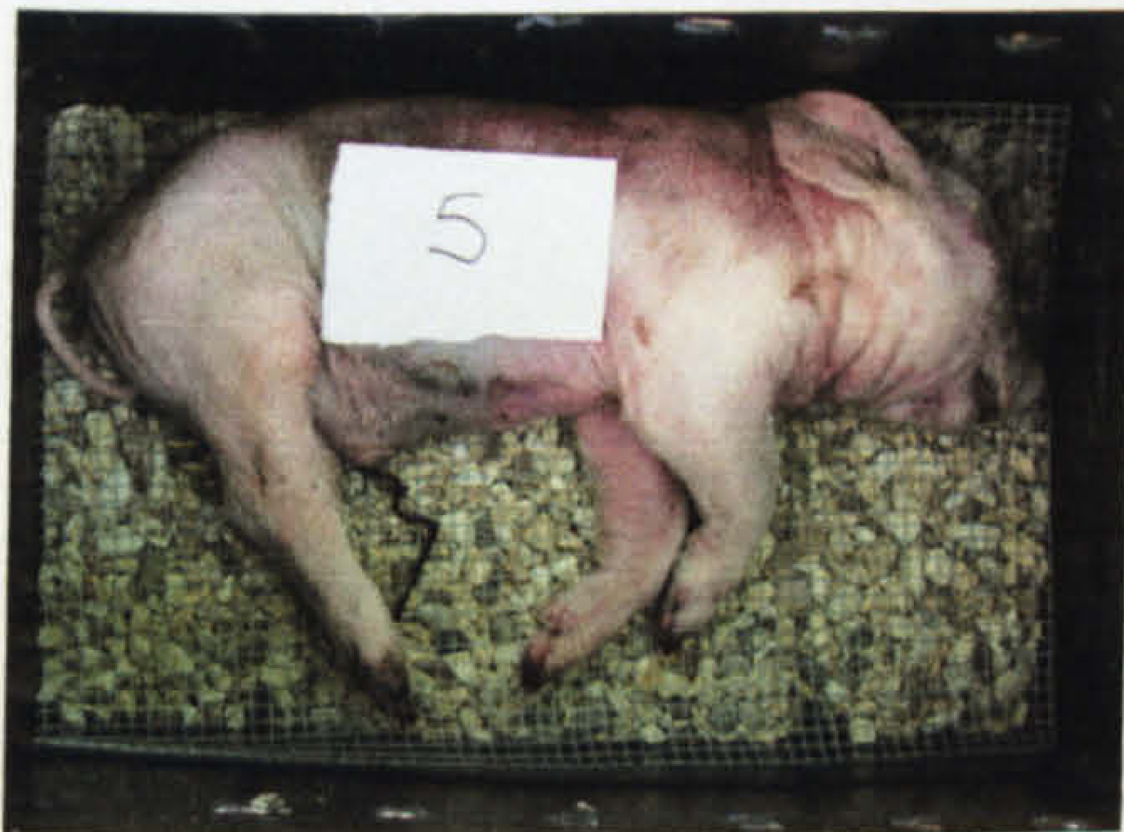


Fig 7.9 Pig no 5

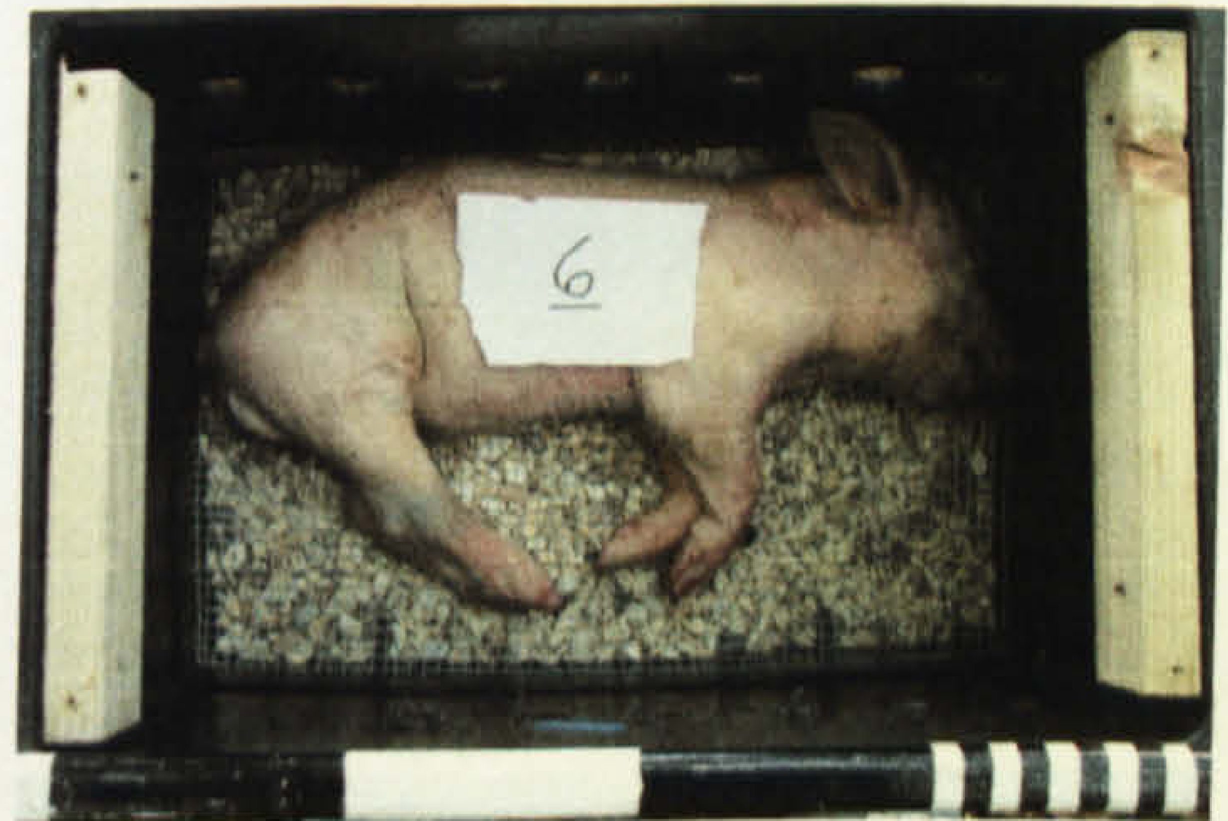


Fig 7.10 Pig no 6,

Both stillborn, No 5 covered with soil, No 6 covered with sand. (Author, 2006)

Fig 7.11 Pig no 7

Neither had any covering. (Author, 2006)

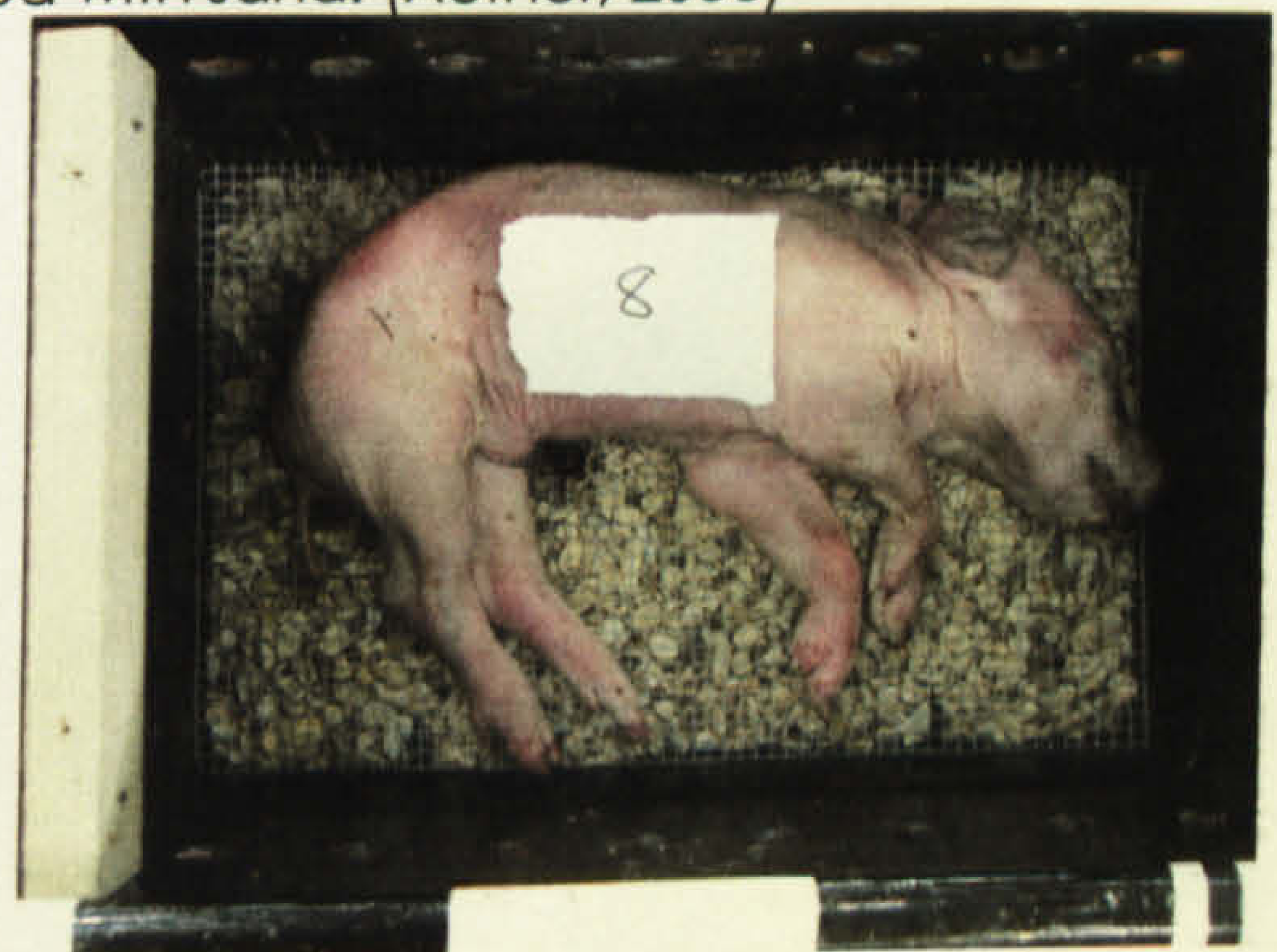
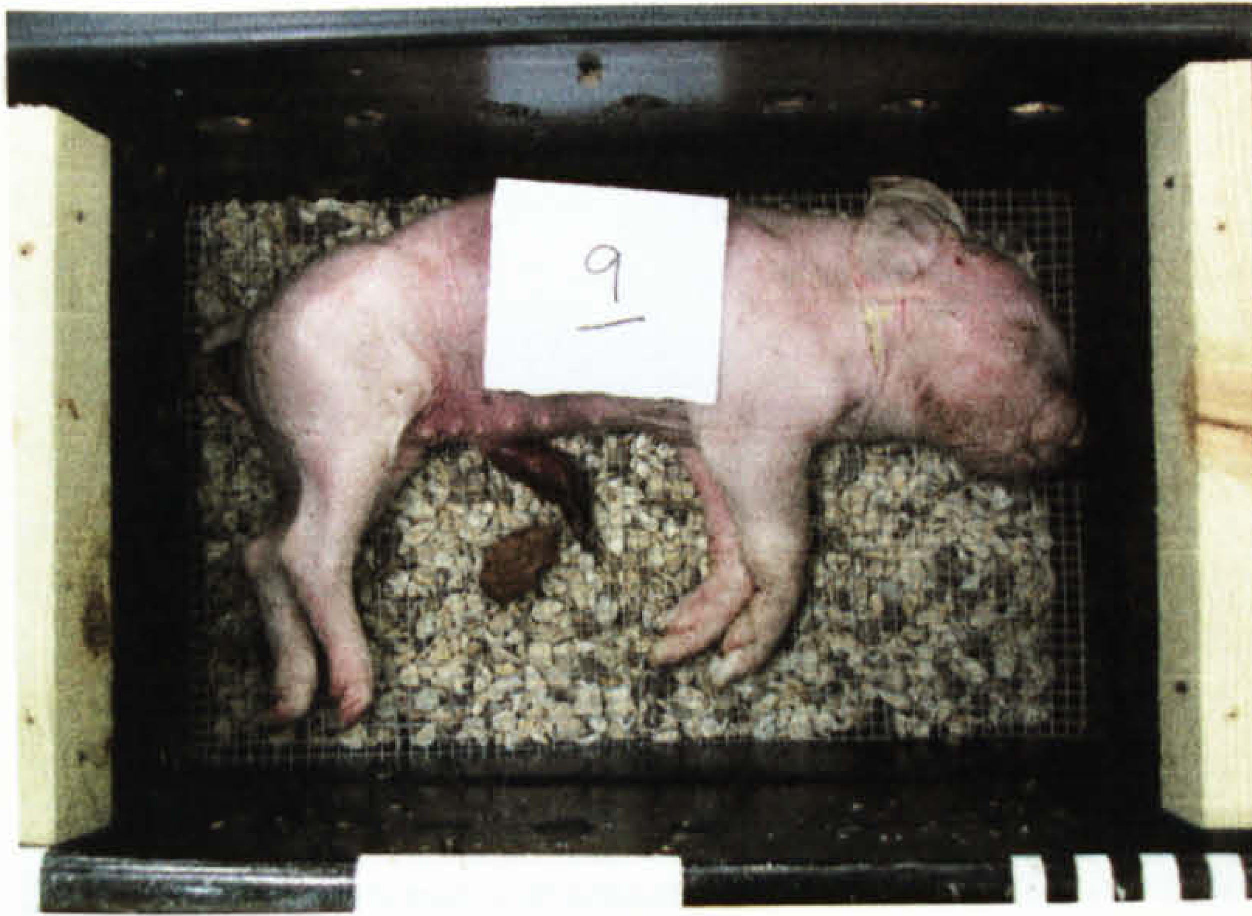


Fig 7.12 Pig no 8.



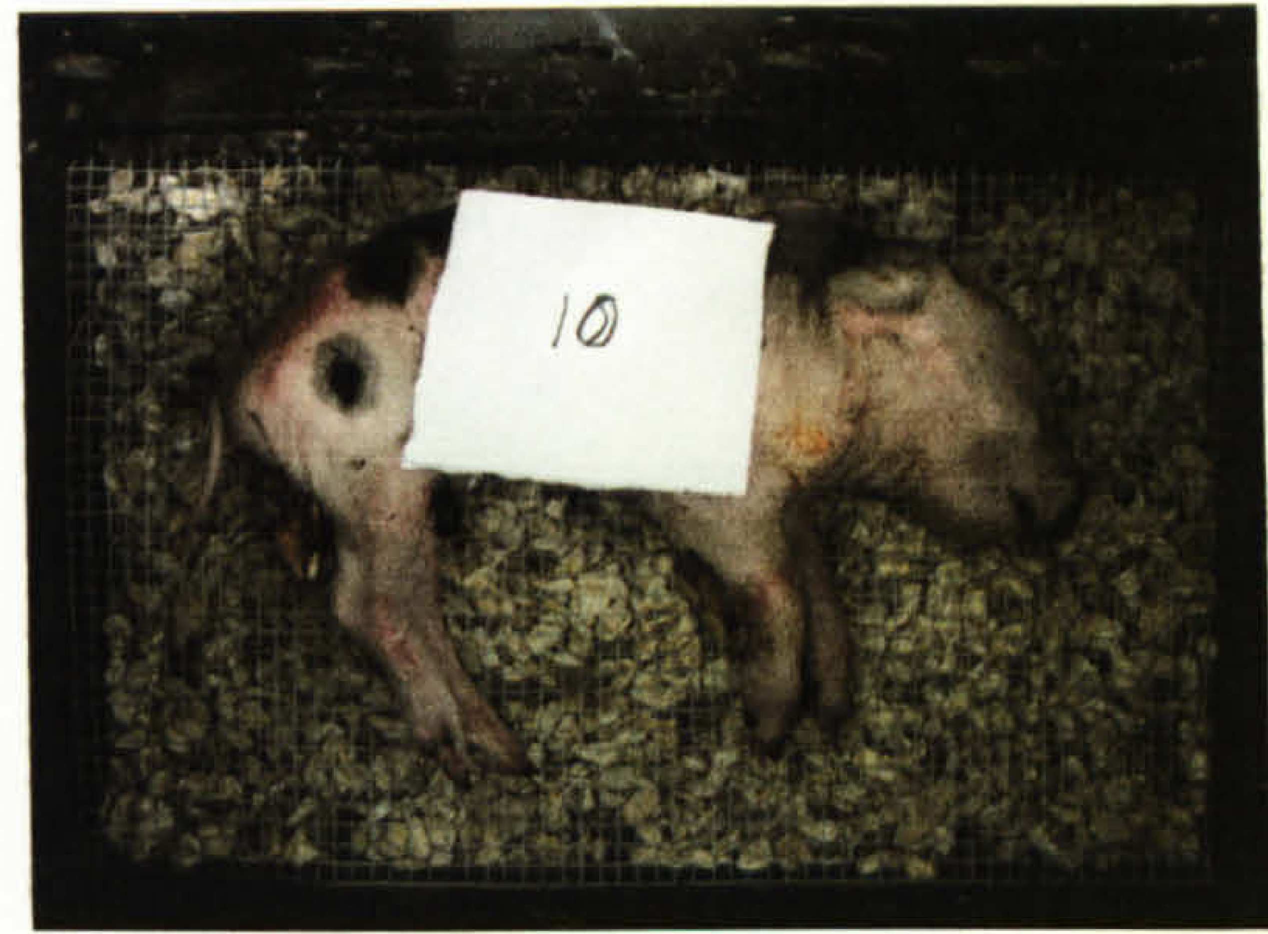


Fig 7.13 Pig no 9

Fig 7.14 Pig no 10

Both had soil added to the box (Author, 2006)

Figs 7.15 Pig no 11

Fig 7.16 Pig no 12.

Both had sand added to the box. (Author, 2006)

The 1st four boxes were originally placed at a different site at Riseholme, but due to vandalism by members of the public (the boxes were overturned) the boxes had to be moved to the location described above which is about 2 miles north of the original site. This was also a wooded area and because of the close proximity of the site this should have little bearing on any climatic or ambient temperature differences. The tanks were placed directly on to the under storey of the woodland about half a meter apart. The remaining eight burials were all located directly to the second site. At this time the newborns were separated from the stillborns by approximately 25 meters in an attempt to detect any potential differences in insect activity.

7.5. Soft Tissue Decomposition

The primary aim of the experiments was to wait until the carcasses had skeletonized and then to remove bone samples at set intervals (1, 3, 6, 9, 12, 18 & 24 months) to look for evidence of bacterial tunnelling by histological analysis. However, to achieve this state the bodies had to be left to putrefy and the decompositional process was tracked throughout. Soft tissue decomposition is often used to determine post-mortem interval when other methods can no longer be applied (entomology, rigor/livor/algor mortis etc)

due to the longer timescale involved. Although there are no hard and fast rules as to how long it takes a body to skeletonize, a reconstruction of climatic data may help in narrowing the possible time that has elapsed before the body is recovered. This research is primarily concerned with the very young child, an area that is almost completely lacking in tangible evidence with reference to the decay process. It is hoped that what is recorded here may aid death scene investigators determine the likely time since death when investigating child deaths rather than adults whose much larger size impacts on how quickly a body can decompose.

A brief but informative description of every pig's decomposition was recorded together with a category for that stage (table 7.2). Photographs were taken at every opening to ensure that a full record was kept. The photographs depict the full state of the body at each point from known time of death that creates an unambiguous record; unlike a simple description that is open to individual interpretation.

Burials nos. 1 & 2 were opened on a daily basis for the first two weeks as they could be recorded with ease and without having to disturb the burial context in any way. It was decided that burials 2 & 3 were not to be recorded at all during this time initially due to their burial context (this would have required the soil/sand to be removed) and subsequently the fact that the burial environment appeared to be inhibiting decomposition (this in itself is important data).

A	*FRESH*
1.	Fresh, no discolouration or insect activity
2.	Fresh burned (cremated)
3.	Fresh, no discolouration, fly eggs present
B.	*EARLY DECOMPOSITION*
1.	Pink-white appearance with skin slippage and some hair loss
2.	Bloating without discolouration
3.	Grey to green discolouration-without bloating some flesh relatively fresh,
4.	Bloating with green discolouration
5.	Appearance of 1st Instar Larvae
6.	Green/grey/black discolouration to most of body
7.	Post bloating following rupture of the abdominal gases with discolouration going from green to dark
8.	Brown to black discolouration of arms and legs, skin having leathery appearance
C.	*ADVANCED DECOMPOSITION*
1.	Decomposition of tissues producing sagging of the flesh, caving in of the abdominal cavity, often accompanied by extensive maggot activity
2.	Moist decomposition in which there is bone exposure
3.	Mummification with some retention of internal structures
4.	Mummification of outer tissues only with internal organs lost/some still intact, through autolysis or insect activity
5.	Mummification with bone exposure of less than one half the skeleton
6.	Adipocere development
D.	*SKELETONIZATION*
1.	Bones with greasy substances and decomposed tissue, sometimes with body fluids still present
2.	Bones with desiccated tissue or mummified tissue covering less than one half the skeleton
3.	Bones largely dry but still retaining some grease
4.	Dry bone
E.	*EXTREME DECOMPOSITION*
1.	Skeletonization with bleaching
2.	Skeletonization with exfoliation
3.	Skeletonization with metaphyseal loss with long bones and cancellous exposure of the vertebrae

Table 7.2 Decompositional data. Modified from Galloway *et al*, 1989.

After this period all four were recorded roughly every other day (although daily at times). The other eight burials were in-situ by the 30-05-06 and at this time all of the twelve burials were recorded on the same days which was either on a daily basis or every other day depending on the speed of post-mortem decay taking place (this became weekly at the point of maximum skeletonization or mummification).

7.6 Further Experimentation

The methods outlined above were devised to obtain data as efficiently as possible with regards to the timescale and origin of bacterial attack in modern and archaeological bone within the regulations set down by DEFRA. By combing multi-factorial methods a clear conclusion is to be expected. However, the nature of the experiments induces the problem of false results due to the unique environment in which the burials were placed (a storage tank that was not subject to normal climatic and entomological conditions, inducing much higher temperatures whilst excluding certain ground dwelling beetles). After lengthy discussions with DEFRA it has now been decided that the pigs could have been placed straight in to the ground from the outset under the derogation within the regulations that clearly states that fallen livestock can be used for 'Diagnostic, Educational and Research Purposes'. Communication with DEFRA (Lincoln) initially concluded that the pigs could not be buried in the soil. Due to this erroneous information the above experiments were carried out. A new methodology was then proposed that involved the shallow burial and ground placement of twelve juvenile pigs to be placed at Riseholme during the spring of 2007. The methods detailed above were employed for the new burials and a detailed methodology is set out below.

7.7 Winter Burials

Because permission was granted from DEFRA in the late autumn it was decided that we would not begin the experiments in earnest until the following spring when decomposition would proceed much quicker at higher temperatures and with insect activity. However, two ground burials were set up in the winter in an attempt to investigate the differences in decompositional time scale and amount of degradation as a result of seasonality. All of the other burials were conducted in early to late spring to achieve skeletonization of the carcasses within a short period. The effect of cooler burials during the coldest winter months on the activity of bacteria is to be assessed.

The pigs were sourced from the same supplier and both were considered fresh on arrival at site. The 1st pig (fig 7.17) weighed 11.7kg and was given a direct soil burial at a maximum grave depth of 40cm. One maggot was seen on the carcass but this was not a true associate due to its large size and was classified as an incidental intruder

from other carcasses that it had been stored with. Otherwise the pig was generally in a fresh condition with a slight tinge of discolouration to the abdominal area. The 2nd pig weighed considerably less at 4.4kgs and was buried at a depth of 25cms (fig 7.18).

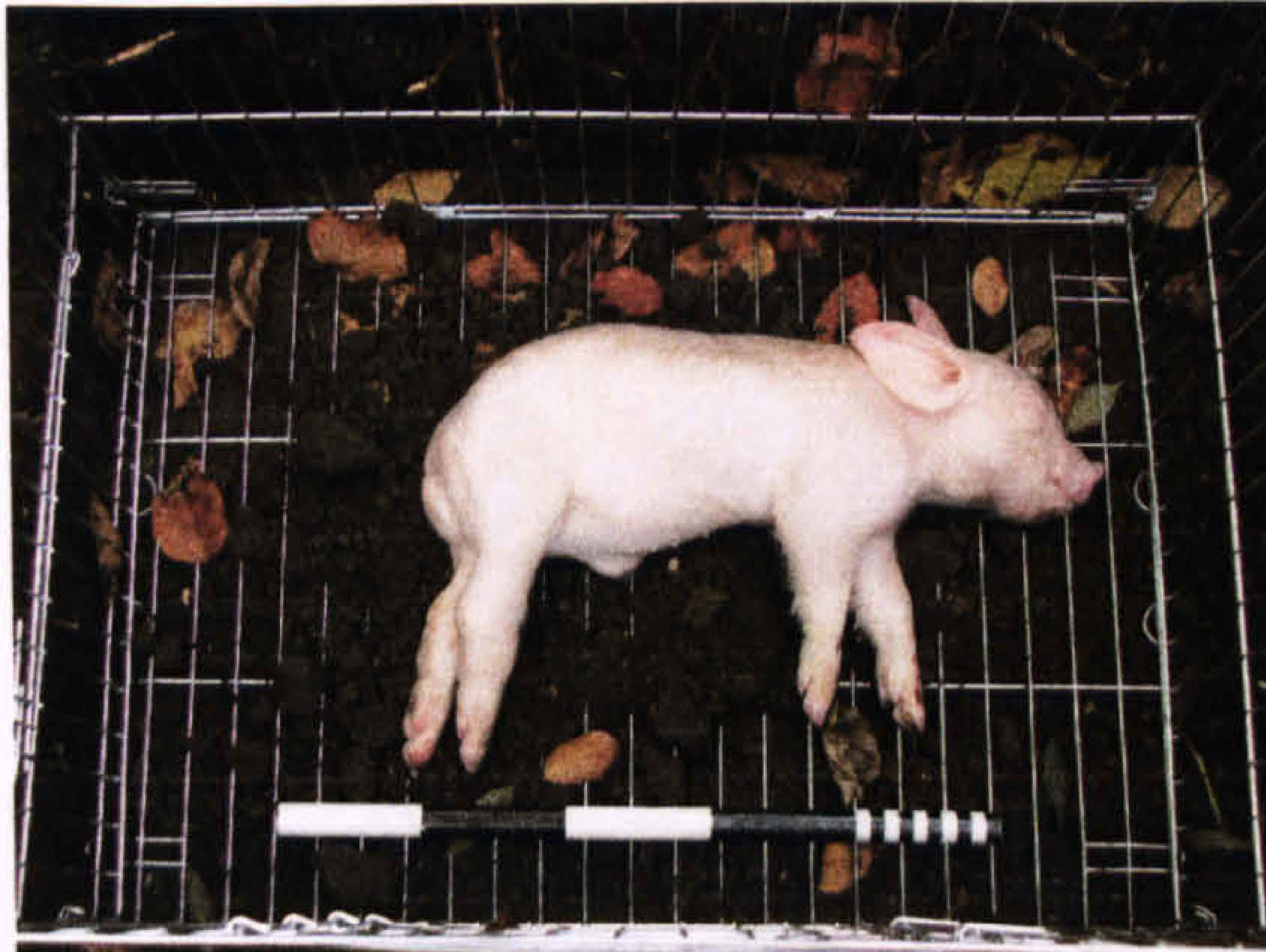


Fig. 7.17 Pig no 13
At Deposition (Author, 2006)

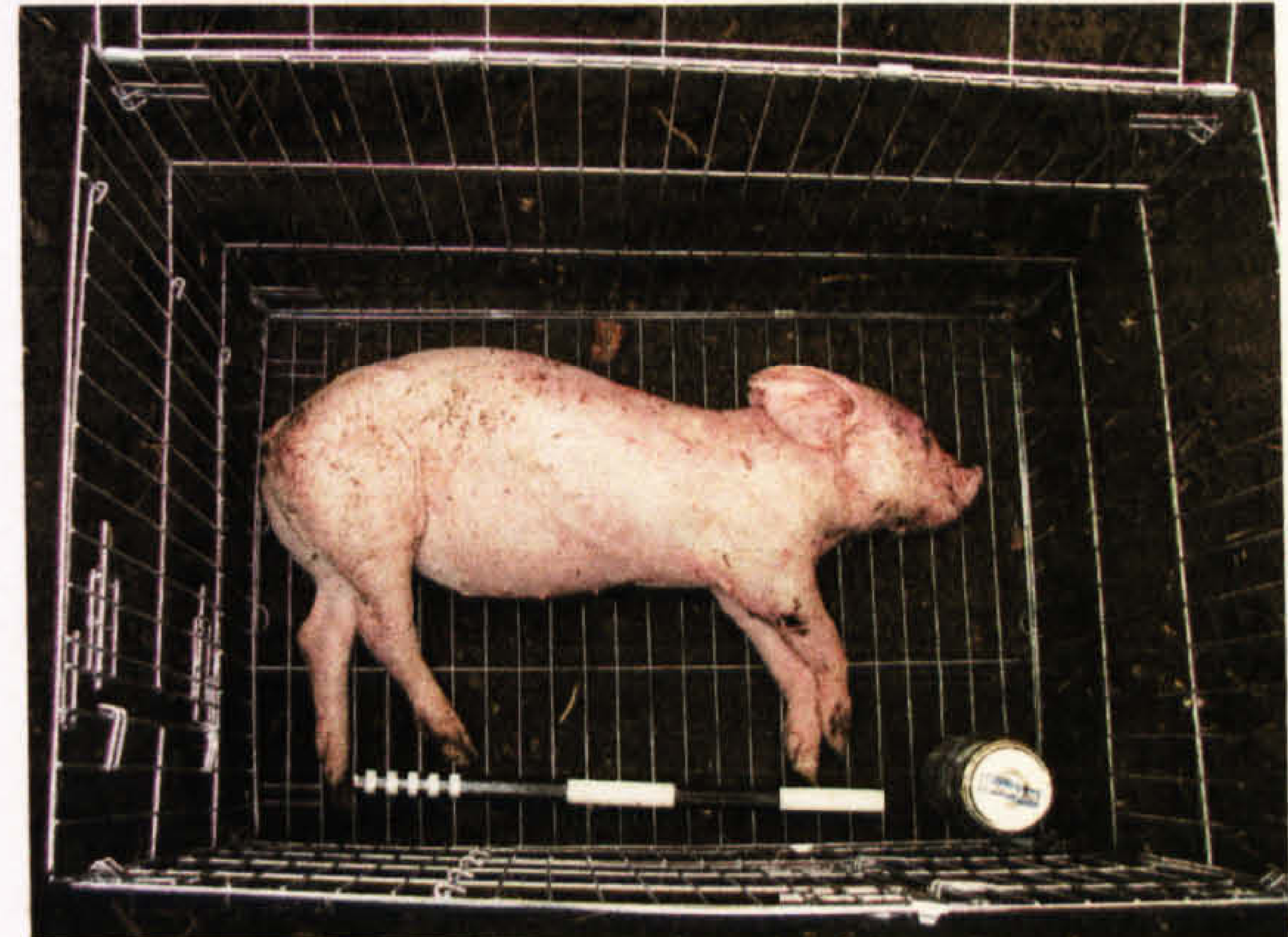


Fig 7.18 Pig no 14

Fig 7.19 Site of Pig no 13 after burial
(Both, Author, 2006)

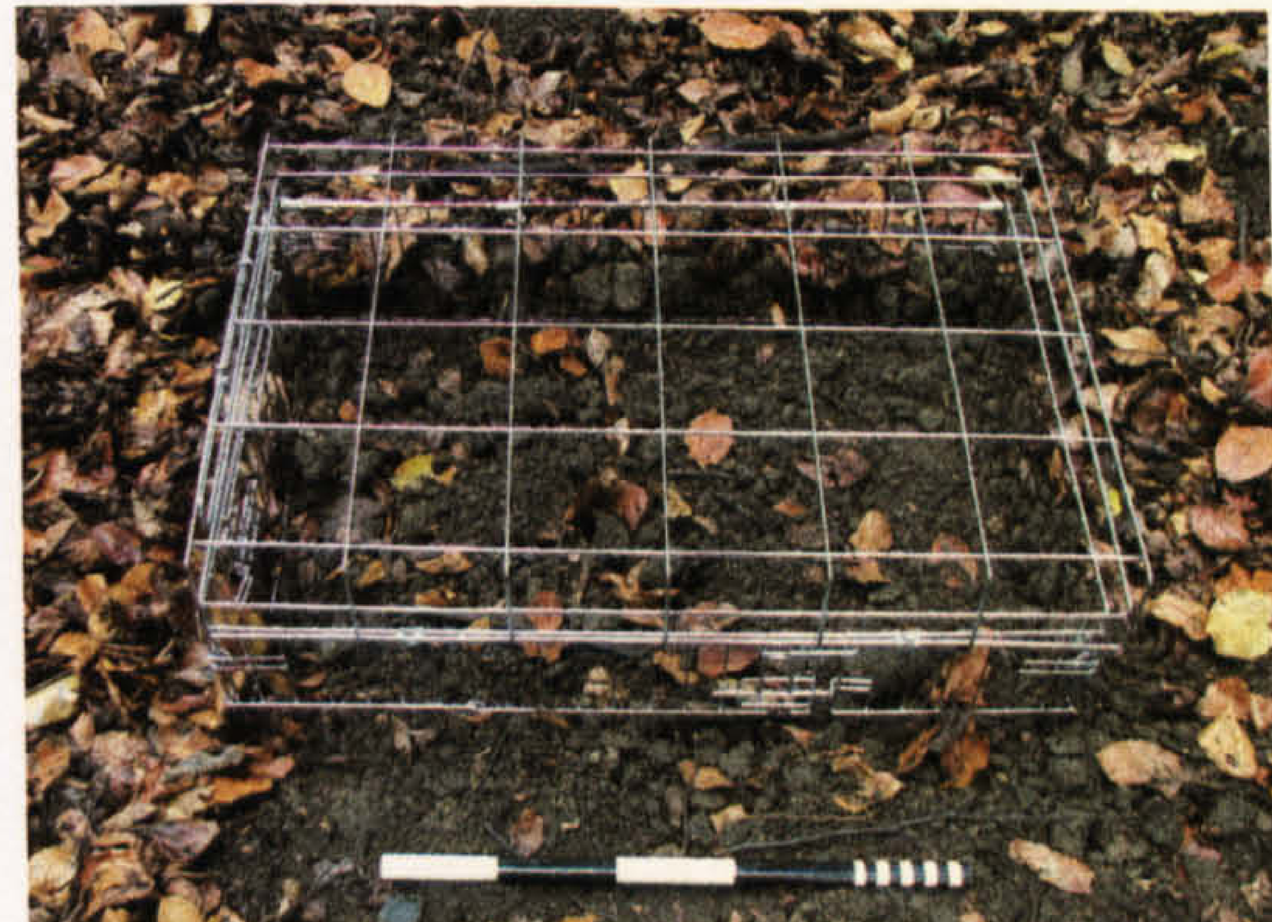


Fig 7.20 Site of Pig no 14 after burial

Both burials were contained within commercially bought cages that were adapted to allow ease of access for inspection and photography (figs 7.19 & 7.20). The cages are zinc plated with dimensions of (H) 64, (W) 77 & (D) 54 cm's. A Hobo data logger was placed in the grave of the larger pig prior to reburial to chart soil temperature.

7.8 Spring Burials

For this part of the experiment 16 domestic pigs (*Sus scrofa)* were given either a soil burial (fig 7.22) or were placed directly on top of the soil (fig 7.21). The burials varied in depth only to a small degree with the shallowest at 30cm (base of pit) and the deepest at 40cms. The soil was placed back in the grave in random fashion, with mixing of topsoil and subsoil resembling a real life scenario in which a body is hastily concealed by shallow burial. They were protected from scavengers by an electric fence that covered approximately 11m x 7m and that comprised a 50m roll of 8 wire single spike Sheep netting (90cm) and an electric fence powered by a 12v Gallagher Batterymaster B75 Wet Battery Energiser and 12v leisure battery. This supplies 6200 volts with stored energy of 0.75 Joules and an output of 0.55J. The ground

depositions were then each protected individually by a custom-made three sided mesh cage.

Fig 7.21 Surface Depositions (Author, 2007)

Fig 7.22 Some of the buried pigs (Author, 2007)

The pigs were again supplied by the local knackerman, who delivered them to the site. They were chosen on the basis of size and freshness although it is impossible to ascertain an exact time or date of death due to the mode in which the knackerman works. His job is merely to collect fallen livestock from outlying farms and these may have been dead for up to three days dependent upon which area they die in. They had all been frozen for a period of 2 days until transfer to Riseholme could be undertaken. They had all died of natural causes and were either stillborn, rolled on by their mother or euthanized (no=1 shot through head/others lethal injection) on medical grounds. On the day of burial no decompositional activity was observed although any previous post-

mortem changes would have been retarded by the freezing process. Despite this, it was considered that by visual inspection of each pig

Condition at Burial/Deposition			
Burials		***Surface***	
Mass Grave	One of the pigs very green at the abdomen, 3 Fresh		
50kg	Skin bright red, Fresh	50kg	*Green Abdomen*
25kg	Green Abdomen	25kg	*Green Abdomen*
15kg	Green Abdomen	15kg	*Very red, gunshot to head, Fresh*
10kg	Green Abdomen	10kg	*Bloated, Postmortem slash to right abdomen*
3-4kg	Fresh	3-4kg	*Fresh*
1-2kg	*Fresh*	*1-2kg*	*Fresh*

Table 7.3 Condition of the pigs at the time of deposition, varying from fresh to early putrefaction.

that nine of the pigs were fresh (table 7.3), six had green discolouration of the abdomen and one was bloated (although no green discolouration of the abdomen was visible). The pigs used in this study ranged from 1.3kg-50kg (2.86-110lbs). The lowest weight is comparable to a fetal child whilst the higher weight roughly equates to a child of around 14years of age.

Girls Age	*Weight Kg*	*Boys Age*	*Weight Kg*	*Mean of Both Weights*	*Weight & Age Chosen*
Fetal	1-2				**1-2Kg (2.2-4.4lbs)**
Birth	3.4	Birth	3.6	3.5 Kg	**3-4Kg (6.6-8.8lbs)**
3 Months	5.4	**3 Months**	6	5.5 Kg	
6 Months	7.2	**6 Months**	7.8	7.5 Kg	
12 Months	9.4	**12 Months**	10.2	9.8 Kg	**10 Kg (22lbs)**
2 Years	11.6	**2 Years**	12.6	12.1 Kg	
3 Years	14	**3 Years**	14.4	14.2 Kg	**15Kg (33lbs)**
4 Years	16	**4 Years**	16	16 Kg	
8 Years	25	**8 Years**	25.2	25.1Kg	**25 Kg (55lbs)**
14 Years	49	**14 Years**	50	49.5Kg	**50Kg (110lbs)**

Table 7.4 Weights chosen for each pig, to roughly correspond with an age range for humans of fetal to 14 year old/small adult.

Six pigs of varying weight (table 7.4) were used to correspond to children from fetal to fourteen years of age (or a small adult). The ages and weights represented are fetal (1-2kg), newborn (3-4kg), 1 year (10kg) 3 years (15kg), 8 years (25kg) and 14 years (50kg). The children's age weights are taken from growth charts and are for those within the 50th

Percentile (i.e. the average child's age/weight ratio) (Charts available @ www.pediatrics.about.com).

Body mass has been chosen as the direct correlate between the pigs and the analogous children. There are two main differences that may affect the experiments. Firstly, piglets grow at a much faster rate than children reaching adult size by around six months of age whereas children develop at a much slower rate and full size is not obtained until the teenage years. The second and more difficult problem is one of weaning. The typical weaning age for piglets raised in intensive conditions is around 25 days and this occurs abruptly. The natural weaning age would normally occur gradually over a period of weeks around the age of 12-15 weeks. This means that from as early as one month old the pig gut flora will change dramatically unlike in a human infant that may not be weaned for 6 months – two years. Any subsequent decomposition is then difficult to reconcile with the breast fed infant. A further problem becomes apparent in that the abrupt weaning is often harsh on the immature digestive system of the baby pig and this often leads to illness such as diarrhoea, failure to thrive or piglets that will require medication on a daily basis.

A mass grave was also set up to investigate whether there is any difference in microbial attack within the individuals (i.e. do those in the middle of the grave contain more bacterial attack?). Four pigs of very similar size (all around 25kg) were thrown at random into a pre-dug pit that reached a depth of 60cms. They were dragged by their hind legs to the edge of the pit and then thrown in. They landed quite neatly and are roughly aligned with the heads all facing in the same direction. Two landed in a supine position, one is laid on its right side and the fourth is laid on its left side (fig 7.23).

Fig 7.23 Mass Grave, four large pigs thrown in at random (Author, 2007).

Burial Type	***Grave Dimensions***		
	Length	**Width**	**Depth**
Mass Grave	1.5m	1.5m	60cm
50KG	1.5m	1m	40cm
25KG	1.5m	80cm	40cm
15KG	75cm	50cm	30cm
10KG	75cm	50cm	30cm
3-4KG	75cm	50cm	40cm
1-2KG	75cm	50cm	30cm

Table 7.5 Grave dimensions for each pig.

7.9 Microscopy

Once skeletonized, bone samples were taken from each pig at set intervals. Full decomposition was not complete in all of the burials and the collection of specimens sometimes necessitated the use of a scalpel and often considerable force to free the bone from the surrounding tissue and ligaments. It was then necessary to remove any tissue by washing and soaking the bones which were subsequently left to dry before being thin sectioned transversely to a width of 50µ with a Leitz 1600 annular saw microtome. Up to three sections were taken of which one was mounted on a glass slide using Euparol mounting medium and covered with a glass cover slip. This was then examined beneath a polarizing light microscope for evidence of microbial activity. Other sections could be used for staining procedures or to check for decomposition throughout the bone length.

7.9.1 Anaerobic Growth of Microbes

Fig 7.24 Anaerobic Jar (www.shharmony.com)

The length of time taken for microbial tunnelling to become evident in bone is not known and although it appears to be something that happens relatively quickly, this could still be in terms of years rather than days or hours. In an attempt to speed up the effect of the microbes, six of the already sectioned bones were put in an anaerobic jar (fig 7.24) and placed in an incubator at 30°C. The optimum temperature for some bacteria would be around 37°C but it was felt that a lower temperature would still suffice and at the same time allow other bacteria without such a high heat tolerance to develop also. This should result in tunnelling at an earlier time than if the skeletons were left outside in the much cooler autumn and winter temperatures that tend to inhibit bacterial growth. However, there is a lack of available moisture within this environment that may inhibit bacterial growth. The anaerobic jar is a very basic system that involves;

the bones (or plates of agar spread with bacteria in most other cases) being placed in the base of the jar with an anaerobic indicator strip that has a drop of water placed on the tip, an anaerobic gas generating sachet is then opened and placed between the interior rack and the outside of the jar. The lid must then be replaced and the valves closed within 30 seconds to ensure that an anaerobic environment will ensue. Once closed the anaerobic indicator strip will, over time, change colour from pink to white giving a visual indication of anaerobiosis.

7.9.2 Staining

Some of the sections were stained with either Methylene Blue, Crystal Violet or by Gram Staining in an attempt to aid the visualisation of bacteria either prior to tunnelling becoming evident or once colonised.

7.9.3 Simple Stain

The first two methods are relatively simple and consist of placing the section on a glass slide, flooding it with either Methylene Blue or Crystal Violet for 30 seconds and then rinsing the section with distilled water to remove excess stain. The section is then blotted dry and can then be examined beneath a microscope where relevant bacteria will be stained blue in the case of Methylene Blue or pink if using Crystal Violet.

7.9.4 Gram Stain

The Gram stain method will separate the bacteria in to two large groups; the Gram-positive bacteria that stain blue and the Gram-negative bacteria that stain pink. Certain bacteria take up stain differently because of a difference in the cell wall composition. Gram-positive bacteria have a thick cell wall layer. Alcohol does not readily penetrate to decolorize the cell wall of the previously applied crystal violet stain. Gram-negative cells have a thinner cell wall through which the alcohol readily penetrates. The crystal violet is removed from these cell walls that are then stained with the safranin counterstain.

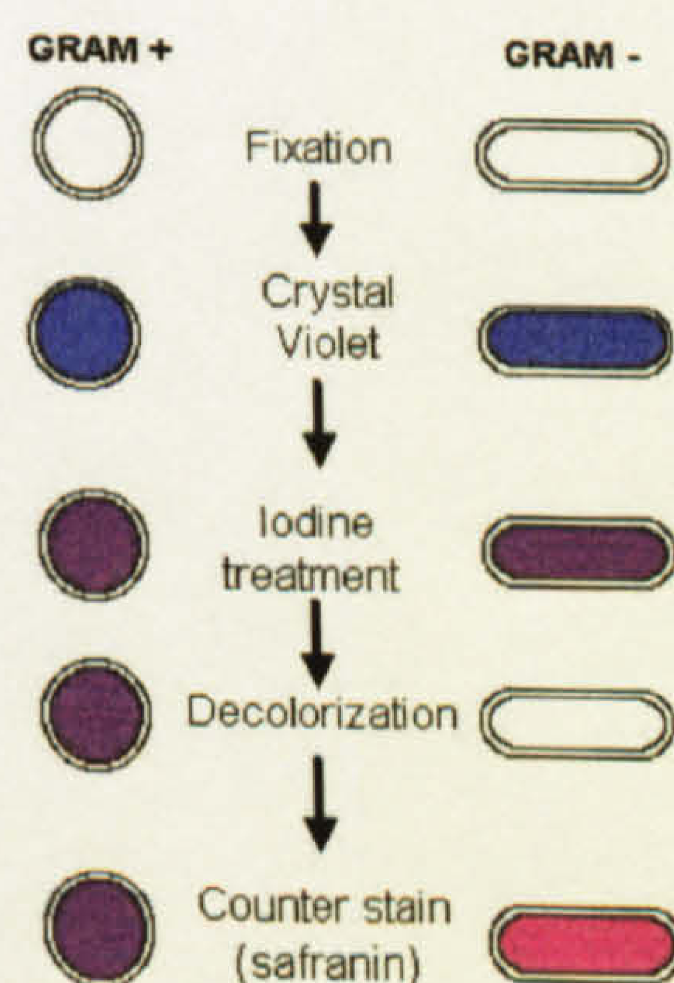


Fig. 7.25 Stages of gram staining with eventual bacterial cell wall colour change to either purple or red. (http:/priede.bf.lu.lv/grozs/Mikrobiologijas/)

The method for Gram staining (fig 7.25) is to place the bone section on a slide, flood with crystal violet for 1 minute, wash off briefly with water for no longer than 5 seconds, flood with Gram's iodine solution and allow to sit for 1 minute, wash off with water and drain off excess liquid, flood slide with 95% alcohol and pour off immediately. Re-flood with 95% alcohol for 10 seconds and wash off with water (the first flooding with alcohol removes the excess water from the slide, so that alcohol used for decolourization is not diluted). Flood the slide with safranin solution and allow it to stain for at least one minute and wash off with water for 5 seconds. Finally, drain the slide and blot dry with bibulous paper. Gram-negative bacteria (fig 7.26) will stain pink (i.e. *E. coli*), while Gram-positive (fig 7.27) bacteria will stain blue (i.e. *Staphylococcus aureus*).

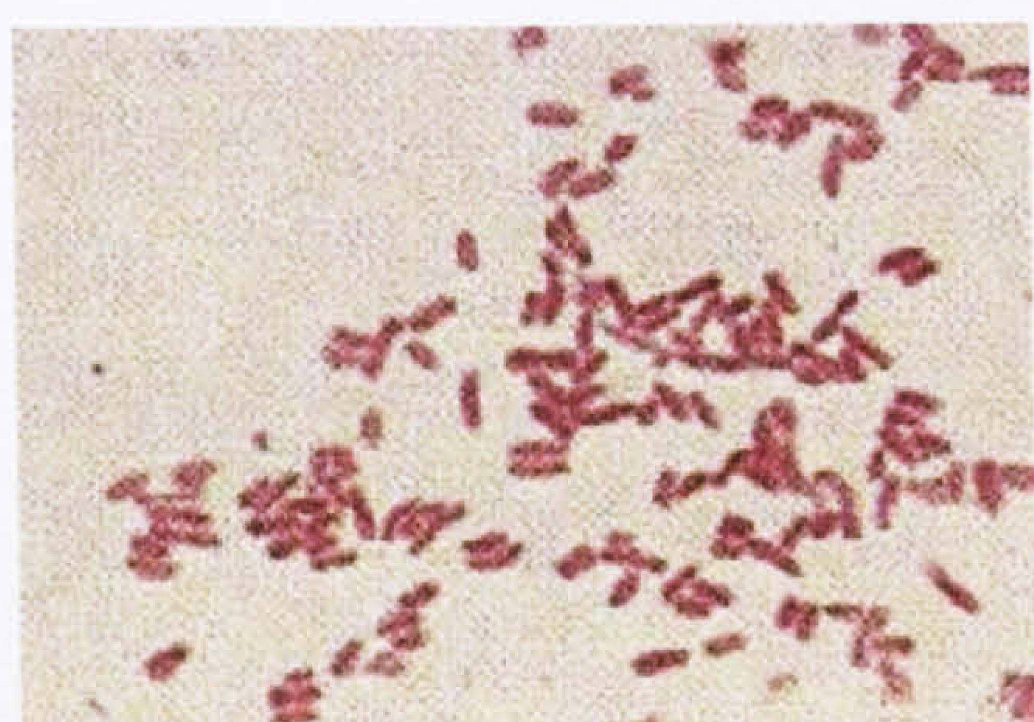


Fig 7.26 Gram negative

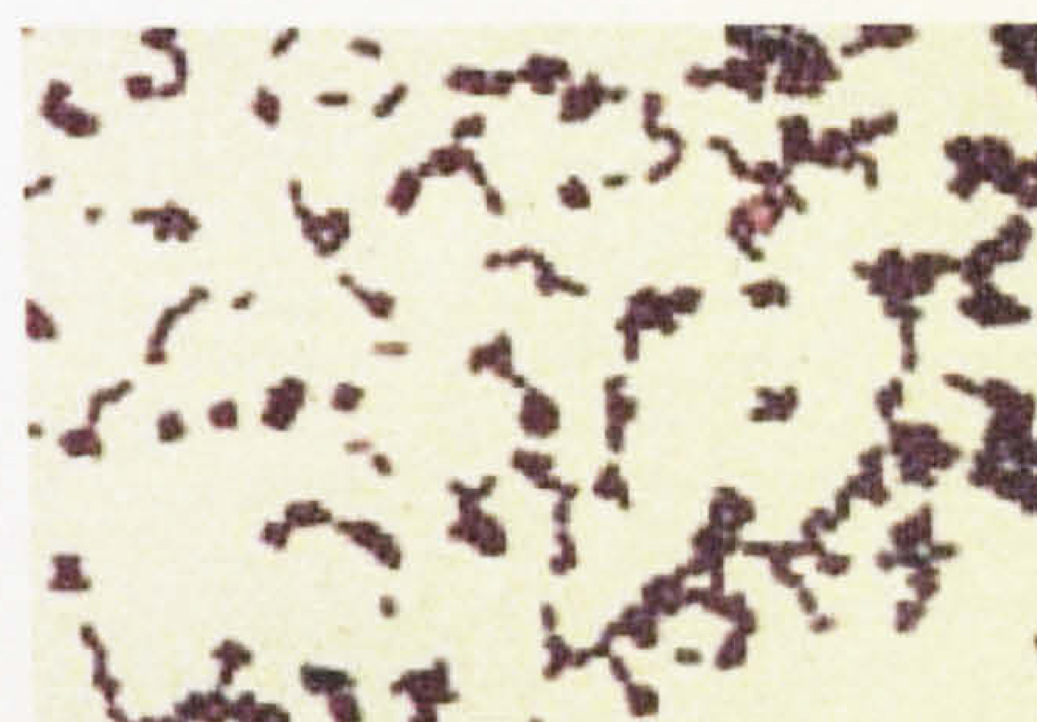


Fig 7.27 Gram positive bacteria

Both images available @ www.nmpdr.org/FIG/wiki/view.cgi/FIG/GramStain

7.10 Archaeological Material (Human and *Pig)*

To test whether bacterial attack can be found in juvenile (especially neonatal) bone, remains from archaeological contexts were also thin sectioned and examined microscopically. Thin sections were already available for the Bolsover site and had been previously analysed (table 7.6) with regards to bacterial attack (Economou, 2003). The Bolsover collection is from the churchyard of the Church of St Mary and St Lawrence that contains burials from the Norman period through to present day. The infants from this site were presumed to be buried in the 19th century due to their context. Most of them were recovered from the north side of the tower area and from a 2-3m wide strip around the foundations. Included within the previous study were three sections from the Blackgate collection (of Anglo-Saxon origin) and two from Carsington Pasture. Budded tunnelling appears to be present from as early as 24 weeks (gestation), but linear longitudinal does not appear until 39 weeks (gestation). After this time both types become increasingly prevalent. This material will be reanalysed as it was previously used for an MSc thesis (Economou, 2003).

	OXFORD HISTOLOGICAL INDEX	LINEAR LONGITUDINAL	BUDDED	AGE
BOL 010	0	A	P	24wks
BOL 008A	5	A	A	26wks
BOL 008B	5	A	A	28wks
BOL 056	1	A	P	30wks
BOL 014	1	A	P	33wks
BOL 009	5	A	A	38wks
BOL 011	0	P	P	39wks
BOL 028	1	P	P	39wks
BOL 017	0	P	P	40wks
BOL 007	5	A	A	1 month
BOL 012	3	P	P	1 month
BOL 021	0	P	P	6 months
BOL 018	0	P	P	4 years
BOL 003	0	P	P	20-40 years
BOL 001	2	P	P	18-30 years
BOL 029	0	P	P	7 years
BOL 002	0	P	P	4 years
BG 3277	3	A	P	27wks
BG 3191	5	A	A	46wks
BG 3215	1	P	P	39 wks
CPCY03	5	A	A	46wks
CPC99-21	5	A	A	46wks

Table 7.6 Data from Bolsover burials. A = Absent P = Present (Data taken from Economou, 2003).

Samples were also taken from the disarticulated human remains in the Blackgate, Newcastle Anglo-Saxon Cemetery. Archaeological pig bone was also sectioned, but this presents problems as in previous studies (Jans *et al*, 2004) microbial attack in disarticulated animal bone has been far less ubiquitous. This is thought to be a counter effect of butchering animals for their meat and possibly also as an effect of cooking. Unfortunately, this involves removal of intestinal organs and the complete disarticulation of the animal. If bacteria are colonising corpses via the vascular system directly from the natural gut flora, these will have been removed, rendering any bones found as effectively sterile. Other archaeological material used for this research include; the Cladh Hallan Mummies, Kilton Hill (possible execution site), Boraray, Stanton, Berinsfield, Exeter, Grantham, Royal Mint, Carsington Pasture and a selection of animal bone from various sites. One hundred and seventy one sections were observed of which one hundred and thirty eight were human and thirty three were animal. This does not include the sections of pig bone from the experimental research.

7.11 Summary

It is hoped that the research carried out within the remit of this study will provide tentative answers to some confusing questions. Bacterial attack exists but its origins and the timescale required are still not known with any certainty. By using animals of differing size, ranging from fetal to young adult and by having above and below ground burials this will be a good starting point in the search for a more sound knowledge of this phenomenon. By combining these results with what is already known from archaeological and forensic cases it may be possible to arrive at some framework of both time and origin.

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Chapter 8. Results

8.1 Introduction

Results of all the experimental and archaeological material are presented here. Soft tissue decomposition has been charted for both the boxed pigs and the second real-time burials up to the point of skeletonization along with climate data for the relevant periods. Bacterial assay results together with the anaerobic jar experiments are also provided. Results of the thin sectioning and microscopy evidence of both the experimental and archaeological material are given in section eight.

8.2 Results of Soft Tissue Decomposition (Boxed Pigs)

The first four pigs went out on the 26th April 2006, whilst the other twelve were not in place until just over a month later on the 30th May 2006. Ideally, it would have been preferable to have all of the burials out at the same time, but due to logistical problems this was not possible. Pigs No's 1-6 were newborns whilst pigs No's 7-12 were considered to be stillborn. As all of the burials were carried out in duplicate, two of the first four (no's 3 & 4) did not have a replicate for some time. It was decided that the spring would be the ideal time to locate the experiments as by this time the temperature would be more suitable to a faster rate of soft tissue decomposition and insect activity would be more substantial (most carrion and blow flies do not fly below 10°C). By April the temperature had risen and daily highs were recorded above 10°C on most days, although daily lows were also recorded as low as -1°C on two separate occasions. By June, daily high temperatures were recorded at up to 27° and at least 16°C on every day throughout the month.

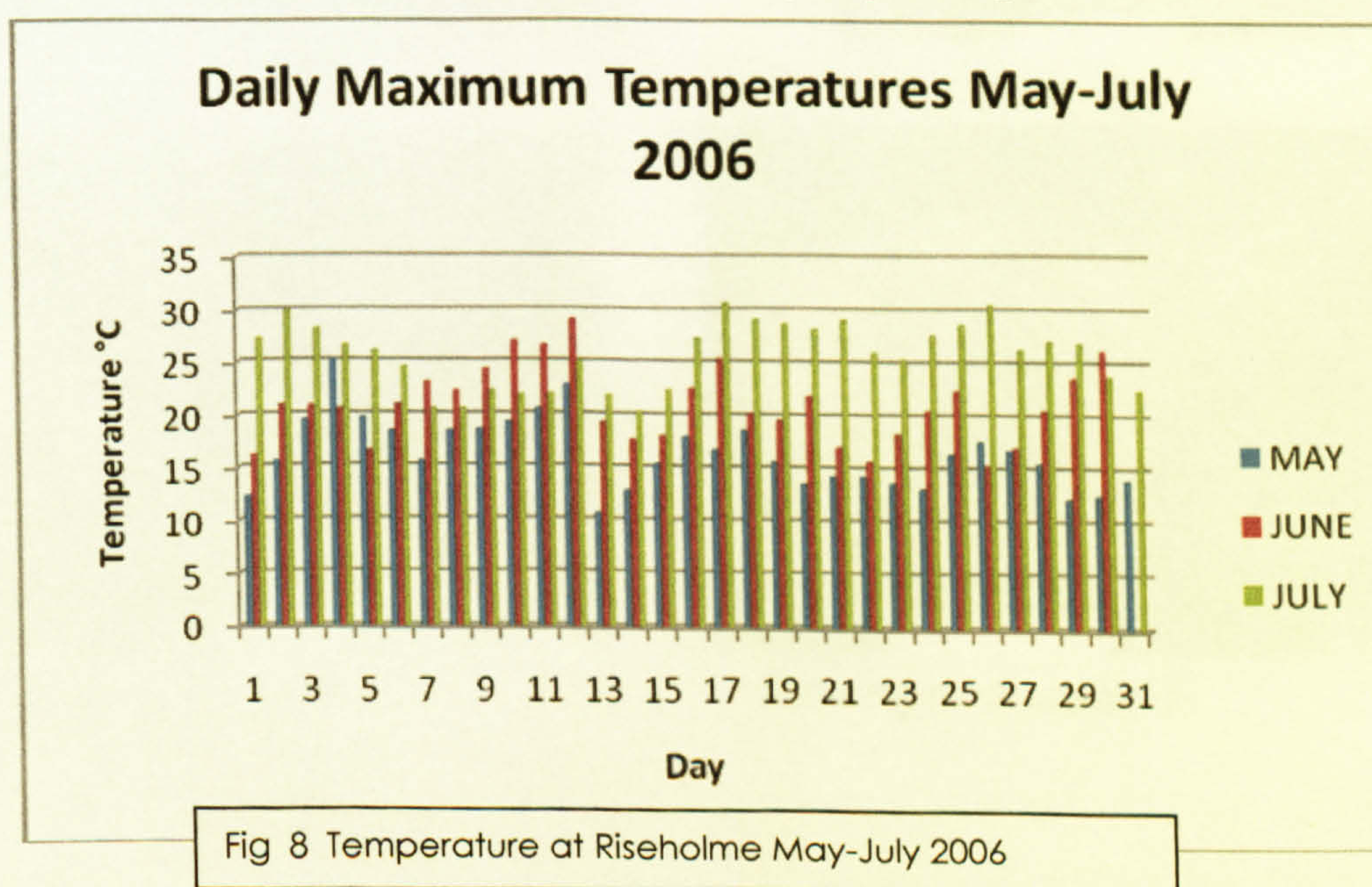

Fig 8 Temperature at Riseholme May-July 2006

The lowest temperature recorded was 6.3°C on the 1st of June but other than this the temperature was almost constantly above 10°C. The difference in temperature (fig 8) had a direct influence on how the two sets of pigs decomposed. The combination of higher temperature (Mean temps: May m =16°C, June m =20°C and July m =26°C) and much greater insect activity throughout the month of June meant that the second set of pigs skeletonized over a very short time period whilst the 1st set of pigs although generally skeletonized retained mummified soft tissue across their entire bodies.

8.2.1 1st Experimental Carcasses:Pigs No's 1-4 Newborns

Of the first four pig burials two were placed in the box without any covering (no 1 & 2) whilst one had a covering of soil (no3) and the other was buried in building sand (no4). They were evaluated on a daily basis but it was decided to leave the two covered ones undisturbed for as long as possible. Table 8.1 charts their decomposition over a number of weeks. Pig 1 (figs 8.1-8.4) was considered fresh and was free from rigor mortis and bloating. Pig 2 (figs 8.5-8.8) was in a state of full rigor mortis with blood present around the facial area.

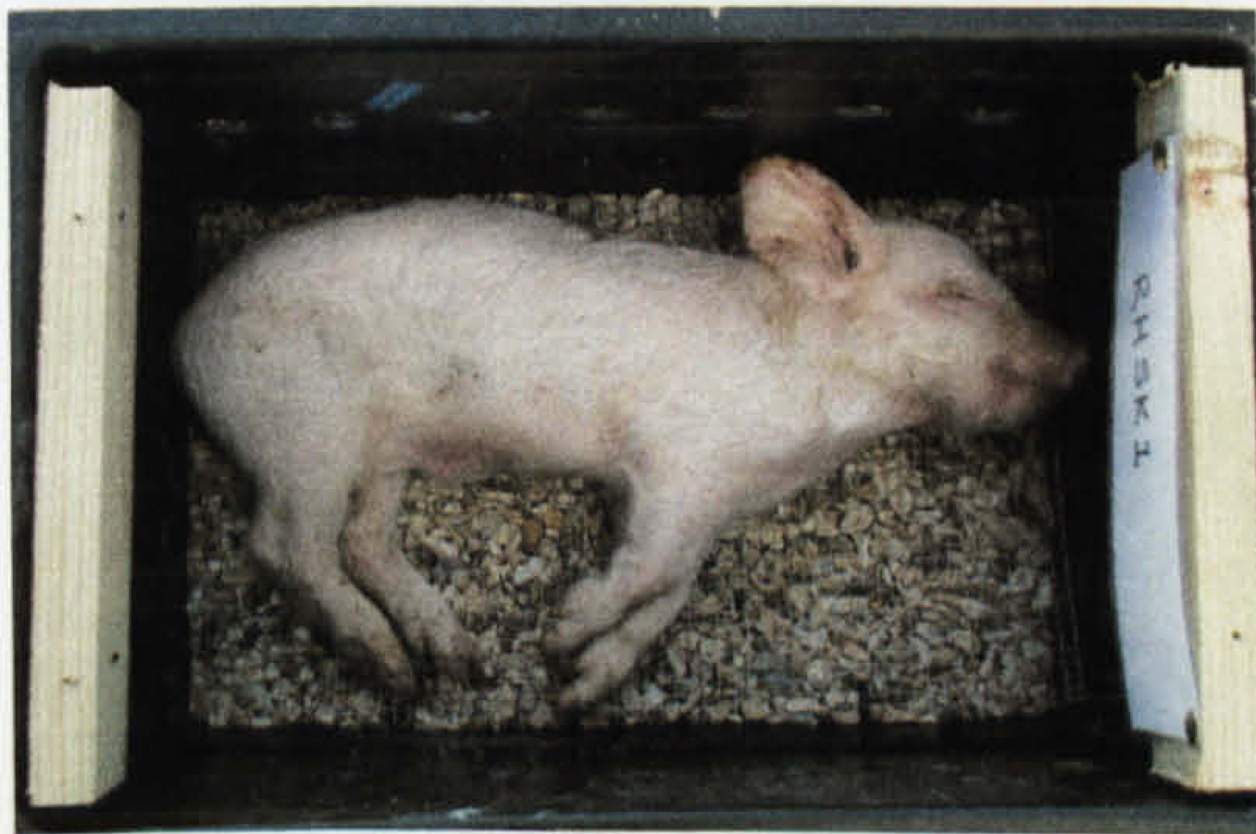

Fig 8.1 Pig 1: At deposition

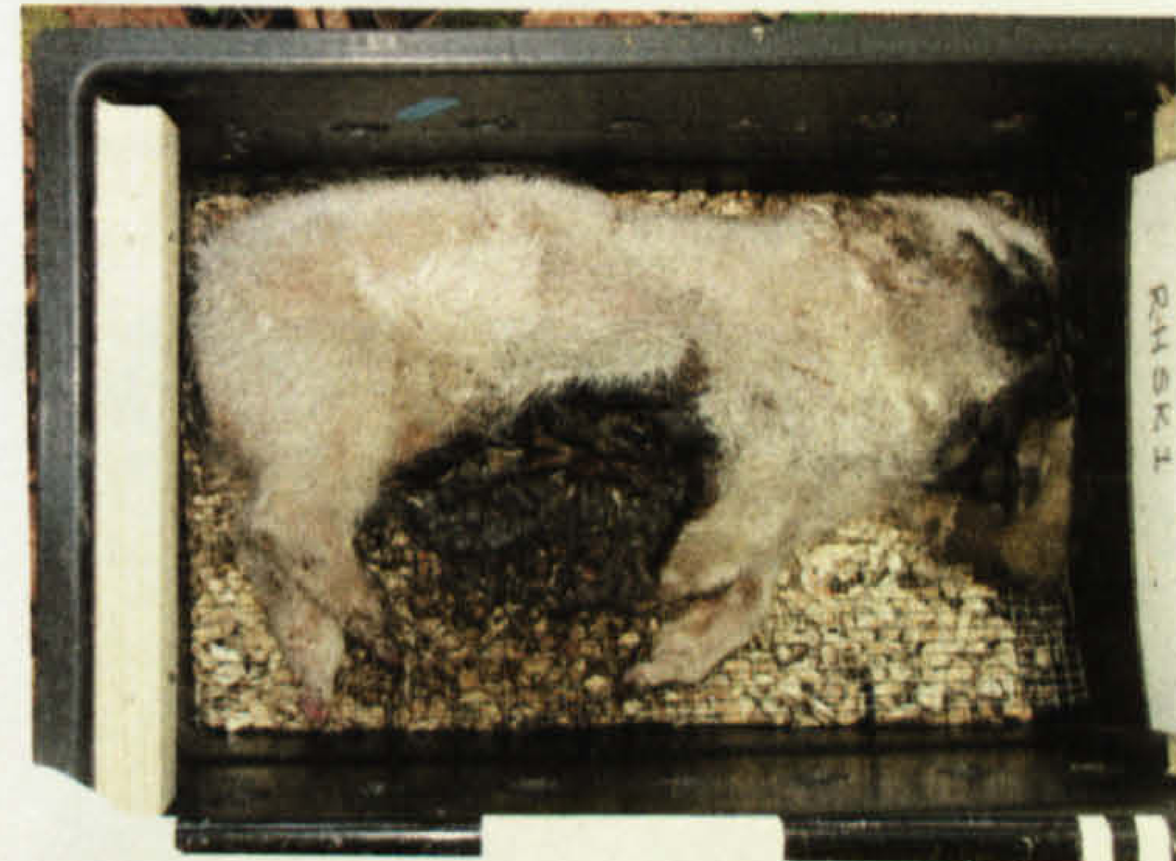

Fig 8.2 Pig 1: 17 days PM

Fig 8.3 Pig 1: One month PM
(All 4 pictures, Author, 2006)

Fig 8.4 Pig 1: Six weeks PM

Pig no1 which was uncovered did not really bloat, maggot activity was absent for the 1st seven days, it took two and a half weeks for the abdominal area to break down and five weeks for the first skeletal elements to become visible. Mummification of the soft tissue was apparent from one month post-mortem and this was retained throughout.

Pig no2 which was also uncovered began to putrefy internally very quickly but decomposition by insects was slower to occur taking nine days for larvae to become evident. The corpse then went through a period of advanced decomposition becoming mummified within five weeks, a state that changed little over the following months.

Fig 8.5 Pig 2: At Deposition

Fig 8.6 Pig 2: 11 days PM

Fig 8.7 Pig 2: Five weeks PM
(All 4 pictures, Author, 2006)

Fig 8.8 Pig 2: Six and a half months PM

Pig no's 3 and 4 both had coverings (soil and sand respectively). Pig no3 (figs 8.9-8.12) was placed in a box that contained soil taken from a cemetery that is currently in use, whilst pig no4 (figs 8.13-8.16) was covered with commercially available builders sand. Both of these animals dried out due to the lack of moisture that could gain access to the box because of their design. Repeated attempts were made to keep the soil/sand moist by introducing water via spray bottles at each visit, but this proved inadequate and was eventually stopped. In the case of pig no1 very little active decomposition could be seen in this pig due to the soil but it is clear that

although not always obvious decomposition was occurring and the internal organs were probably being destroyed within one month of deposition and by two months were completely absent, leaving just bones surrounded by mummifying soft tissue.

Unfortunately, at some time the tank became waterlogged and when a site visit took place in November the pig was completely covered in water. As much of this fluid was drained as possible and it became clear that the skin had taken on the appearance of that found in bog bodies.

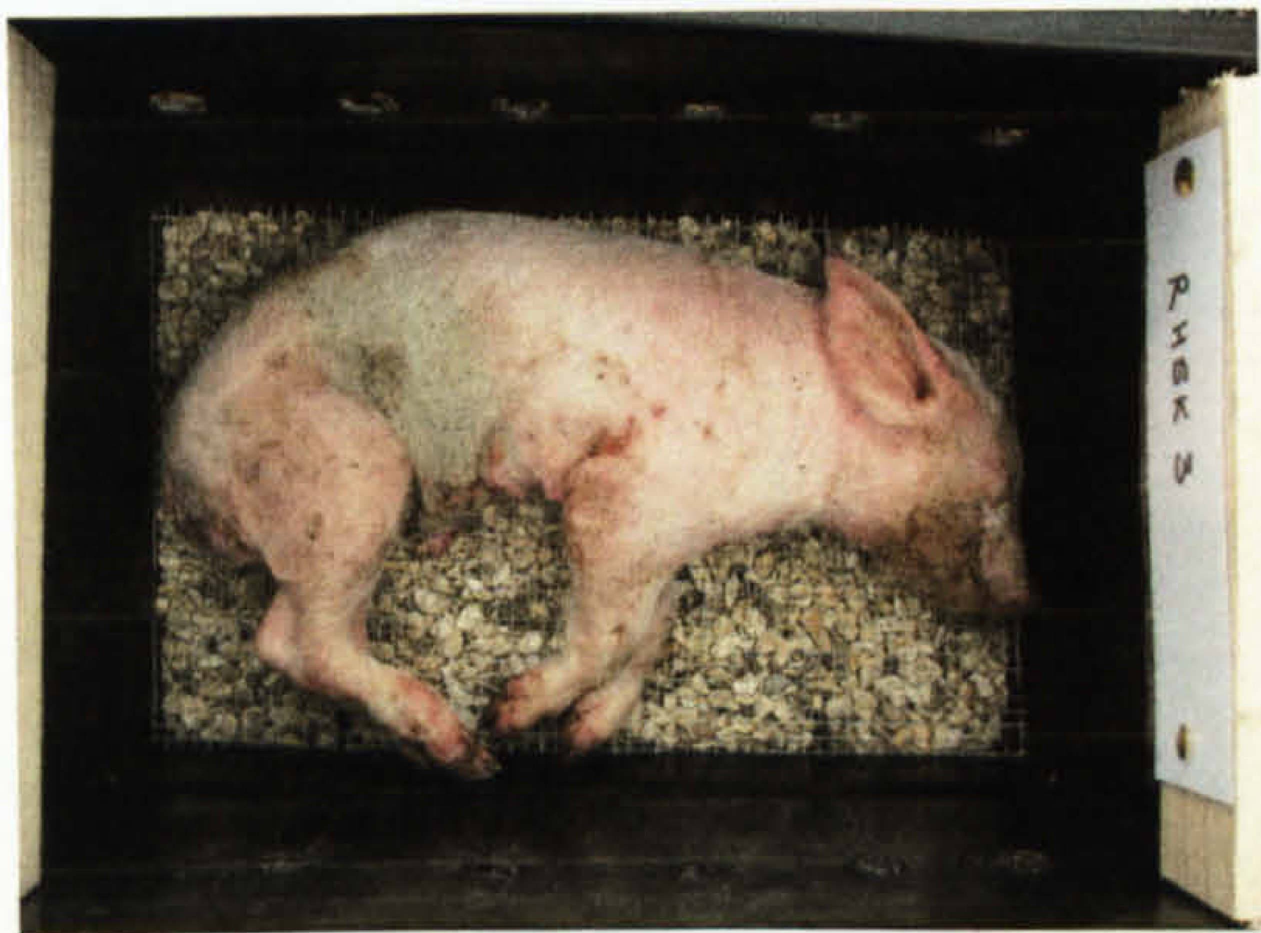

Fig 8.9 Pig 3: At Deposition

Fig 8.10 Pig 3: Four weeks Pm

Fig 8.11 Pig 3: Seven weeks PM
(All 4 pictures, Author, 2006)

Fig 8.12 Pig 3: Six and a half months PM

Pig no 4 suffered from the same drying problems as pig No3. The carcass was considered fresh and was free of bloating and rigor mortice at burial. Again, this was a burial that did not outwardly appear to be decomposing but was actually decaying on the inside unobserved. Two and a half weeks passed before maggots were introduced and then it was a slow process over another three weeks until all that remained was skin and bone. At the site visit in November the pig was practically unchanged apart from some further hair loss.

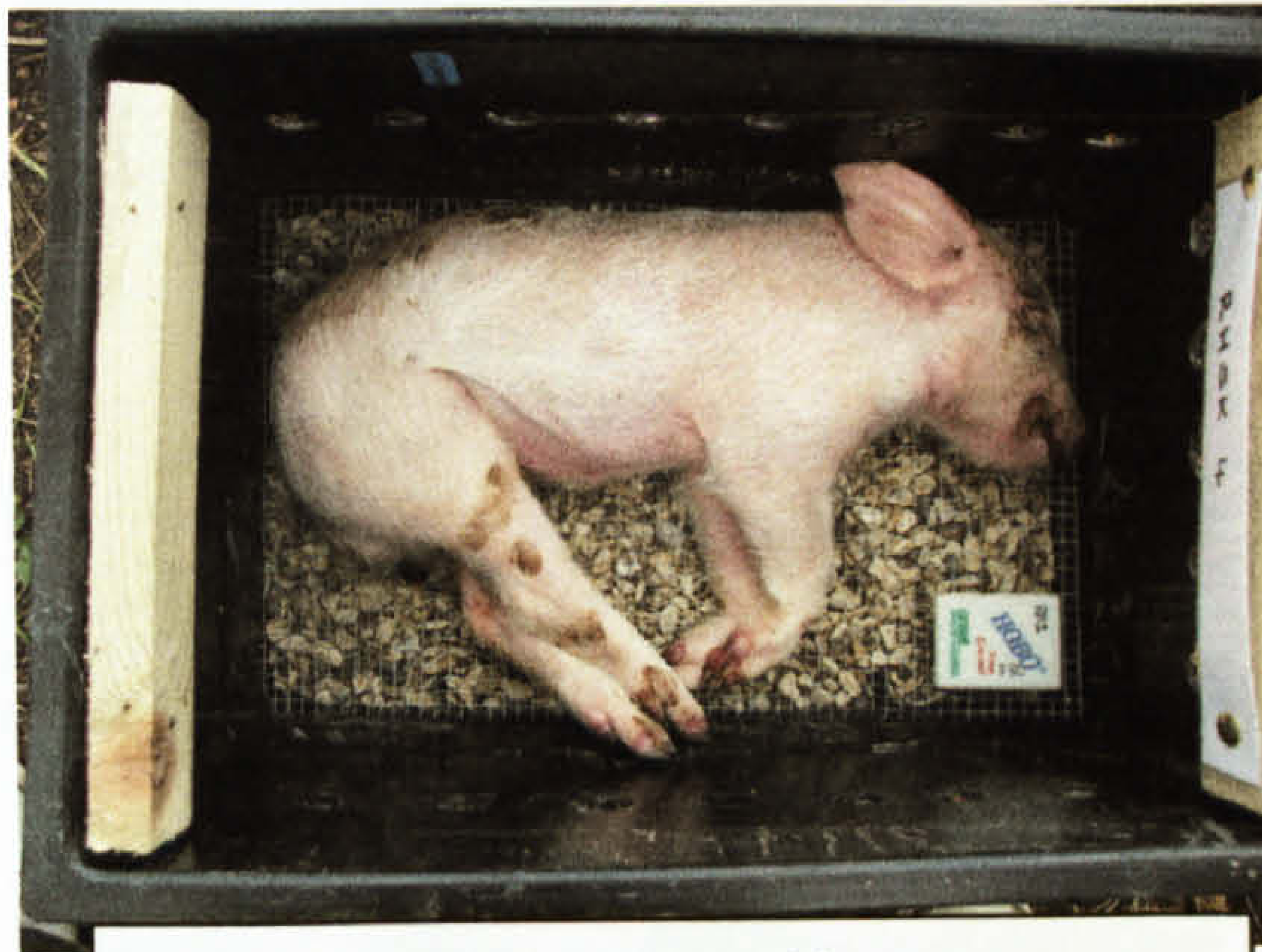
Fig 8.13 Pig 4: At Deposition

Fig 8.14 Pig 4: Three weeks PM

Fig 8.15 Pig 4: Four weeks PM
(All 4 pictures, Author, 2006)

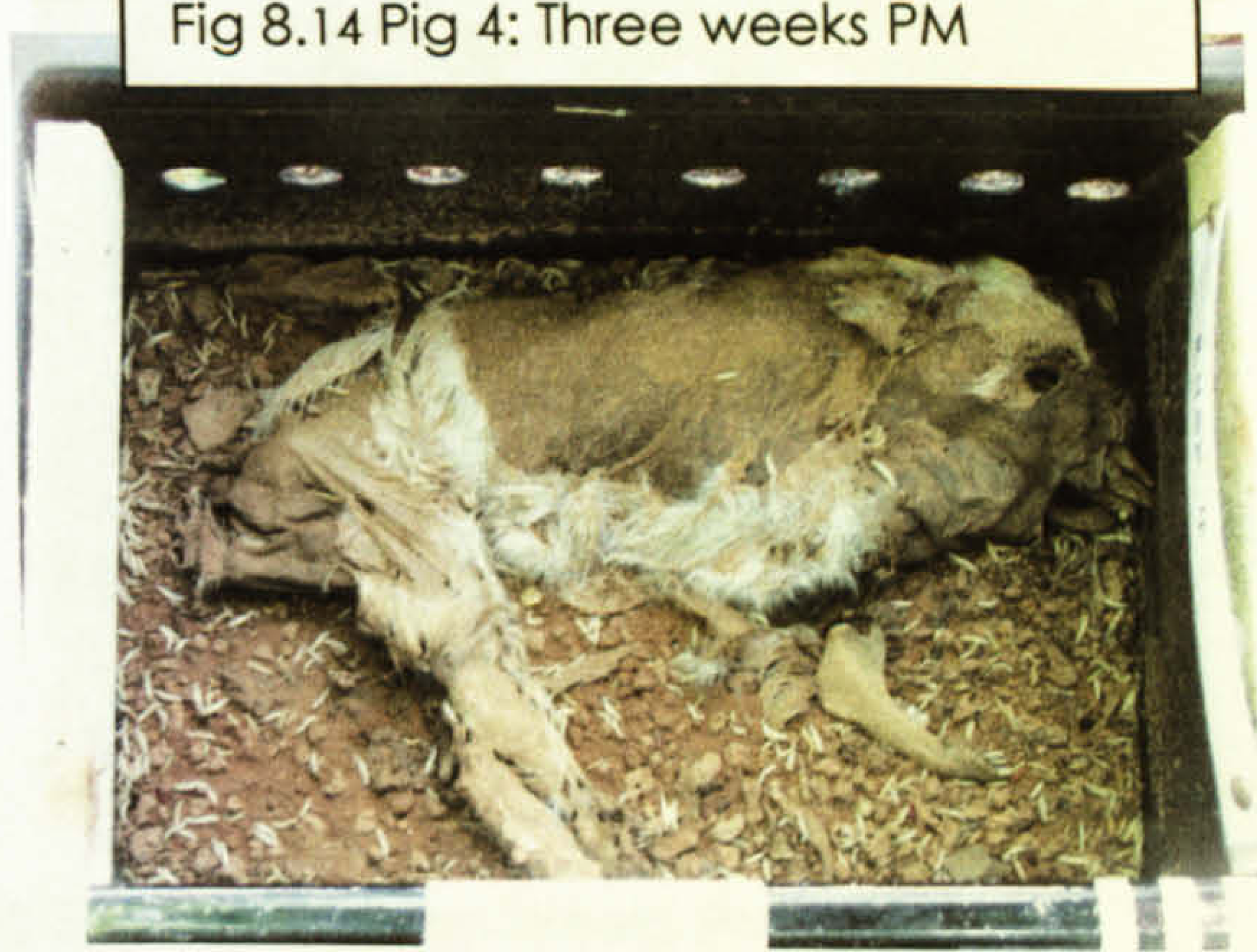
Fig 8.16 Pig 4: Six and a half wks PM

8.2.2 Experimental Carcasses: Pigs 5-12 (Boxed)

Pigs 5-12 all arrived at site on the 30.05.06, roughly one month after the first set. By this time average daily temperatures were slightly higher. Pigs 5 & 6 were the last of the newborns whilst pigs 7-12 were considered to be stillborn. Tables 8 - 8.28 chart their decomposition over a number of weeks. Pig 5 (figs 8.17-8.20) was covered with soil and pig 6 with sand. Pig no5 decomposed extremely rapidly reaching an almost skeletonized state within 11 days. The amount of insect activity at this pig was phenomenal, with thousands of maggots literally spilling out of the box. This level of insect activity was restricted to this pig and was not seen at this scale in any of the other animals.

Fig 8.17 Pig 5: Four Days PM

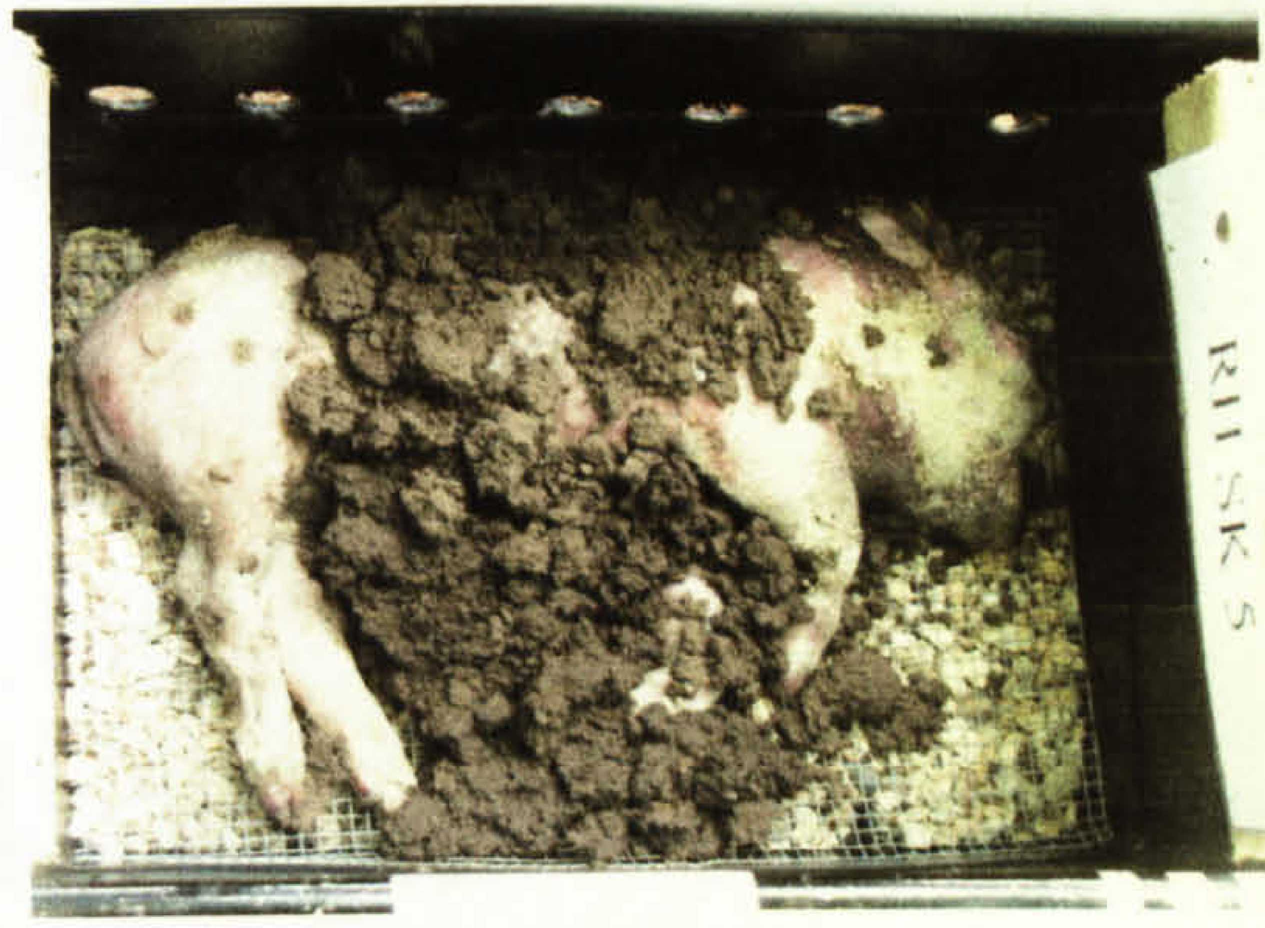

Fig 8.18 Pig 5: Six Weeks PM

Fig 8.19 Pig 5: Six & a Half Weeks PM
(All 4 pictures, Author, 2006)

Fig 8.20 Pig 5: Seven Weeks PM

Pig no6 (figs 8.21-8.22) took slightly longer to skeletonize (20 days). Within 24 hours of burial putrefaction was evident at the abdomen and hind leg areas and many fly eggs were present. Green putrefaction progressed until 90% of the body was affected at eight days post burial. Within two days of this the abdomen had opened, maggot activity was extensive and the corpse had become deflated. From this point on, the internal soft tissues were rapidly destroyed, the skin became much drier and at each visitation more bones became visible. The corpse remained in a static condition from the end of June to the next examination in November where it was found that the bodily condition was almost identical except for further drying of the skin and the onset of some type of fungal/mould growth also to the skin. Pig no6 remained mummified whilst pig no5 eventually became waterlogged.

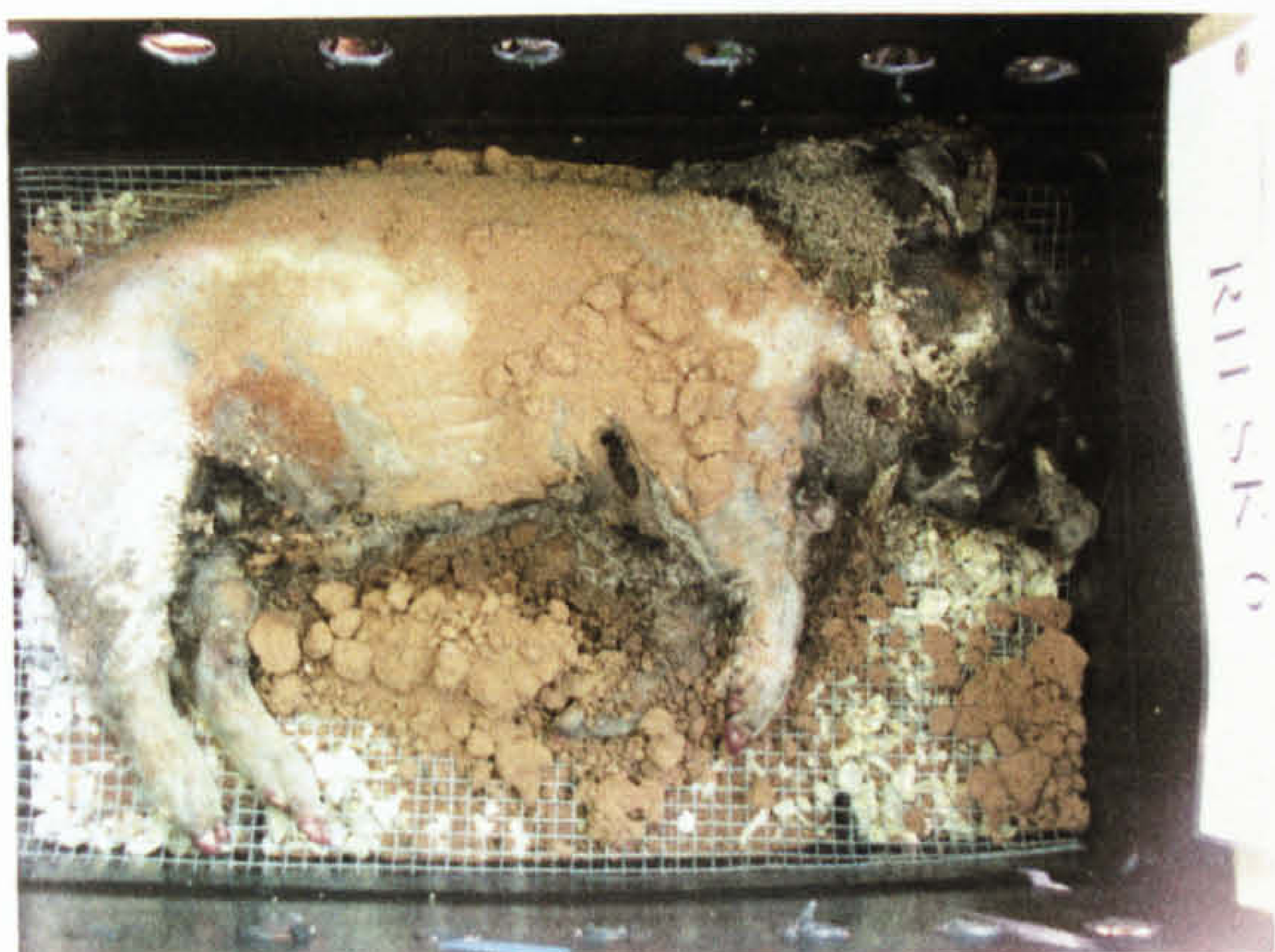

Fig 8.21 Pig 6: Ten Days PM

Fig 8.22 Pig 6: Eleven Days PM

All of the pigs numbered 7-12 were considered to be stillborn and all went out at Riseholme together on the 30th May 06. The length of time taken to skeletonize was very similar in all cases and ranged from 14-20 days. Bloating was not observed in four of the carcasses as would be expected if the animals had a sterile intestinal tract. The two that did bloat may perhaps have been born alive only to die within the first hours/days after birth and subsequent to contamination by exogenous bacteria. Carcasses 7 & 8 (figs 8.23-8.26) were not given a covering, whilst 9 & 10 (figs 8.27-8.30) had a layer of soil and 11 & 12 (figs 8.31-8.34) had sand added to the boxes. By November of 2006 pigs 7 & 8 remained mummified, 9, 11 & 12 became waterlogged and pig 10 was skeletonized in a dry state.

Fig 8.23 Pig 7 Bloated one week PM
(All 4 pictures, Author, 2006)

Fig 8.24 Pig 7 Three weeks PM Mummifying

Fig 8.25 Pig 8: One week PM Bloated

Fig 8.26 Pig 8: Two weeks PM Mummifying

Fig 8.27 Pig 9: At Deposition 30.05.06

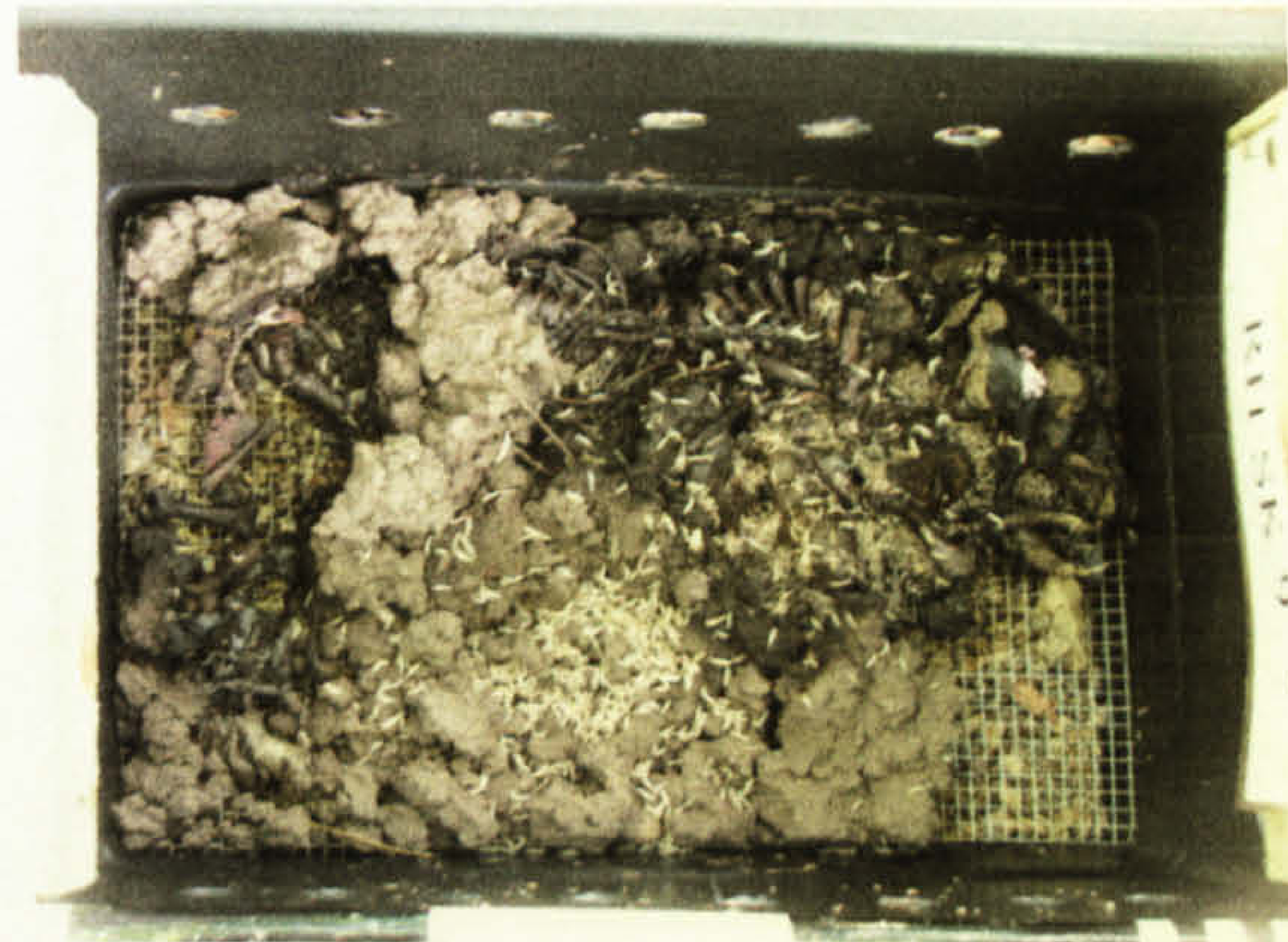

Fig 8.28 Pig 9: Two weeks PM, Skeletonizing

Fig 8.29 Pig 10: At Deposition
(All 6 pictures, Author, 2006)

Fig 8.30 Pig 10: Two Weeks PM Skeletonizing

Fig 8.31 Pig 11: Five days PM

Fig 8.32 Pig 11: Three Weeks PM Skeletonized

Fig 8.33 Pig 12 Five days PM

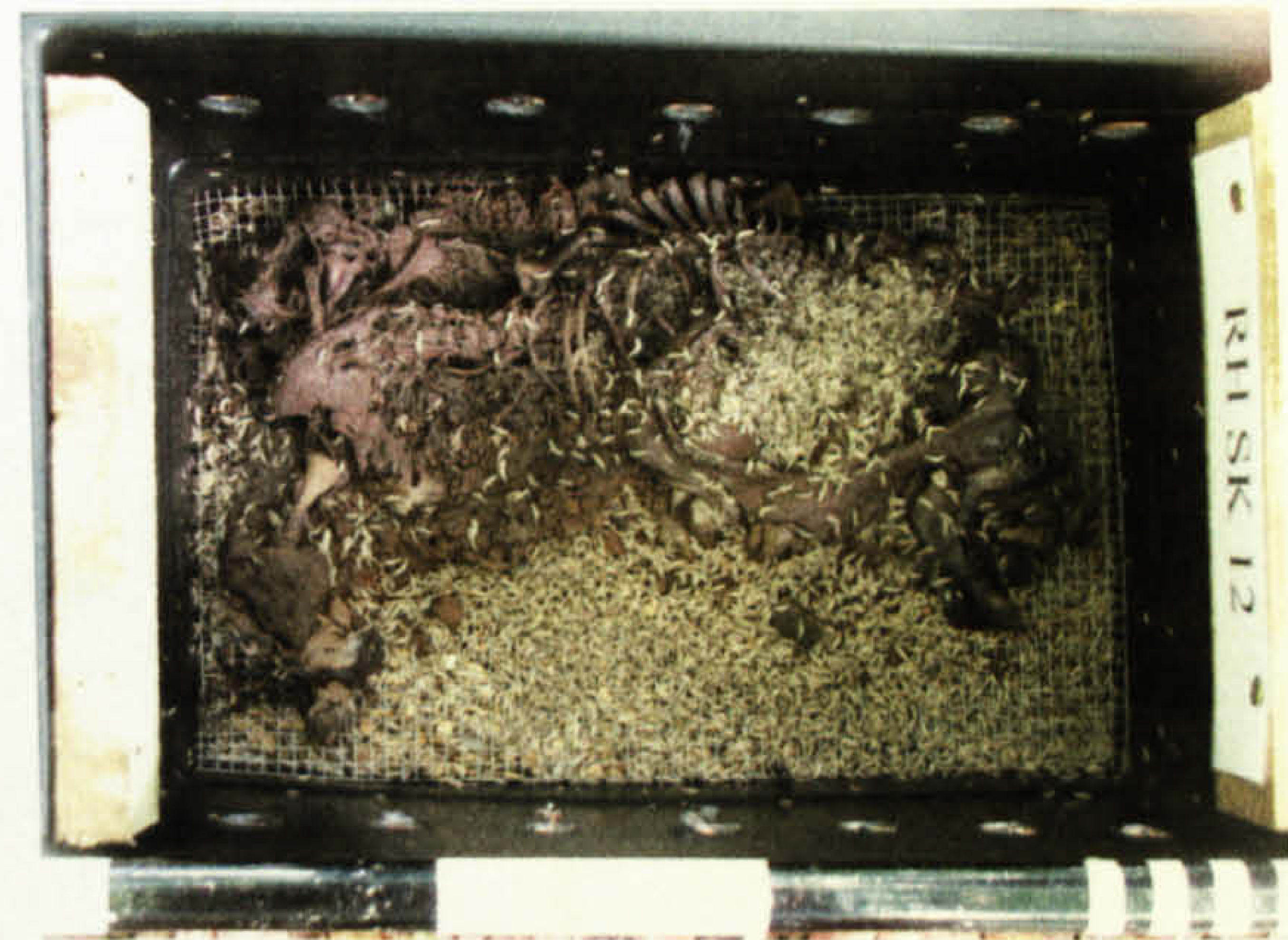

Fig 8.34 Two Weeks PM Almost skeletonized

(All 4 pictures, Author, 2006)

	PIG No 1 (newborn) no cover				PIG No 2 (newborn) no cover				PIG No 3 (newborn) soil				PIG No 4 (newborn) sand			
	1 wks	2 wks	3 wks	4 wks	1 wks	2 wks	3 wks	4 wks	2 wks	4 wks	6 wks	8 wks	2 wks	4 wks	6 wks	8 wks
Fresh (A)																
1. No discolouration or insect activity																
2. Fresh burned																
3. Fresh, no discolouration, fly eggs present																
Early Decomposition (B)																
1. Pink-white appearance, with skin slippage and some hair loss																
2. Bloating without discolouration																
3. Grey-green discolouration without bloating, some flesh relatively fresh																
4. Bloating with green discolouration																
5. Appearance of 1st instar larvae																
6. Grey/green/black discolouration to most of body																
7. Post-bloating following rupture of abdomen with discolouration darkening																
8. Brown/black discolouration of arms and legs, skin having leathery appearance																
Advanced Decomposition (C)																
1. Decomposition of the tissues, producing sagging of the flesh, caving in of the abdominal cavity, often accompanied by extensive maggot activity																
2. Moist decomposition in which there is bone exposure																
3. Mummification with some retention of internal structures																
4. Mummification of outer tissues only with internal organs lost through autolysis or insect activity/or with internal organs still visible																
5. Mummification with bone exposure of less than one half the skeleton																
6. Adipocere development																
Skeletonization (D)																
1. Bones with greasy substances and decomposed tissue, sometimes with body fluids still present																
2. Bones with desiccated/mummified tissue covering less than one half the skeleton																
3. Bones largely dry but still retaining some grease																
4. Dry bone																
Extreme decomposition (E)																
1. Skeletonization with bleaching																
2. Skeletonization with exfoliation																
3. Skeletonization with metaphyseal loss of long bones and cancellous exposure of the vertebrae																
	Mummified				Mummified				Mummified				Waterlogged			

Table 8 Decomposition Data Pigs 1-4 (Boxed)

Stage	PIG No 5 (newborn) soil				PIG No 6 (newborn) sand				PIG No 7 (fetal) no cover				PIG No 8 (fetal) no cover			
	1 wks	2 wks	3 wks	4 wks	1 wks	2 wks	3 wks	4 wks	1 wks	2 wks	3 wks	4 wks	1 wks	2 wks	3 wks	4 wks
Fresh (A)																
1. No discolouration or insect activity																
2. Fresh burned																
3. Fresh, no discolouration, fly eggs present																
Early Decomposition (B)																
1. Pink-white appearance, with skin slippage and some hair loss																
2. Bloating without discolouration																
3. Grey-green discolouration without bloating, some flesh relatively fresh																
4. Bloating with green discolouration																
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4. Mummification of outer tissues only with internal organs lost through autolysis or insect activity/or with internal organs still visible																
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4. Dry bone																
Extreme decomposition (E)																
1. Skeletonization with bleaching																
2. Skeletonization with exfoliation																
3. Skeletonization with metaphyseal loss of long bones and cancellous exposure of the vertebrae																
	Waterlogged				Mummified				Mummified				Mummified			

Table 8.1 Decomposition Data Pigs 5-8 (Boxed)

	PIG No 9 (fetal) soil				PIG No 10 (fetal) soil				PIG No 11 (fetal) sand				PIG No 12 9fetal) sand			
	1 wks	2 wks	3 wks	4 wks	1 wks	2 wks	3 wks	4 wks	1 wks	2 wks	3 wks	4 wks	1 wks	2 wks	3 wks	4 wks
Fresh (A)																
1. No discolouration or insect activity																
2. Fresh burned																
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1. Pink-white appearance, with skin slippage and some hair loss																
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5. Appearance of 1st instar larvae																
6. Grey/green/black discolouration to most of body																
7. Post-bloating following rupture of abdomen with discolouration darkening																
8. Brown/black discolouration of arms and legs, skin having leathery appearance																
Advanced Decomposition (C)																
1. Decomposition of the tissues, producing sagging of the flesh, caving in of the abdominal cavity, often accompanied by extensive maggot activity																
2. Moist decomposition in which there is bone exposure																
3. Mummification with some retention of internal structures																
4. Mummification of outer tissues only with internal organs lost through autolysis or insect activity/or with internal organs still visible																
5. Mummification with bone exposure of less than one half the skeleton																
6. Adipocere development																
Skeletonization (D)																
1. Bones with greasy substances and decomposed tissue, sometimes with body fluids still present																
2. Bones with desiccated/mummified tissue covering less than one half the skeleton																
3. Bones largely dry but still retaining some grease																
4. Dry bone																
Extreme decomposition (E)																
1. Skeletonization with bleaching																
2. Skeletonization with exfoliation																
3. Skeletonization with metaphyseal loss of long bones and cancellous exposure of the vertebrae																
	Waterlogged				Skeletonized Dry				Waterlogged				Waterlogged			

Table 8.2 Decomposition Data Pigs 9-12 (Boxed)

8.3 Results Soft Tissue Decomposition Real Burial Pigs.

It was only possible to monitor soft tissue decomposition in those pigs that were deposited on the ground surface. The buried pig carcasses were not disturbed until the point of examination at one year postmortem.

In 2007, unusually high amounts of rainfall (fig 8.35) were recorded. In the months of May, June and July 482.4mm of rain fell. This compares to 180.5 in 2006 and 208.9 in 2008, although most of the difference is accounted for in June and July. Average maximum daily temperatures (fig 8.36) were lower during the months of May, June and July of 2007 when compared to the same months in both 2006 and 2008. This meant that over several of the most pertinent periods of decomposition the site was often waterlogged and decomposition was slowed. This also had a detrimental effect on the decomposition of the buried cadavers.

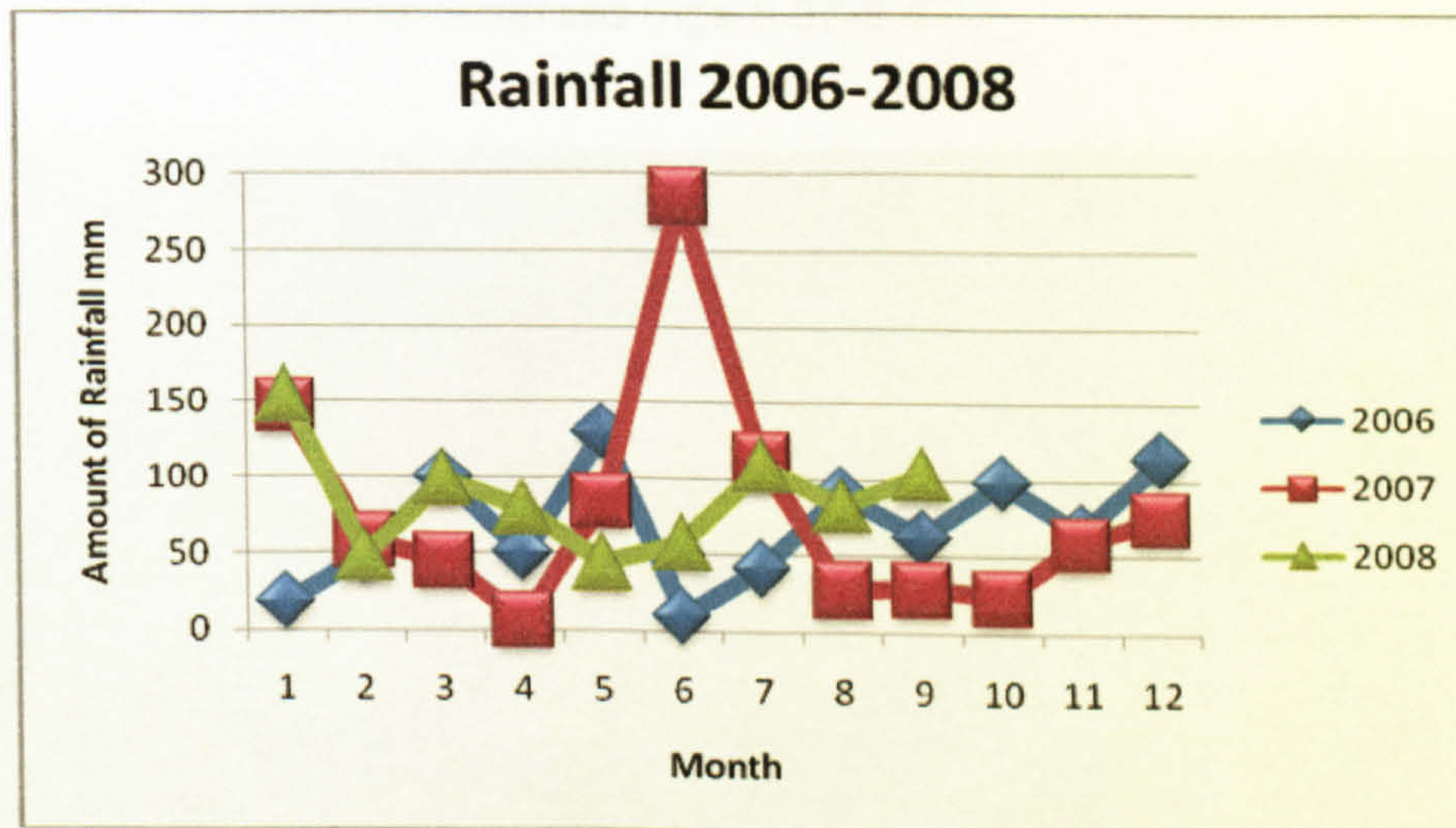

Fig 8.35 Average monthly rainfall for the three years 2006-08 inclusive

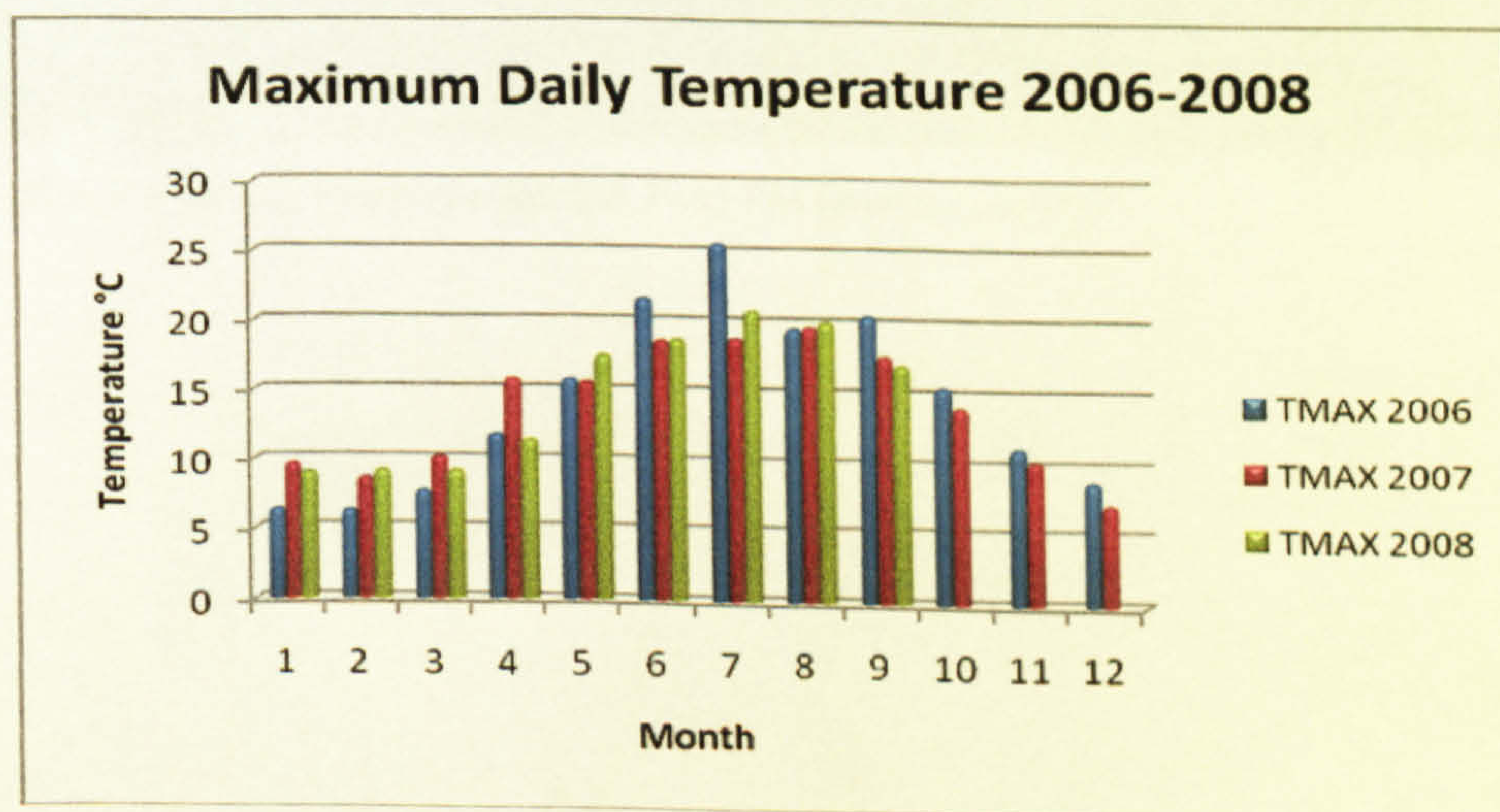

Fig 8.36 Monthly temperature maximums 2006-08 inclusive

8.3.1 Pigs 15-21 (surface depositions)

With the exception of pigs no 20 (fetal) and 15 (50kg) all of the carcasses followed a similar path of decomposition. Pig No's 16-19 inclusive decayed quite rapidly in the first 2-4 wks. There was then a slowing of the decompositional rate coinciding with extremely wet weather. Weeks 6-14 eventually led to the skeletonization of the remains. Over this time period the skin became repeatedly waterlogged, impeding the decay process. Without the extreme rainfall the pigs would probably have achieved skeletonization by around weeks 6-8. The fetal pig (no 21) achieved skeletonization by around week 8 and decomposition advanced much quicker in the early postmortem period. The largest of the pigs (no 15/50kg) was slowest to decompose retaining much of its mass for the first 6-7wks. Again there was a slowing between weeks 6-11 and skeletonization was eventually reached in week 14. A selection of photographs has been provided that show some of the different decomposition phenomena observed (figs 8.37-8.47).

Fig 8.37 50kg pig during extensive rainfall 7wks PM (Author, 2007)

Fig 8.38 50kg pig. Beetle larvae only observed on this pig and only during the very wet period seven weeks PM

Fig 8.39 50kg pig. Skin hangs around the cranial area after rain. Seven weeks PM (Both pictures Author, 2007)

Fig 8.40 Foetal pig two weeks PM

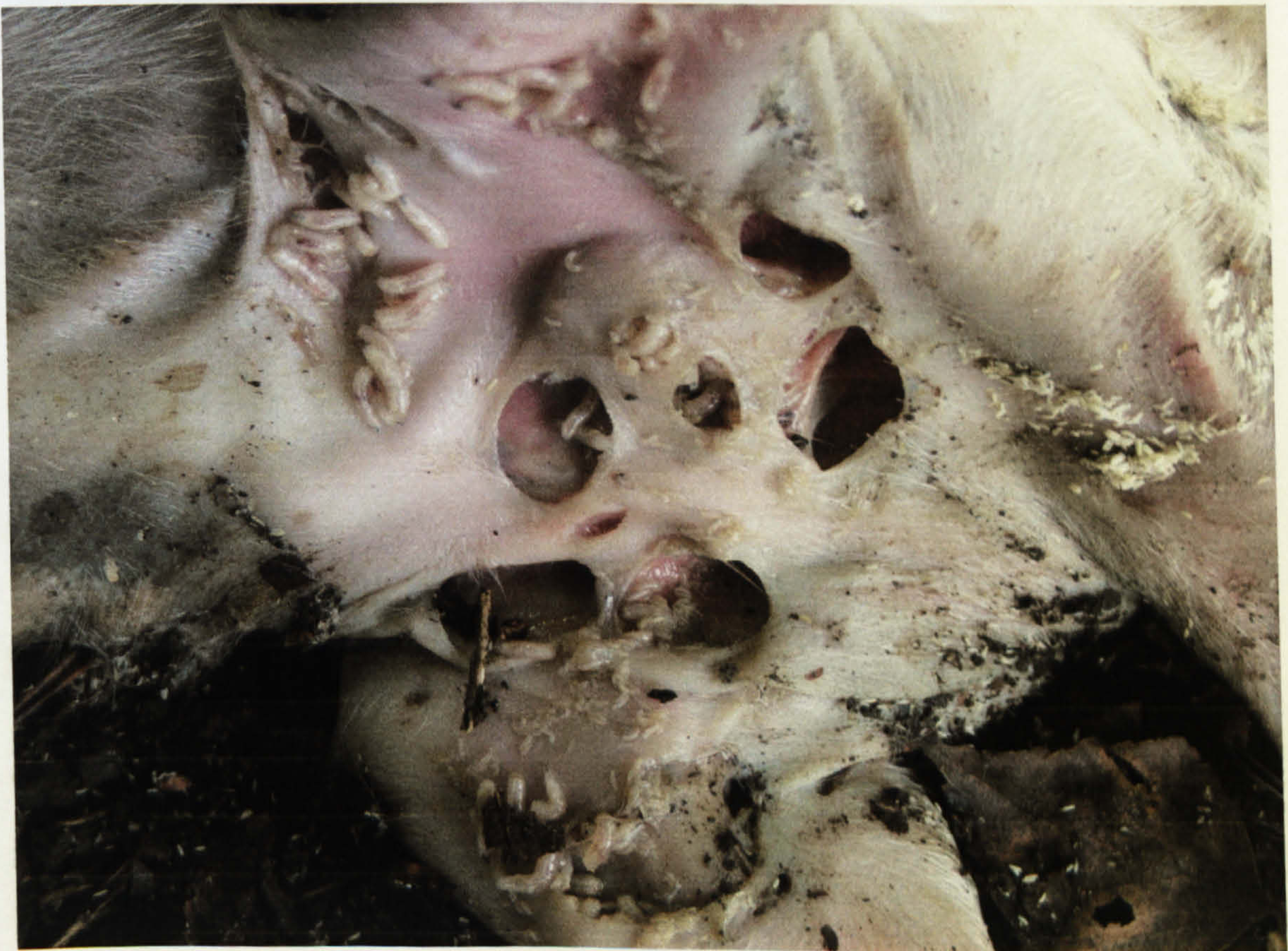

Fig 8.41 Foetal pig (2wks PM) between front legs (Both pictures Author, 2007)

Fig 8.42 Foetal pig four weeks PM

Fig 8.43 Foetal pig six weeks PM

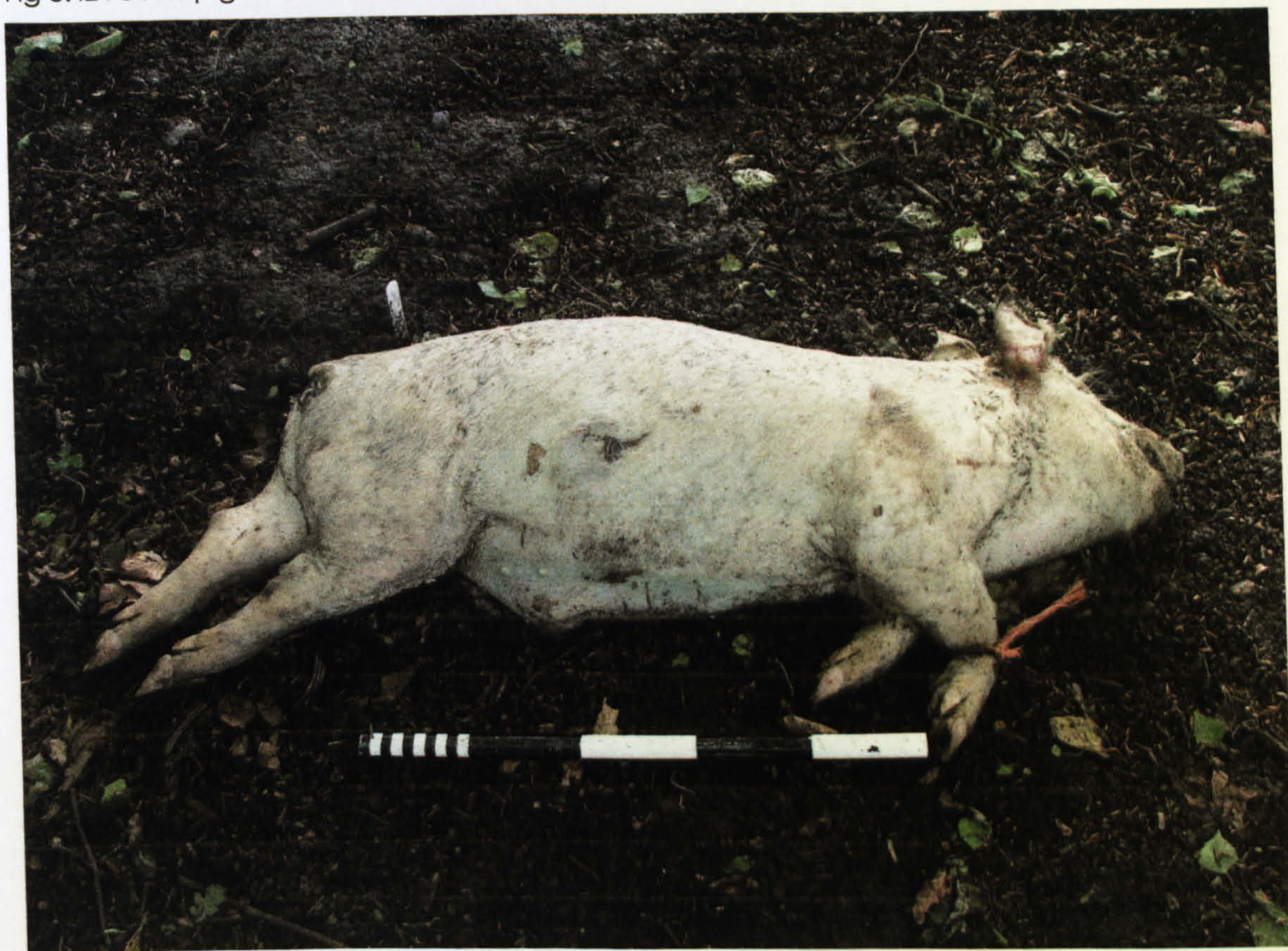

Fig 8.44 Pig 16 (25kg) Very little outward decomposition but many maggots are within the carcass two weeks PM
(All three pictures Author, 2007)

Fig 8.45 Pig 16 Little remains of the pig except for skin and bone. Maggots can be seen migrating backwards towards the bigger 50kg pig which is still fleshed. Four weeks PM

Fig 8.46 Pig 16 Six weeks PM mummified skin attached to the skull
(Both pictures Author, 2007)

Fig 8.47 Pig 16 Eight weeks PM Bleaching of the skeleton (Author, 2007)

Tables 8.3 & 8.4 recording the first ten weeks are provided below that present a concise form of the early postmortem decomposition. All of the surface depositions were skeletonized before the first samples were taken for sectioning at 6 months. They were sampled again at 12mths postmortem.

8.3.2 Buried Pigs 21-30

Samples were taken from all of the buried pigs at one year postmortem. At this time the carcasses had not skeletonized and large amounts of soft tissue were still present. This is probably due to the fact that such high amounts of rain fell over the early decomposition period. There was also adipocere formation and a black sludge type of putrefaction with a really quite offensive odour. It was impossible to photographically evidence the putrefaction as the heavy wet soil was firmly adhered to the remains (fig8.48). Maggot activity was also noted even though the burials were 30-40cm in depth. Samples were also taken from the mass grave that contained four pigs. Two bones were taken from two separate pigs.

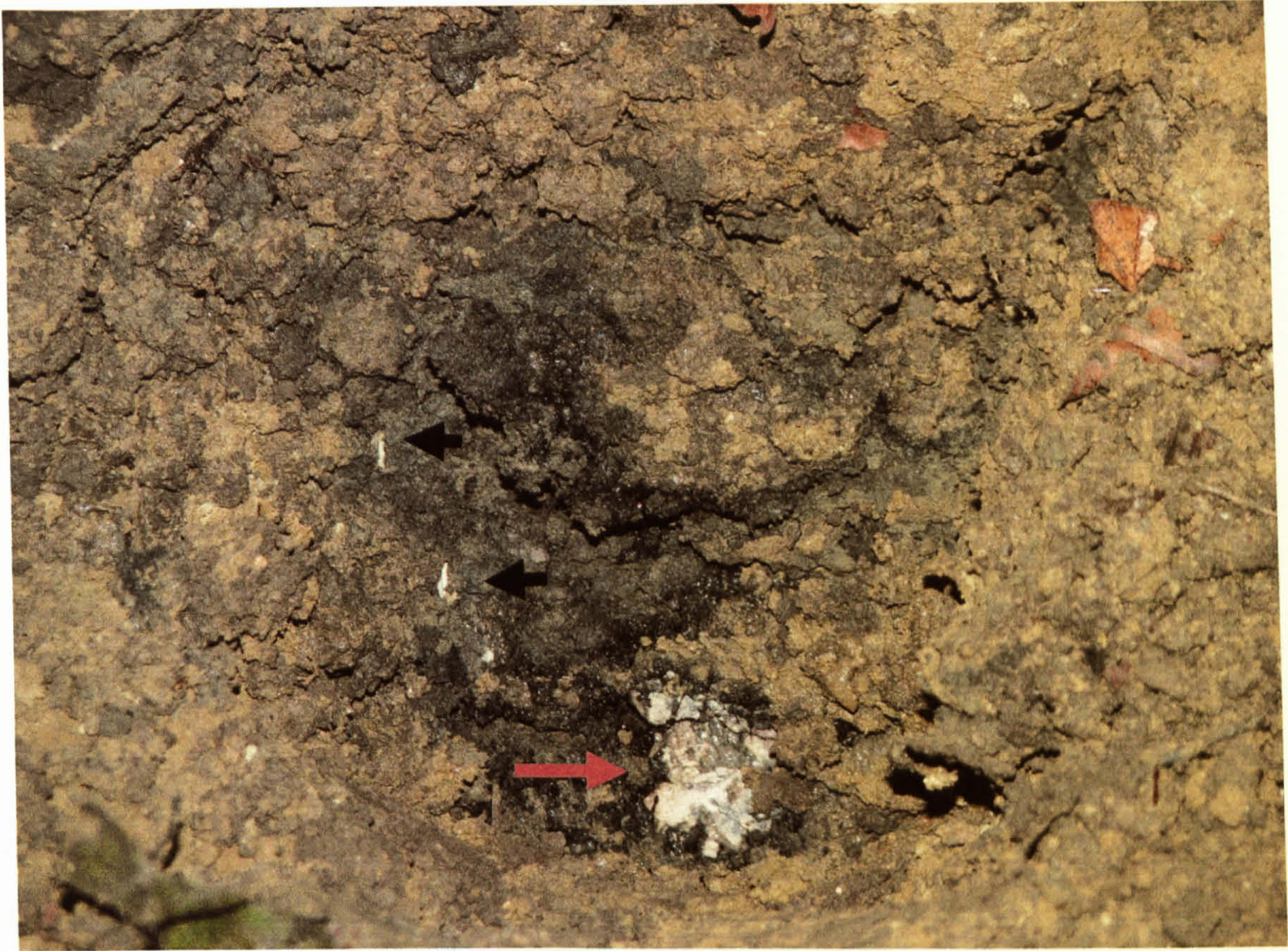

Fig 8.48 Buried pig One year Post-burial. Black arrows denote maggots, whilst the red arrow shows an area of adipocere formation. (Author, 2008)

	PIG No 20 (1-2kg)							PIG No 19 (3-4kg)							PIG No 18 (10kg)						
	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks	14 wks	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks	14 wks	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks	14 wks
Fresh (A)																					
4. No discolouration or insect activity																					
5. Fresh burned																					
6. Fresh, no discolouration, fly eggs present																					
Early Decomposition (B)																					
9. Pink-white appearance, with skin slippage and some hair loss																					
10. Bloating without discolouration																					
11. Grey-green discolouration without bloating, some flesh relatively fresh																					
12. Bloating with green discolouration																					
13. Appearance of 1st instar larvae																					
14. Grey/green/black discolouration to most of body																					
15. Post-bloating following rupture of abdomen with discolouration darkening																					
16. Brown/black discolouration of arms and legs, skin having leathery appearance																					
Advanced Decomposition (C)																					
7. Decomposition of the tissues, producing sagging of the flesh, caving in of the abdominal cavity, often accompanied by extensive maggot activity																					
8. Moist decomposition in which there is bone exposure																					
9. Mummification with some retention of internal structures																					
10. Mummification of outer tissues only with internal organs lost through autolysis or insect activity/or with internal organs still visible																					
11. Mummification with bone exposure of less than one half the skeleton																					
12. Adipocere development																					
Skeletonization (D)																					
5. Bones with greasy substances and decomposed tissue, sometimes with body fluids still present																					
6. Bones with desiccated/mummified tissue covering less than one half the skeleton																					
7. Bones largely dry but still retaining some grease																					
8. Dry bone																					
Extreme decomposition (E)																					
4. Skeletonization with bleaching																					
5. Skeletonization with exfoliation																					
6. Skeletonization with metaphyseal loss of long bones and cancellous exposure of the vertebrae																					

Table 8.3 Decomposition Data Pigs 18-20 (Surface)

	PIG No 17 (15kg)							PIG No 16 (25kg)							PIG No 15 (50kg)						
	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks	14 wks	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks	14 wks	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks	14 wks
Fresh (A)																					
7. No discolouration or insect activity																					
8. Fresh burned																					
9. Fresh, no discolouration, fly eggs present																					
Early Decomposition (B)																					
17. Pink-white appearance, with skin slippage and some hair loss																					
18. Bloating without discolouration																					
19. Grey-green discolouration without bloating, some flesh relatively fresh																					
20. Bloating with green discolouration																					
21. Appearance of 1st instar larvae																					
22. Grey/green/black discolouration to most of body																					
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24. Brown/black discolouration of arms and legs, skin having leathery appearance																					
Advanced Decomposition (C)																					
13. Decomposition of the tissues, producing sagging of the flesh, caving in of the abdominal cavity, often accompanied by extensive maggot activity																					
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10. Bones with desiccated/mummified tissue covering less than one half the skeleton																					
11. Bones largely dry but still retaining some grease																					
12. Dry bone																					
Extreme decomposition (E)																					
7. Skeletonization with bleaching																					
8. Skeletonization with exfoliation																					
9. Skeletonization with metaphyseal loss of long bones and cancellous exposure of the vertebrae																					

Table 8.4 Decomposition Data Pigs 15-17 (Surface)

8.4 Note on Description of Histological Preservation

Previous studies have classified micro focal destruction (MFD) in to four separate categories on the basis of their appearance in microscopic thin section and as previously discussed these are called Wedl (fungal), linear longitudinal, lamellate and budded (bacterial). Hackett (1981) who originally described the typical lesions believed that there were probably more than 4 types and even in his own work does not classify lesions to type. During this research there have been times when destruction has taken place in bone that cannot be categorised using the previous terms. In some instances the bone may be very degraded by bacteria but discrete tunnels cannot be seen due to the severity of the damage. In these cases the terms 'bacterial attack' or 'generalised attack' have been used. If the bone is damaged but bacterial attack does not seem to be responsible then the term 'generalised destruction' has been used. Further to this, in some of the archaeological sections and in the pigs there is destruction in the form of black dots (that resemble budded tunnelling) that coincides with collagen loss that can be seen optically as a loss of birefringence under polarized light. As this is seen in sections where clear tunnelling also occurs it appears to be the origin of tunnelling and has been termed early stage tunnelling.

8.5 Results of Bacteria Based Experiments (Boxed Pigs)

The first bone sections were taken approximately one month post-mortem and after skeletonization of most of the carcasses had occurred (29th June 2006). In four cases (pigs no's 1-4) most of the soft tissue had mummified although the internal organs were by this point completely corrupt leaving remains that consisted of very tough skin and the skeleton. A cross section of the bones was taken for thin sectioning (Pigs no's 1, 2, 3, 5, 7 & 8) and two transverse cuts were taken from each bone. The second samples were taken on the 11th November 2006 (6 months post-mortem) and by this time the majority of the pigs had almost completely skeletonized apart from mummified tissue that was still evident in a few of the burials. The first four pigs that went out were still bone that was almost completely covered by mummified soft tissue. The skin of pig No 1 was almost entirely covered with what appeared to be a white coloured mould and further traces of this substance was observed on pigs no's 2 & 4. The box containing pig No3 had somehow become waterlogged and the skin had lost the associated hair seen previously and the skin had the appearance of a bog body. It was decided not to take samples from waterlogged specimens. As the experiments are in duplicate

only six bones were removed for sectioning (one from each type of experiment). Further samples were taken at 12 months PM (no's 5, 6, 8, 10 & 12), 18 months (no's 2, 7 & 10) and 24 months (all of the pigs). At this time the pigs were mainly either skeletal with retained mummified tissue or waterlogged.

8.5.1 Bacterial Assays

From the 1st samples two of the sections were taken for experimental staining, the 1st (pig 1) was subjected to gram staining (taken to the University of Lincoln, where they have the necessary materials) whilst the other (pig 7) was stained with Methylene Blue. Large colonies of bacteria could clearly be seen within both sections. Neither had any covering (i.e. sand or soil) but no7 was considered to be stillborn. This means that either the pig was older than previously thought or that there was environmental contamination. It was considered that this was true colonisation rather than surface contamination due to the fact that the bacteria could be seen throughout the depth of the section. In pig No1 that was used for gram staining and both gram positive and gram negative bacteria were clearly visible. In the second samples (pigs 2, 5, 6, 7, 9 & 12) all of the bones sectioned were subjected to simple staining with Methylene Blue and bacterial colonies were visualized in 4 of the sections (table 8.5). Bacteria were evident in pigs 2, 5, 6 & 7, the 1st three being newborns and no7 the stillborn. Of these 2 had no covering, 1 had a covering of soil and 1 was covered by sand. The sections from no's 9 & 12 which had coverings of soil and sand respectively and were considered stillborn did not have visible bacterial colonies. If tunnels are present these will also take up the stain but none were apparent for the sections taken at one month or for those taken at 6 months.

Date	Pig No	Age	Covering	Section	Staining Method Applied	Bacteria	Tunnels
29.06.06	SK1	Newborn	No	T	GS	Yes	No
29.06.06	SK2	Newborn	No	T	N/A		No
29.06.06	SK3	Newborn	Soil	T	N/A		No
29.06.06	SK5	Newborn	Soil	T	N/A		No
29.06.06	SK7	Fetal	No	T	MB	Yes	No
29.06.06	SK8	Fetal	No	T	N/A		No
11.11.06	SK2	Newborn	No	T/L	MB	Yes	No
11.11.06	SK5	Newborn	Soil	T/L	MB	Yes	No
11.11.06	SK6	Newborn	Sand	T	MB	Yes	No
11.11.06	SK7	Fetal	No	T	MB	Yes	No
11.11.06	SK9	Fetal	Soil	T/L	MB	No	No
11.11.06	SK12	Fetal	Sand	T	MB	No	No

Table 8.5 Results of staining thin sections (boxed) Key: Section, T = Transverse, L = Longitudinal, Staining method, GS= Gram Stain, MB = Methylene Blue

8.5.2 Anaerobic Jar Results

Pig No	Destruction	Collagen Loss	Tunnels
1	No Destruction	Small amount of collagen loss	No
2	No Destruction	Some collagen loss	No
3	No Destruction	Significant collagen loss	No
5	No Destruction	Some collagen loss	No

Table 8.6 Results of collagen destruction via Anaerobic jar

Of the bones placed in the anaerobic jar (table 8.6) and left for a period of one year no tunnelling was observed in any case. There was however some collagen loss that was visualized as a loss of birefringence under polarized light within the bones, but this could not be attributed directly to bacterial attack as no tunnels were seen.

8.5.3 Results of Histology (Boxed Pigs)

The sections taken at one month and six months post-mortem were examined under normal microscopy and no evidence for bacterial tunnelling could be seen and under polarized light microscopy the collagen content of the bone appeared to be well preserved. However, in the bones that were stained by either Methylene Blue or Gram staining (figs 8.49-8.50) it became clear that the sections were heavily colonized by bacteria at one month postmortem. The pig bones were not sectioned until they had almost skeletonized and so it is highly possible that the bacteria were within the bone at a much earlier time. As previously noted bone is highly vascularised and bacteria are extremely mobile and colonization would ensue very soon after death. Both Gram positive and Gram negative bacteria are present and there are different types of bacteria visible in the form of both rods and cocci. The bacteria present are not contamination from the microtome saw or the bone surface as the colonies are very large and they can be visualised 'through' the section rather than just on the surface.

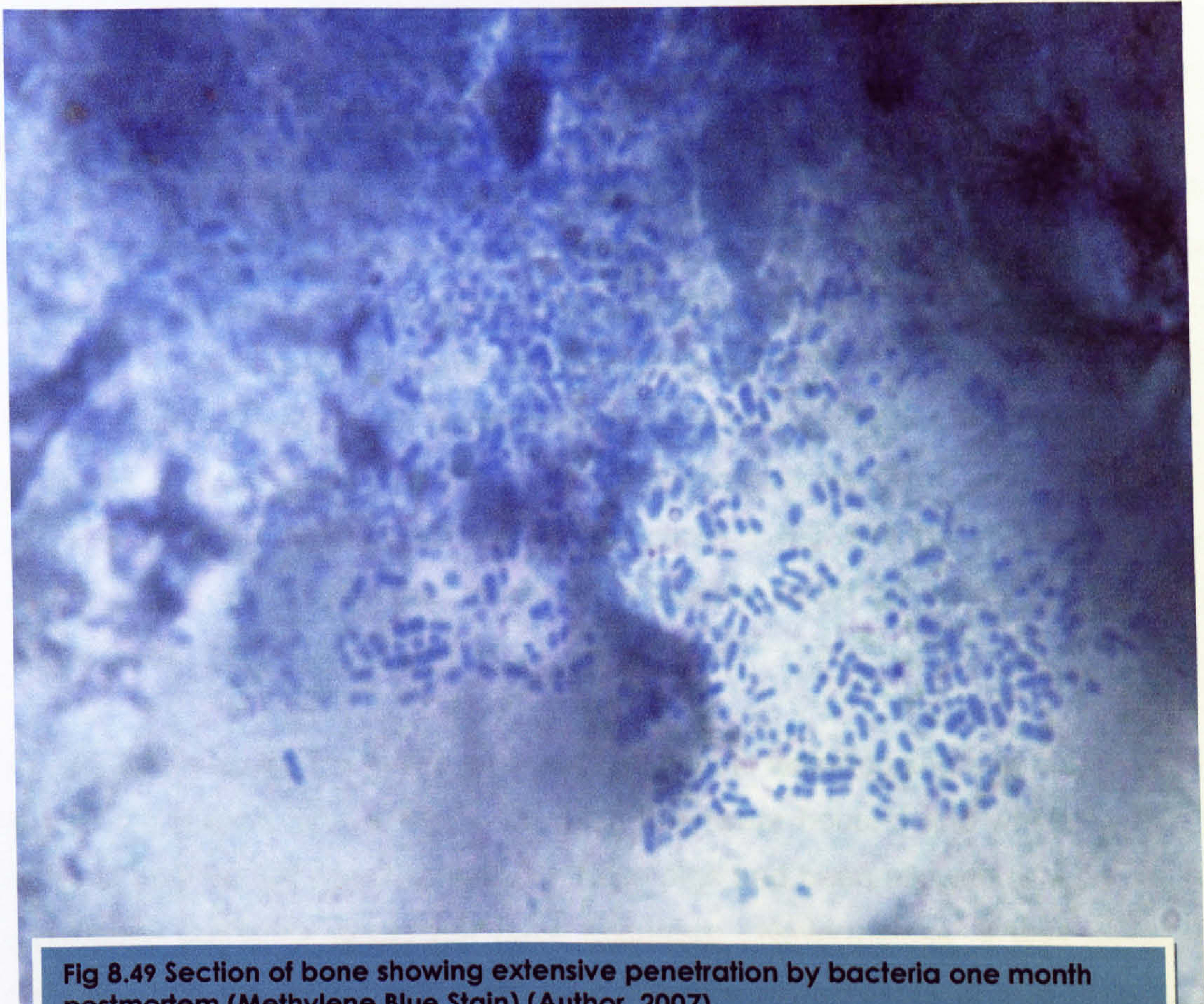


Fig 8.49 Section of bone showing extensive penetration by bacteria one month postmortem (Methylene Blue Stain) (Author, 2007)

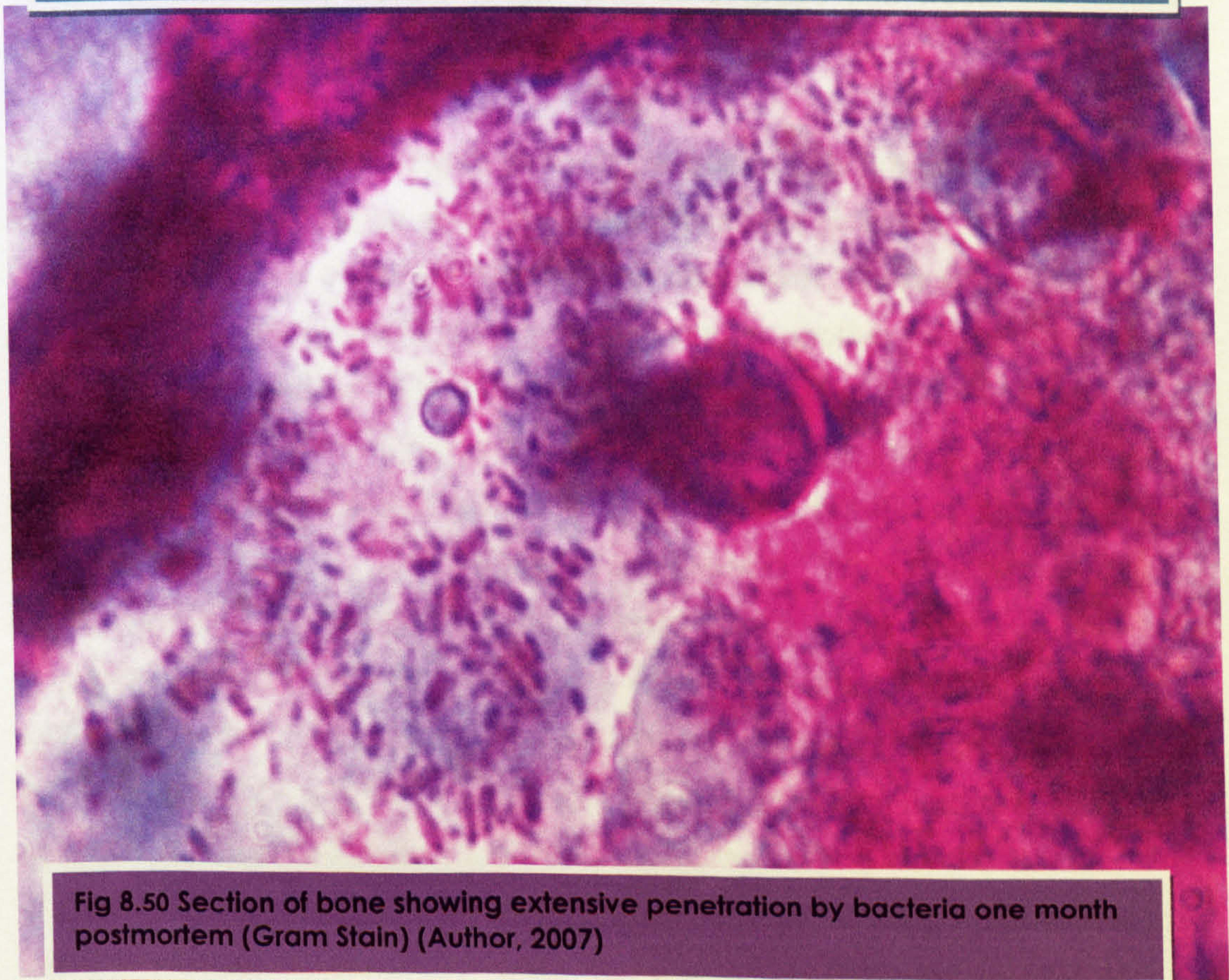


Fig 8.50 Section of bone showing extensive penetration by bacteria one month postmortem (Gram Stain) (Author, 2007)

For their continued survival and possible destruction of the bone collagen a favourable environment would be necessary. Unfortunately due to the nature of the boxes, five of the twelve pigs became waterlogged and a further six suffered some degree of mummification. Neither of these conditions is sympathetic to the type of microbial attack being researched here. It is clear that in cases of extreme dryness bacterial attack will be arrested and in waterlogged conditions MFD most often takes the form of an 'outside-in' mode of destruction caused by cyanobacteria. At the intervals set for sectioning it became necessary to select bones from pigs that may yield results, and not all of the pigs were sectioned at the same time. Because by this time the real burials had been put in place it was felt that this was an easy sacrifice as it was hoped that much better results would be achieved with the 2nd experiments.

As discussed above the boxed pigs were subject to waterlogging and mummification due to the specific environments in which they were housed and which formed the basis of DEFRA set specifications. If these pigs had been allowed real burials then the above problems would not have occurred. Nevertheless, it was decided to continue with the boxed pigs even after the new experiments of true burials were started as they would have a longer time to degrade and would act as 'backup' should anything jeopardise the real burials.

The full results of the histological study of the boxed pigs are presented below (table 8.7). No real histological changes were observed in either the stillborn or the newborn pigs during the initial 18months of deposition. There was some staining to one bone that was probably due to the soil and in another case there was a small amount of pink coloured infiltration that appeared to be of fungal origin as there was a corresponding loss of collagen at that area. The bone that was dark stained also appeared to have suffered from some collagen loss but this is more suggestive of chemical hydrolysis as no tunnelling was observed. When the pigs were sampled for the final time at 2 years postmortem there was a visible difference in histological structure. All twelve pigs were sectioned at this time and all but one had early stage tunnelling visible. In four of the newborns the early tunnelling was extensive and large enough to be defined as pre-tunnelling and in a further case there was evidence of fungal invasion in the form of Wedl tunnelling. Of the four with evidence of pre-tunnelling the type of covering varied with two having a sand covering, one soil and one had no covering at all. None of the six stillborns showed any signs of either pre-tunnelling or tunnelling. Although in one case (pig no 8, highlighted) there

was some loss of collagen. On reflection this pig was probably not stillborn due to two reasons. Firstly, it was rather heavy to be stillborn and secondly after deposition this piglet bloated and there was green staining of the abdomen suggestive of an animal that had lived long enough to have gut bacteria present. Unfortunately, it is impossible to be certain whether any of the designated stillborns died either prior to or at the time of birth as they were simply selected on size, weight and presence of umbilical cord as they were chosen directly from the processing room floor out of a selection of around a ton of animal waste.

All of the above would suggest that any MFD found was a direct consequence of gut microbes both because the stillborns were not affected and in the newborns that were affected, at least one was not subjected to soil bacteria and two were in sand.

The results of the boxed pigs are far from conclusive mainly due to the deficiencies of the study, namely the mummification and waterlogging. Whether or not this had a direct effect is debateable, but in the cases of pre-tunnelling two of the pigs were waterlogged and two mummified. As discussed in a previous chapter it is possible that the bacteria had time to cause damage within the bone before the microclimate became hostile. It is entirely possible therefore that the tunnelling is a true reflection of gut microbes in the newborns which correlates with the lack of tunnelling in the stillborns that should have been sterile and not subject to any MFD arising from this source. The experiments were not a perfect model and the author is fully aware of their limitations.

Postmortem interval	*Pig no*	*New/Still Born*	*Description*	*Collagen Loss*	*Tunnelling*
1 Month	1	Newborn	No Destruction	No	No
	2	Newborn	No Destruction	No	No
	3	Newborn	No Destruction	No	No
	5	Newborn	No Destruction	No	No
	7	Stillborn	No Destruction	No	No
	8	Stillborn	No Destruction	No	No
6 Months	2	Newborn	No Destruction	No	No
	5	Newborn	No Destruction	No	No
	6	Newborn	No Destruction	No	No
	7	Stillborn	No Destruction	No	No
	9	Stillborn	No Destruction	No	No
	12	Stillborn	No Destruction	No	No
12 Months	5	Newborn	No Destruction	No	No
	6	Newborn	No Destruction	No	No
	8	Stillborn	No Destruction	No	No
	10	Stillborn	Very dark staining	Significant	No
	12	Stillborn	No destruction	No	No
18 Months	2	Newborn	Small area of decomp	Minimal	No
	7	Stillborn	Pink area Fungal?	At pink area	No
	10	Stillborn	No Destruction	No	No
24 Months	1	Newborn	Areas of black dots	Minimal	Possible Wedl?
	2	Newborn	Areas of black dots	Minimal	Early stage tunnelling
	3	Newborn	Areas of black dots	Minimal	Early stage tunnelling
	4	Newborn	Few black dots	No	Early stage tunnelling
	5	Newborn	No destruction	No	No
	6	Newborn	Areas of black dots	Minimal	Early stage tunnelling
	7	Stillborn	Very few black dots	No	No
	8	Stillborn	Areas of black dots	Minimal	No
	9	Stillborn	Very few black dots	No	No
	10	Stillborn	Very few black dots	No	No
	11	Stillborn	Very few black dots	No	No
	12	Stillborn	Very few black dots	No	No

Table 8.7 Results of Histology (Boxed pigs)

8.6 Results of Microscopy: Real Burials (2nd Burials)

Sections were taken from the real burials at selective intervals. Bones could be removed with ease from the surface depositions but only with some difficulty from the buried remains. A literature review had suggested that bacterial attack would not commence until after skeletonization and even then would probably not occur until 2-5 years postmortem. It was therefore decided to allow sufficient time to pass for the buried remains to skeletonize. The poor weather conditions linked to the excessive rainfall during the early period of burial suggested that the buried pigs would not be skeletonized within the first year. In addition to this, femurs are the bone of choice for this research due to their proximity to the pelvic area (and therefore the intestinal tract) and because each pig only provides two of these elements it was decided to sample the surface pigs only twice (6mths & 12mths) and the buried pigs only once at 12mths. The surface pigs were all skeletonized by the 1st sampling period (6mths), but the buried pigs were not skeletonized at 12mths.

8.6.1 Surface Depositions Summary

The results of the surface depositions were quite interesting. Research had suggested that the decomposer ability of insect larvae may lessen the chances of bacteria surviving and therefore reducing the chance of bacterial attack in bones from this type of environment. However, at the first sampling six months postmortem, three of the pigs had suffered some degree of bone degradation. In the bones that were affected there was change in the form of black spots within the bone structure with a correlation to collagen loss as evidenced by loss of birefringence when viewed under polarised light.

Tunnelling of bone has previously been recorded and classified from archaeological bone that has had hundreds of years to degrade and therefore does not record how tunnelling begins or how it would appear visually. There must be an initial point at which bone collagen is being used by bacteria but without the requisite archetypal tunnels having developed. Where the black spots have not joined together this has been termed early stage tunnelling and where they have coalesced to form larger entities the term tunnelling has been applied (the latter most closely resembling what is seen in archaeological material). In some archaeological material (examined by the author) this phenomenon has been noted. The Royal Mint sections provided much of this data where the black spots are seen individually, coalescing, and then finally merging to form characteristic tunnels.

Within the 1st sample the foetal pig, and the 25kg pig were both unaffected whilst the 10kg pig had a very few black spots. By the time of the 2nd sampling all of the bones were affected to some degree with the exception of the foetal pig, which remained unique in its lack of tunnelling/pre-tunnelling. This would suggest that there may be a link between intestinal microbes and MFD.

Postmortem Interval	*Pig No*	*Weight (kg)*	*Buried/ Surface*	*Description*	*Collagen Loss*	*Tunnelling*
6 Months	15	50	Surface	Dark band to central section	Minimal	Early stage tunnelling
	16	25	Surface	Well preserved	No	No
	17	15	Surface	Many black dots	Minimal	Early stage tunnelling
	18	10	Surface	Very few black dots	Minimal	No
	19	3-4	Surface	Destruction endosteal	Loss endosteal	Tunnelling
	20	1-2 (Fetal)	Surface	No destruction	No	No
12 Months	15	50	Surface	Black dots	Collagen loss	Early stage tunnelling
	16	25	Surface	Black dots	Collagen loss	Early stage tunnelling
	17	15	Surface	Black dots	Collagen loss	Early stage tunnelling
	18	10	Surface	Black dots	Collagen loss	Tunnelling
	19	3-4	Surface	Very few black dots	Minimal collagen loss	Early stage tunnelling
	20	1-2 (Fetal)	Surface	No Destruction	No collagen loss	No
	21	50	Buried	Many black dots	Collagen loss	Tunnelling
	22	25	Buried	Many black dots	Collagen loss	Tunnelling
	23	15	Buried	Many black dots	Collagen loss	Tunnelling
	24	10	Buried	Many black dots	Minor collagen loss	Early stage tunnelling
	25	3-4	Buried	Many black dots	Minor collagen loss	Early stage tunnelling
	26	1-2	Buried	Perfect preservation	Minor collagen loss	No
(Mass)	27-30	25	Buried	Band of destruction	Minimal	Possible
(Mass)	27-30	25	Buried	Black dots	Minimal	No

Table 8.8 Results of Histology Real Burial Pigs

8.6.2 Buried Pigs Summary

Because the pigs had not skeletonized at exhumation it was expected that no bacterial attack would have taken place if skeletonization was a precondition of MFD. Of the eight bones sampled from the buried remains (6 discrete burials and 2 from the mass grave) all but one (foetal) had suffered collagen loss. Again, tunnelling took the form of black spots and coalescing black spots with a corresponding loss of collagen (figs 8.51-8.70) which manifested in all of the bones except for the foetal pig. This degradation was much more advanced than in the surface depositions with many more spots and larger amounts of bone affected. Because the pigs had not skeletonized it is unlikely that soil bacteria were the perpetrators as a good deal of soft tissue remained on the corpses and adipocere was also present which prevented contact with the soil. It was also mentioned in chapter 4 that some soils have both less bacteria and less collagenolytic strains. This is true of spruce soils which are of the type found at Riseholme where considerably less Collagenase producing microbes would be present. This associated with the lack of destruction in the foetal pig, also points towards an intestinal origin of MFD.

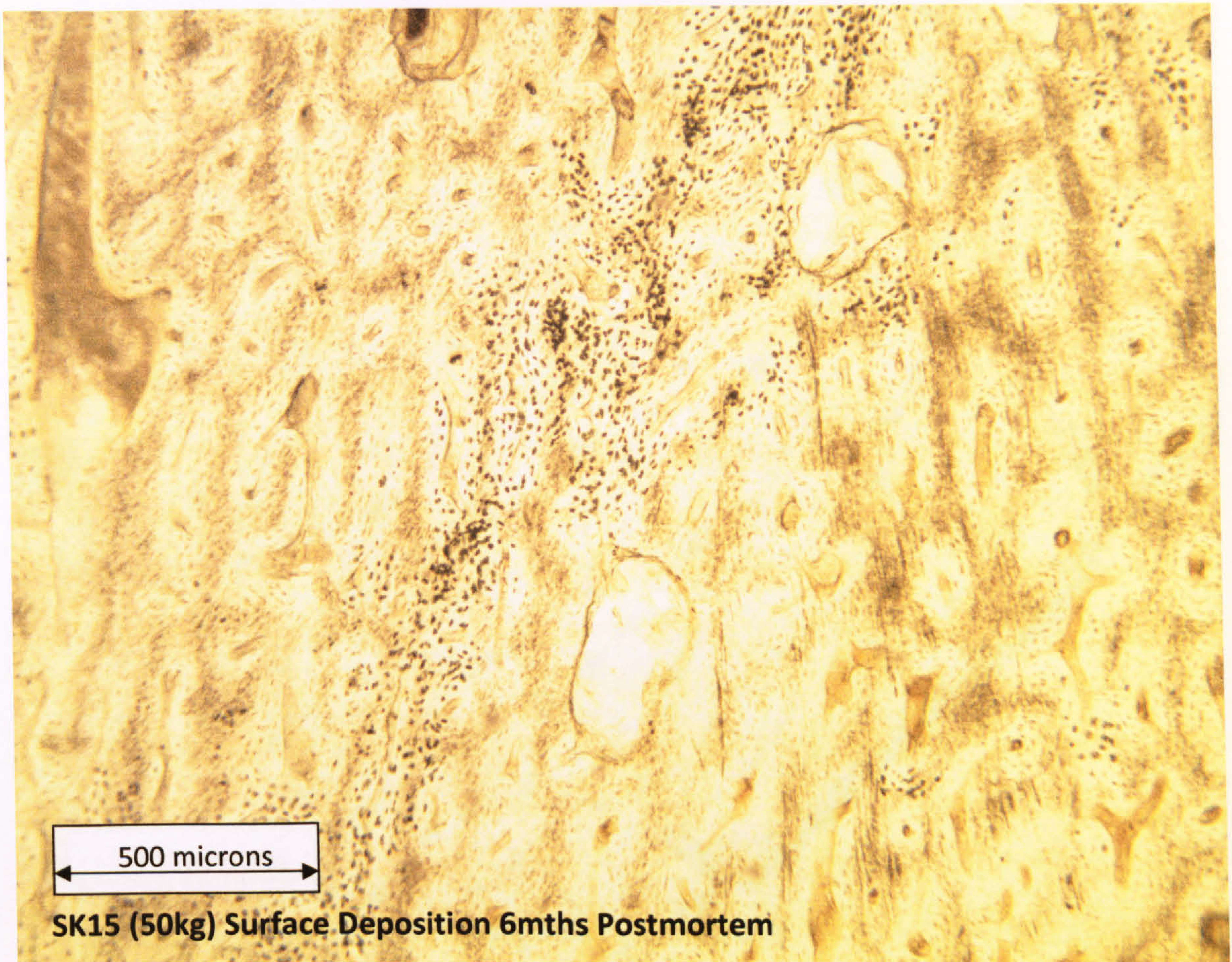


Fig 8.51

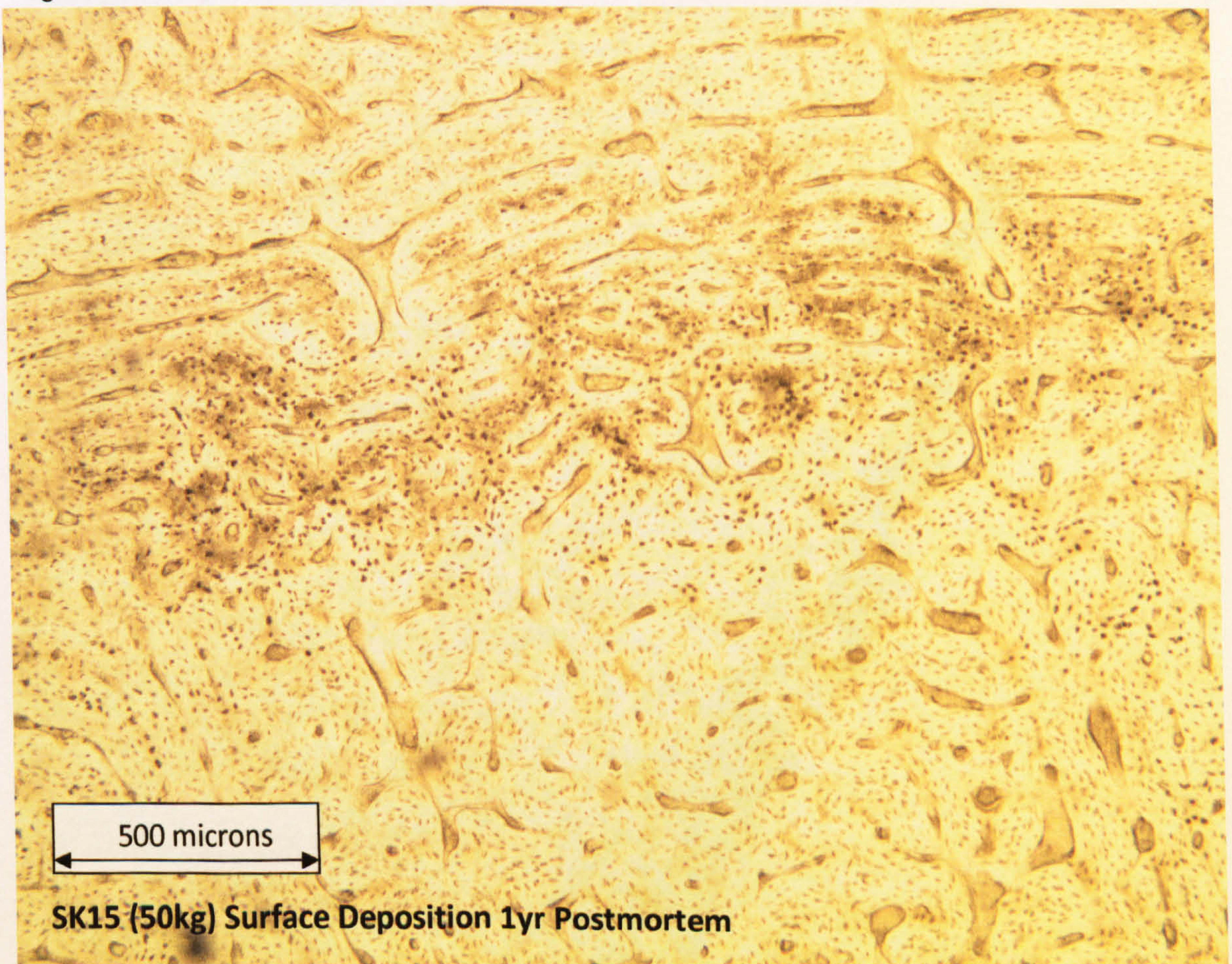


Fig 8.52

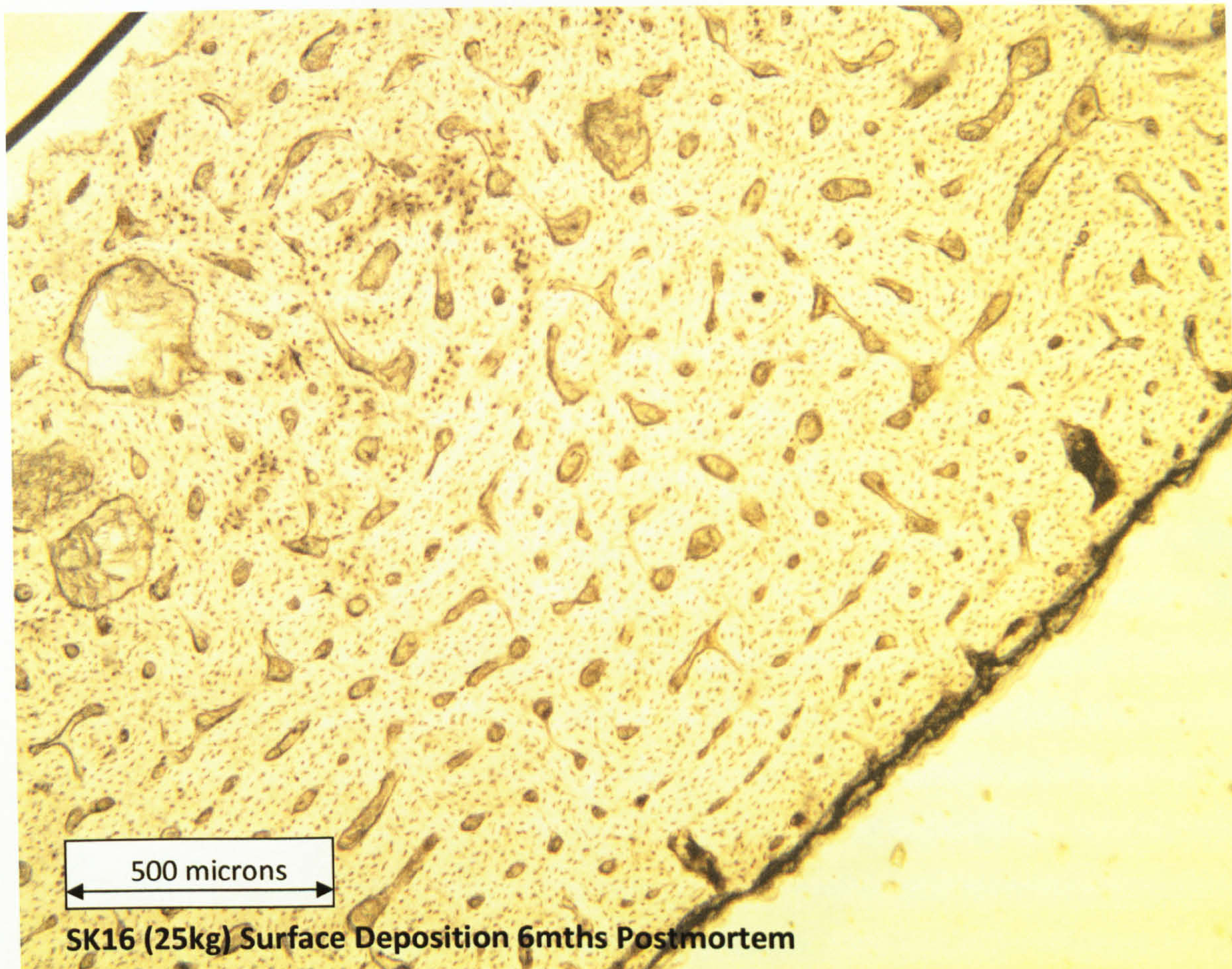
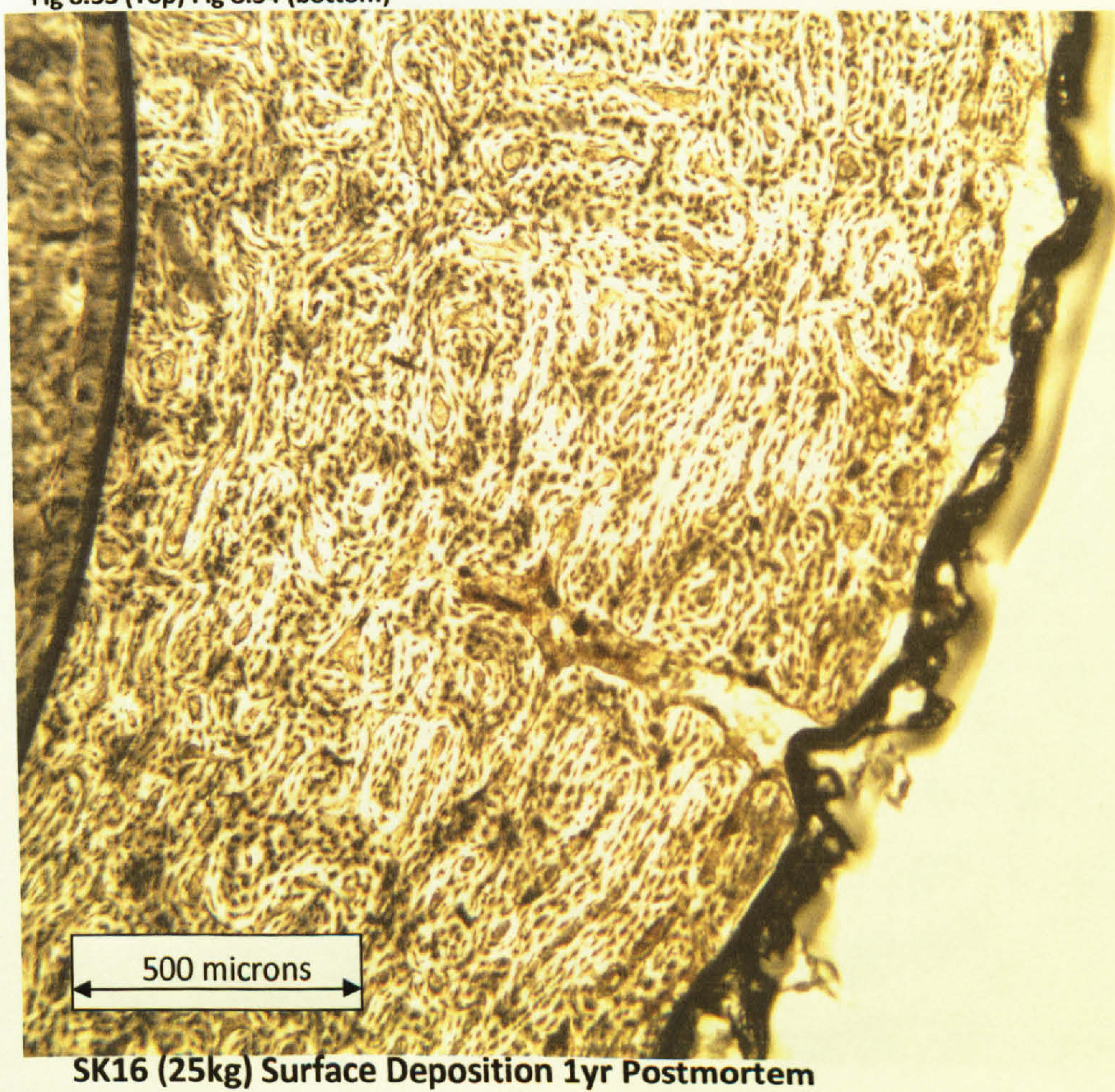
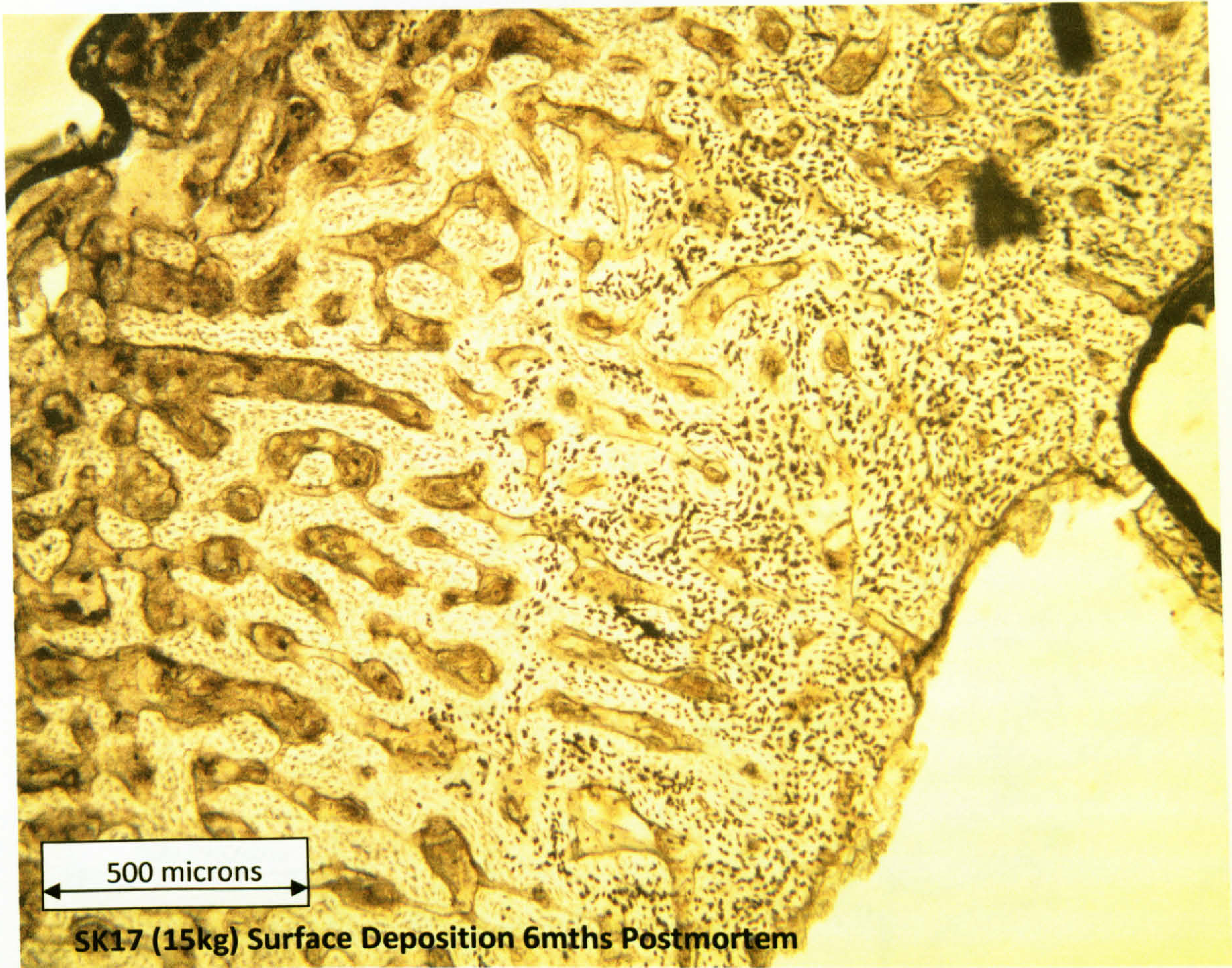


Fig 8.53 (Top) Fig 8.54 (bottom)



SK16 (25kg) Surface Deposition 1yr Postmortem



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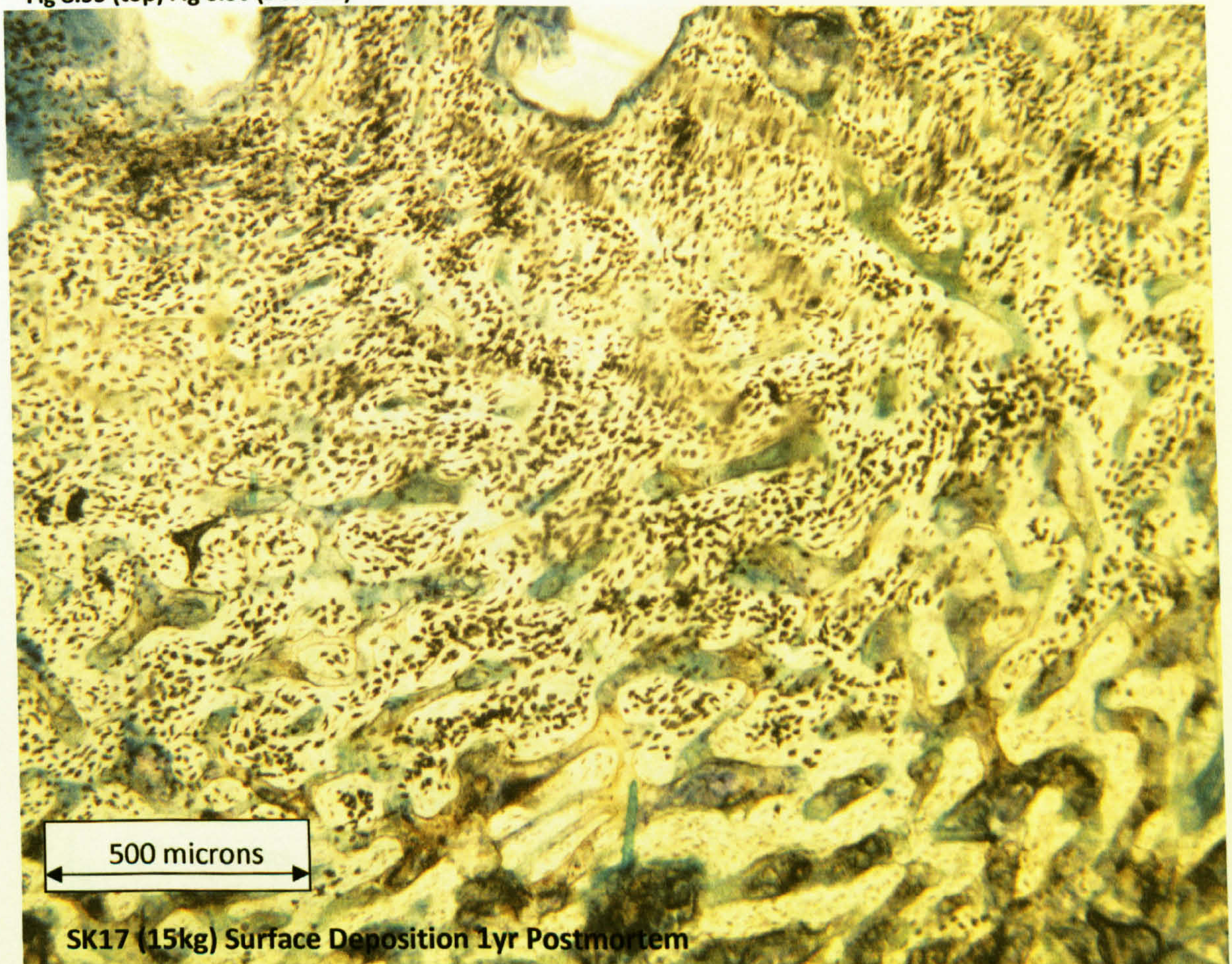


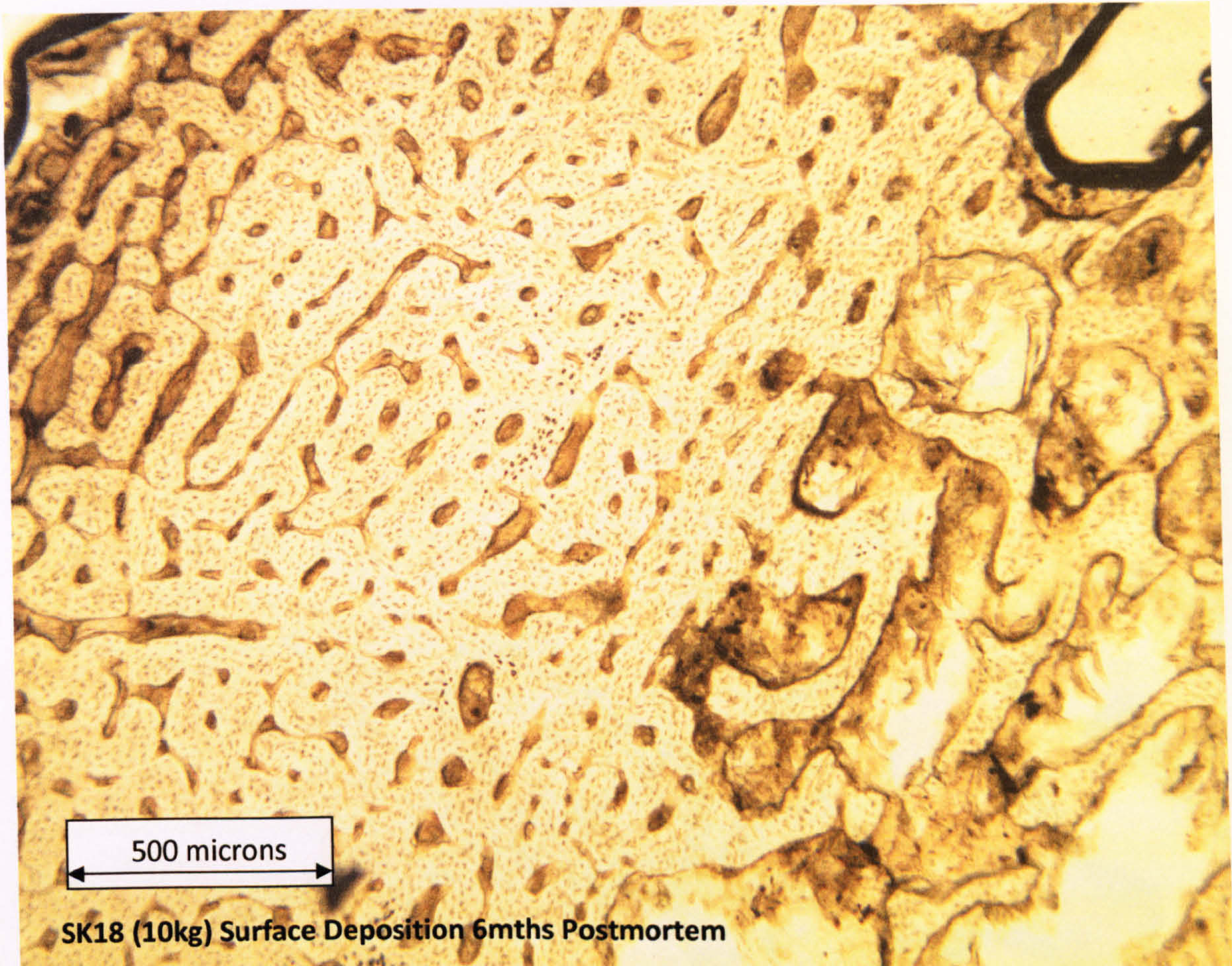
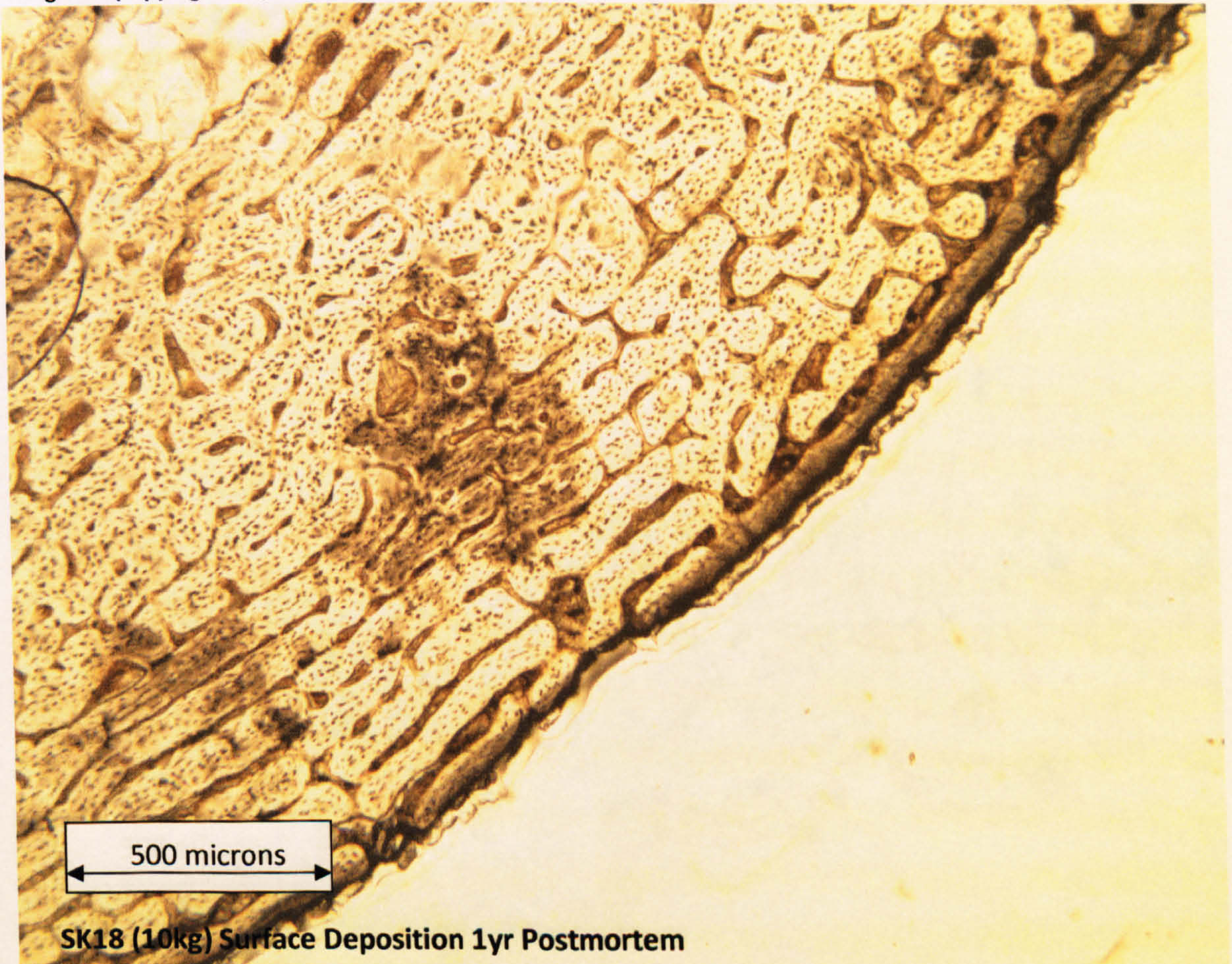
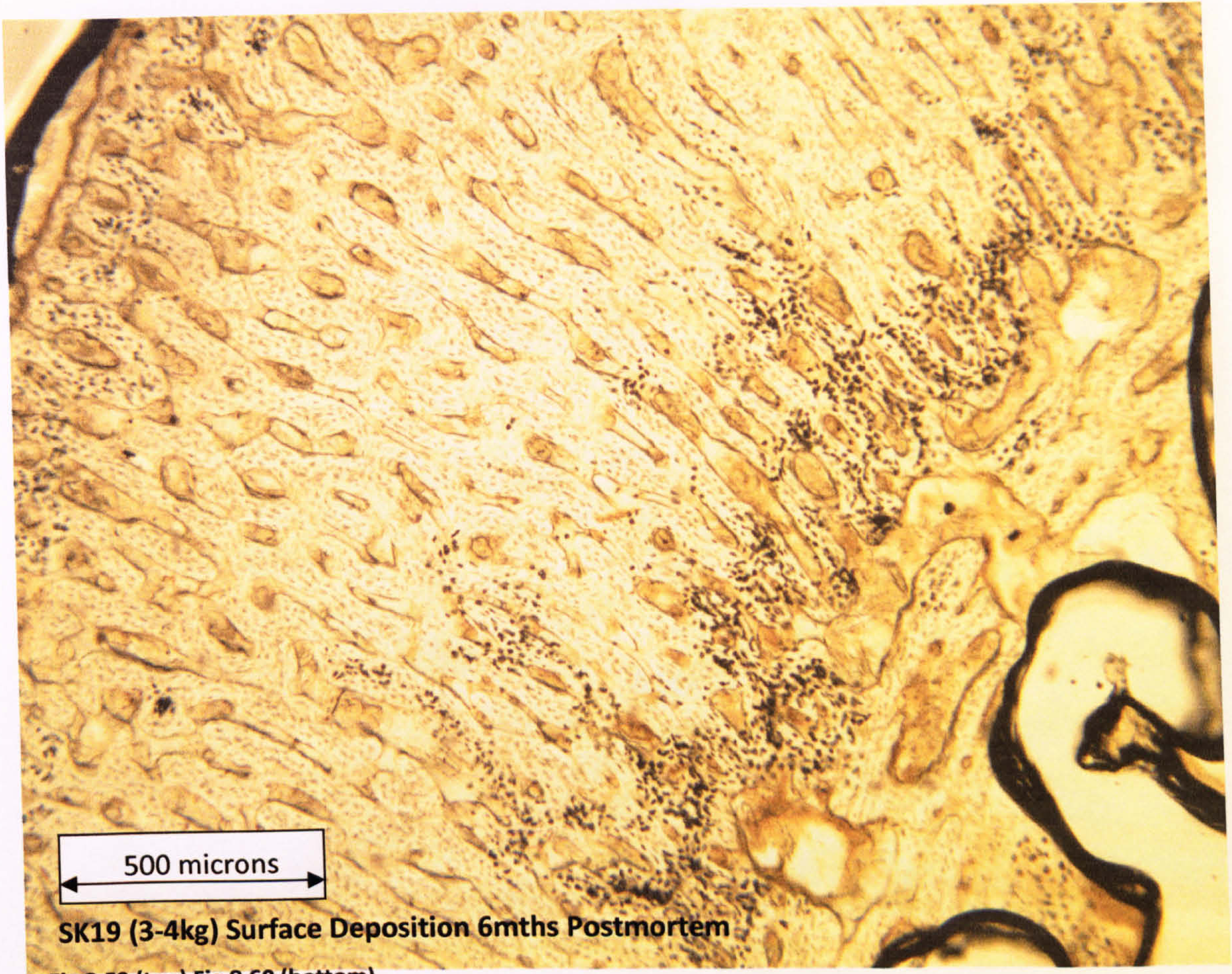


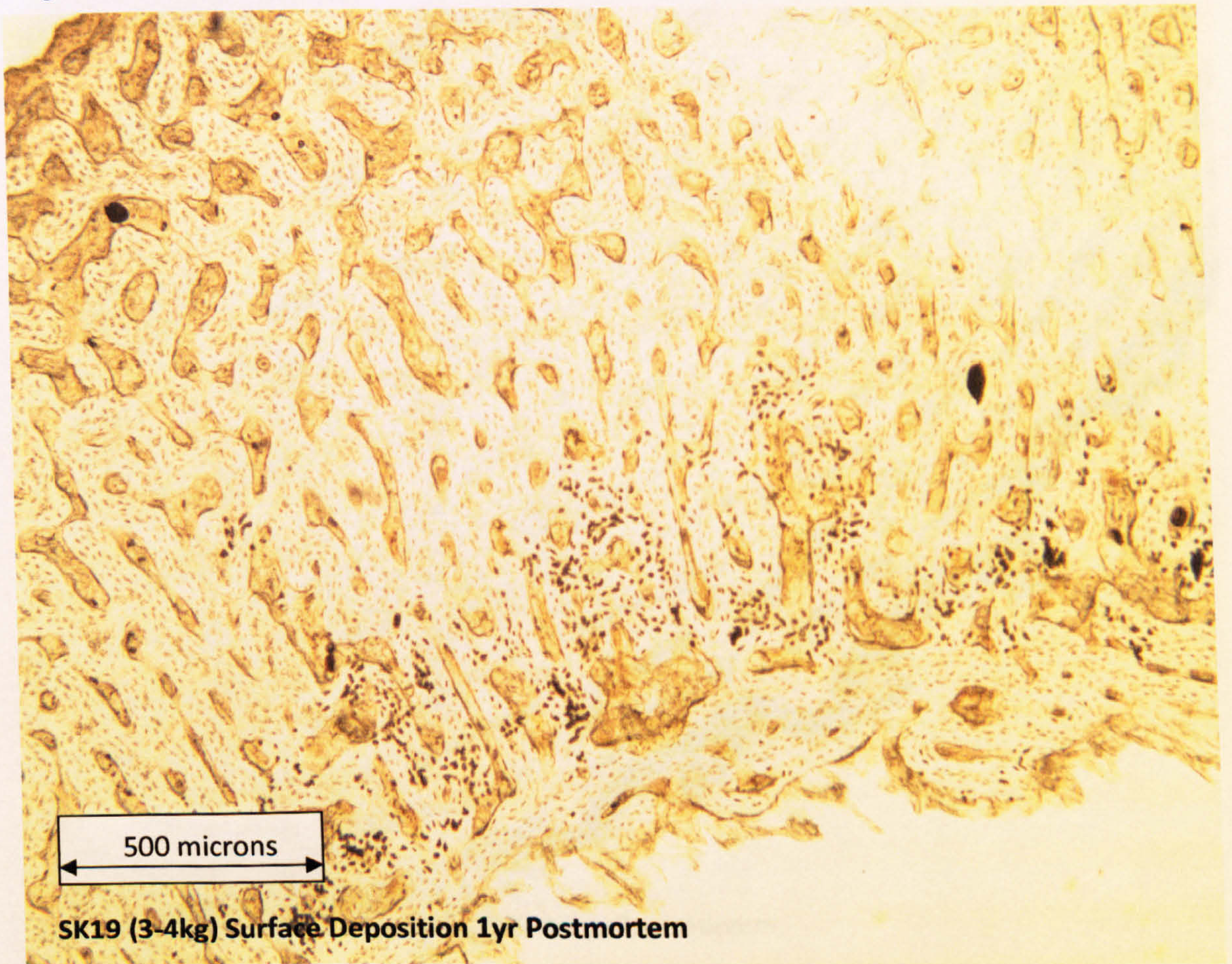
Fig 8.57 (top) Fig 8.58 (bottom)



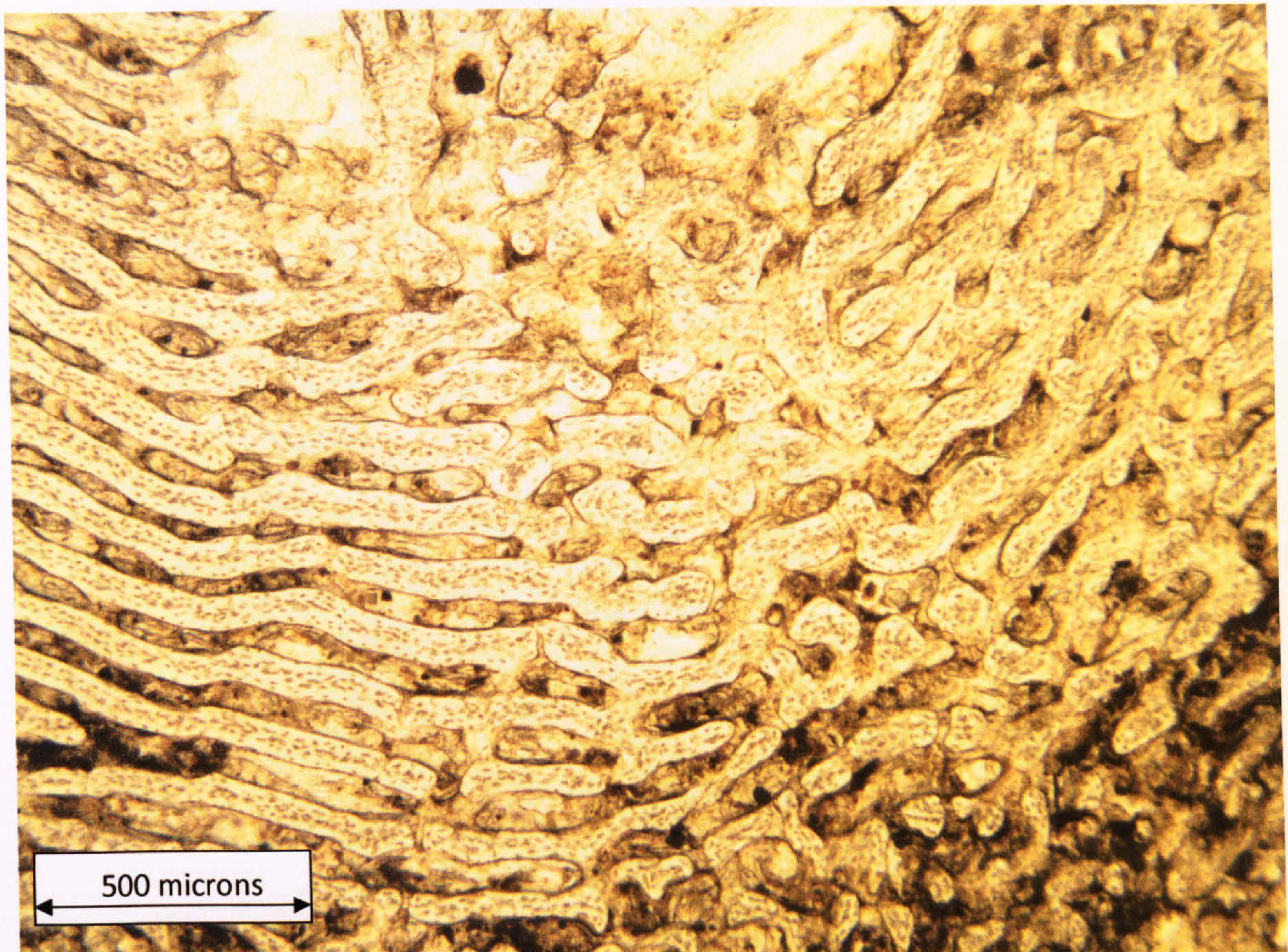


SK19 (3-4kg) Surface Deposition 6mths Postmortem

Fig 8.59 (top) Fig 8.60 (bottom)

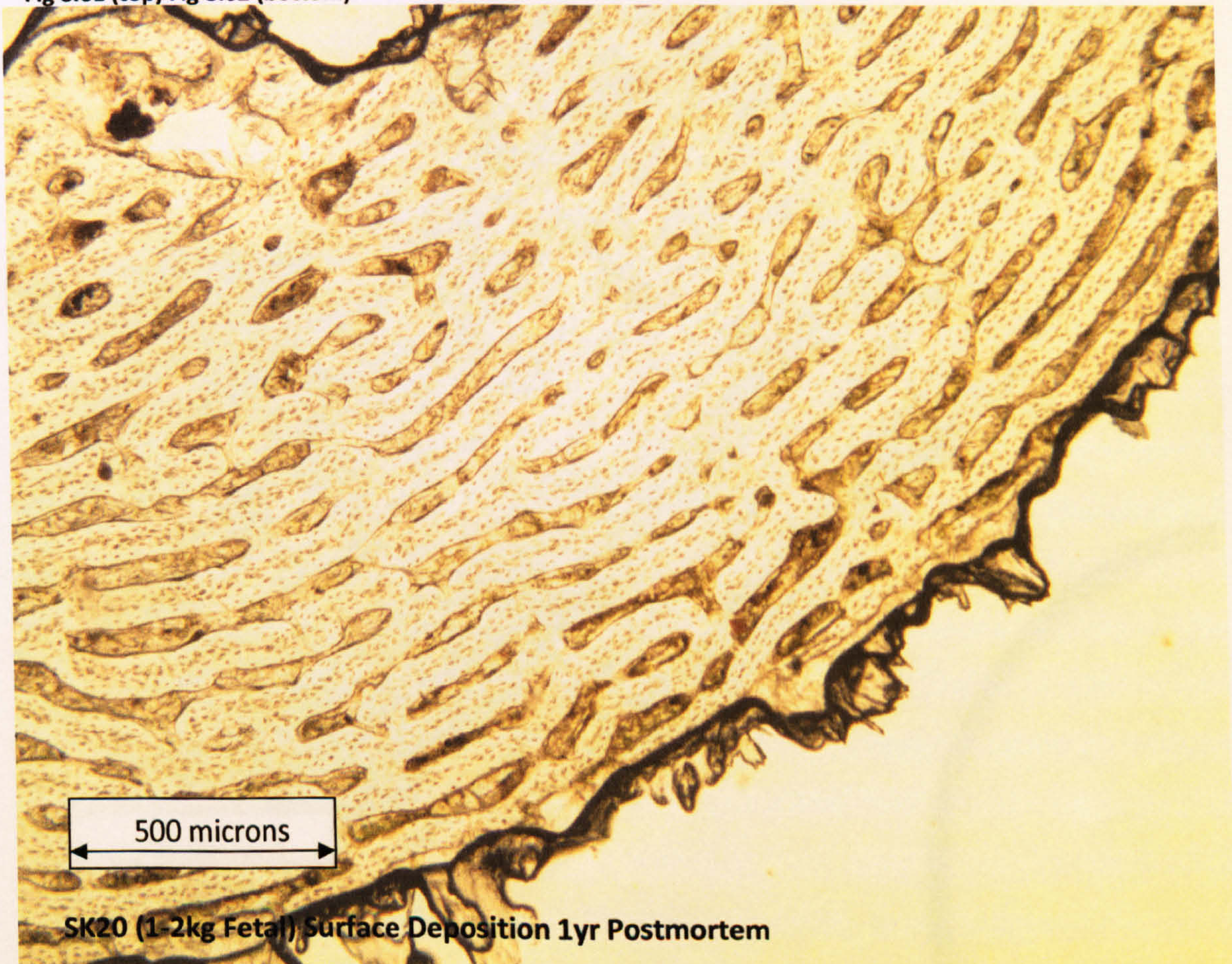


SK19 (3-4kg) Surface Deposition 1yr Postmortem



SK20 (1-2kg Fetal) Surface Deposition 6mths Postmortem

Fig 8.61 (top) Fig 8.62 (bottom)



SK20 (1-2kg Fetal) Surface Deposition 1yr Postmortem

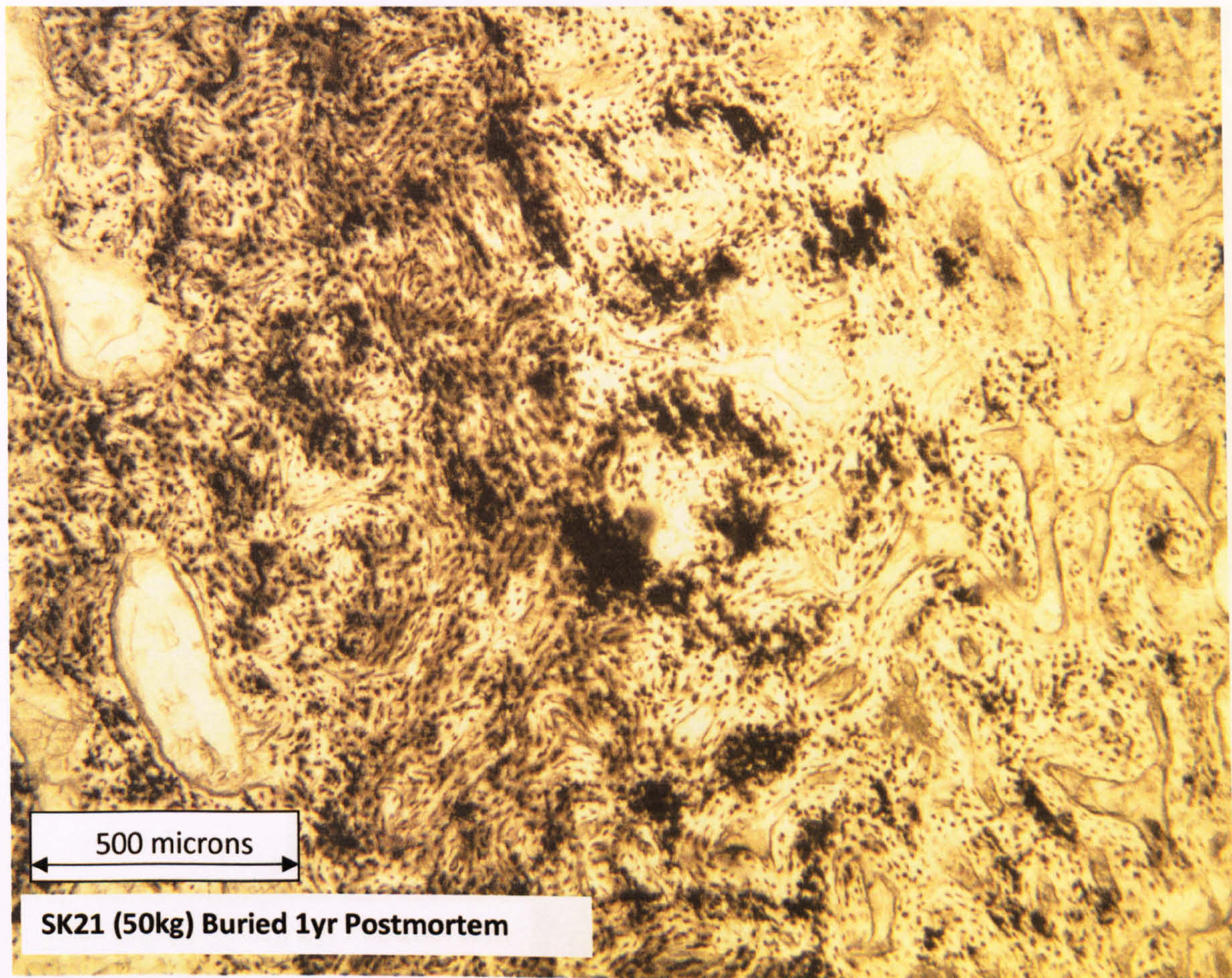
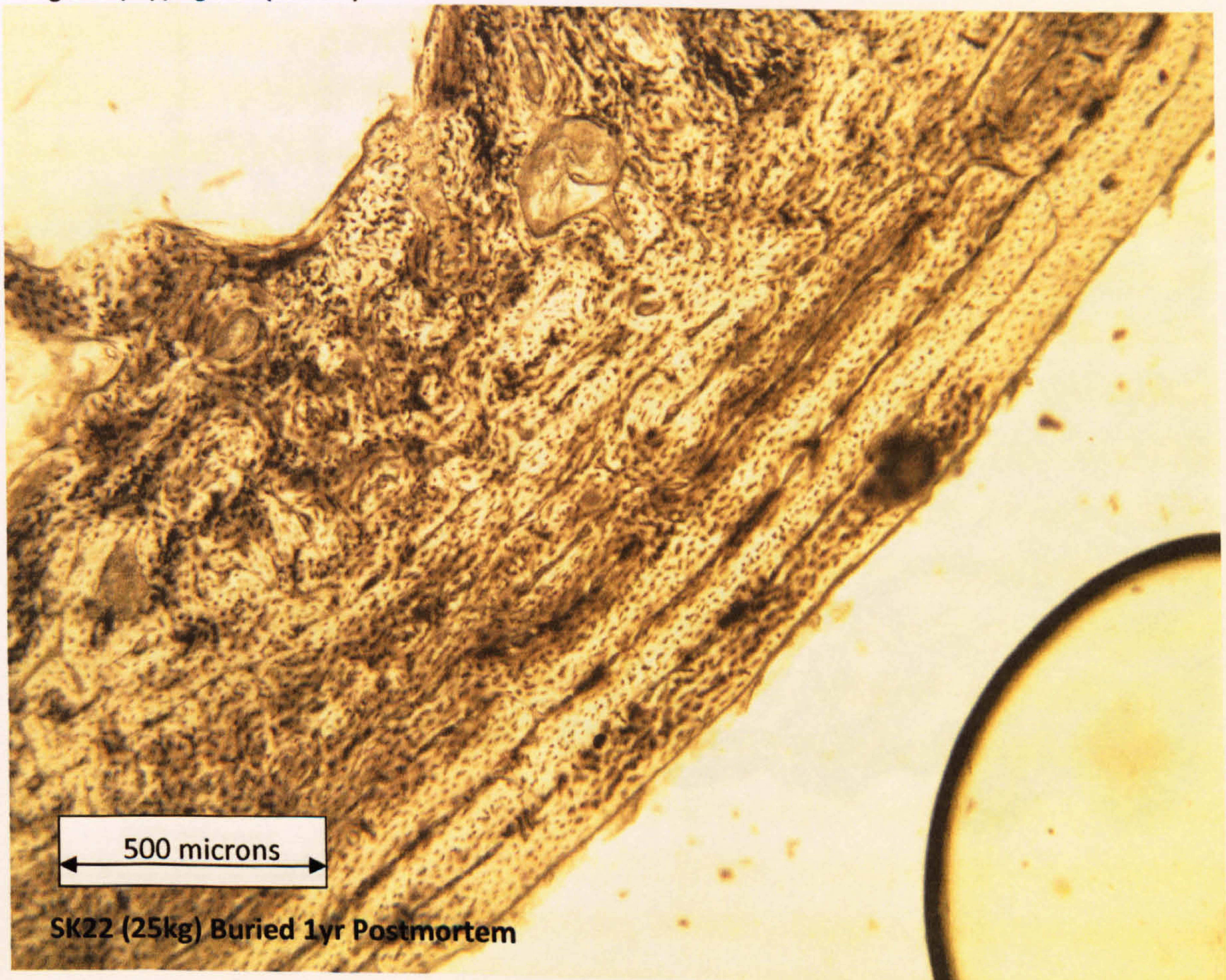


Fig 8.63 (top) Fig 8.64 (bottom)



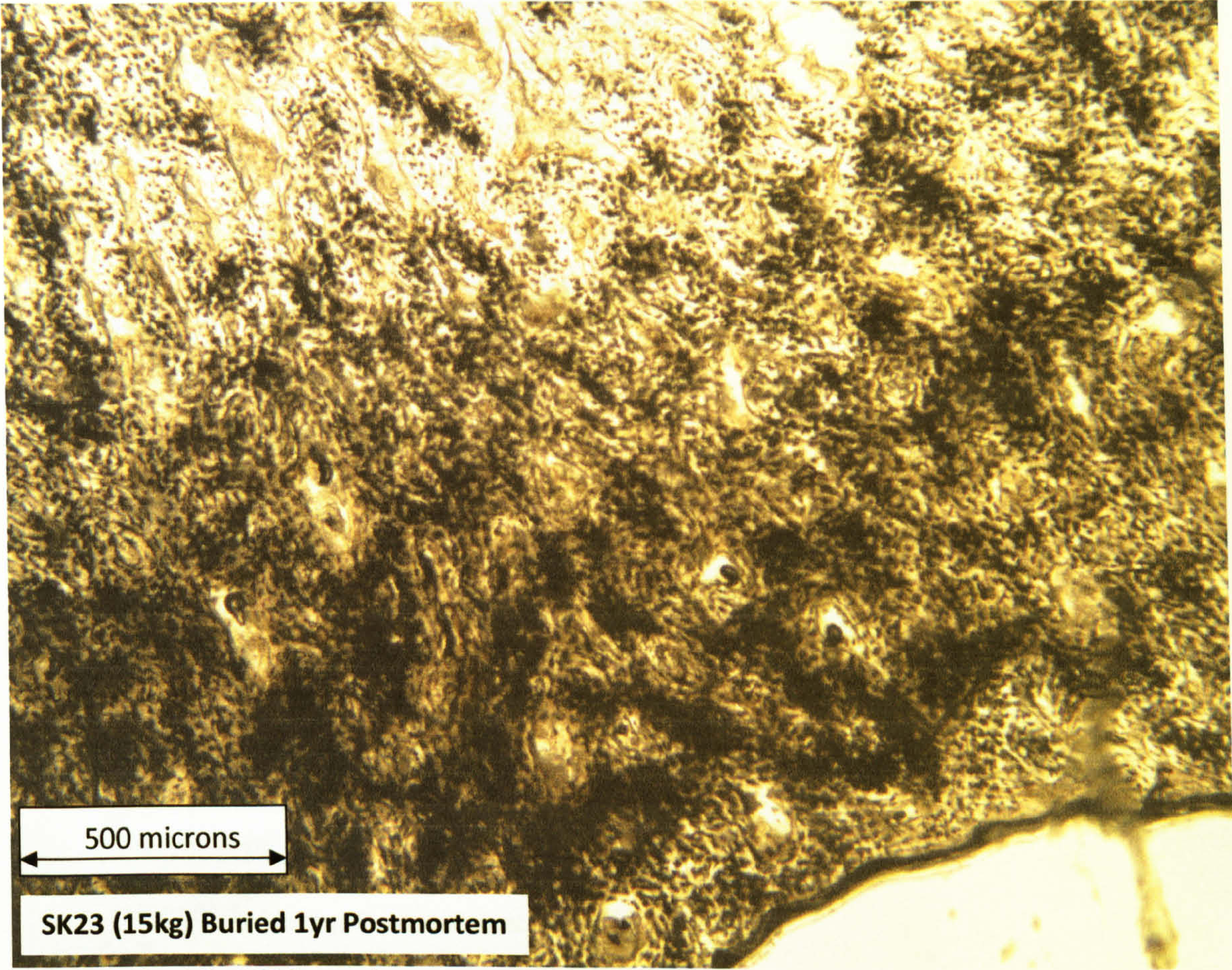
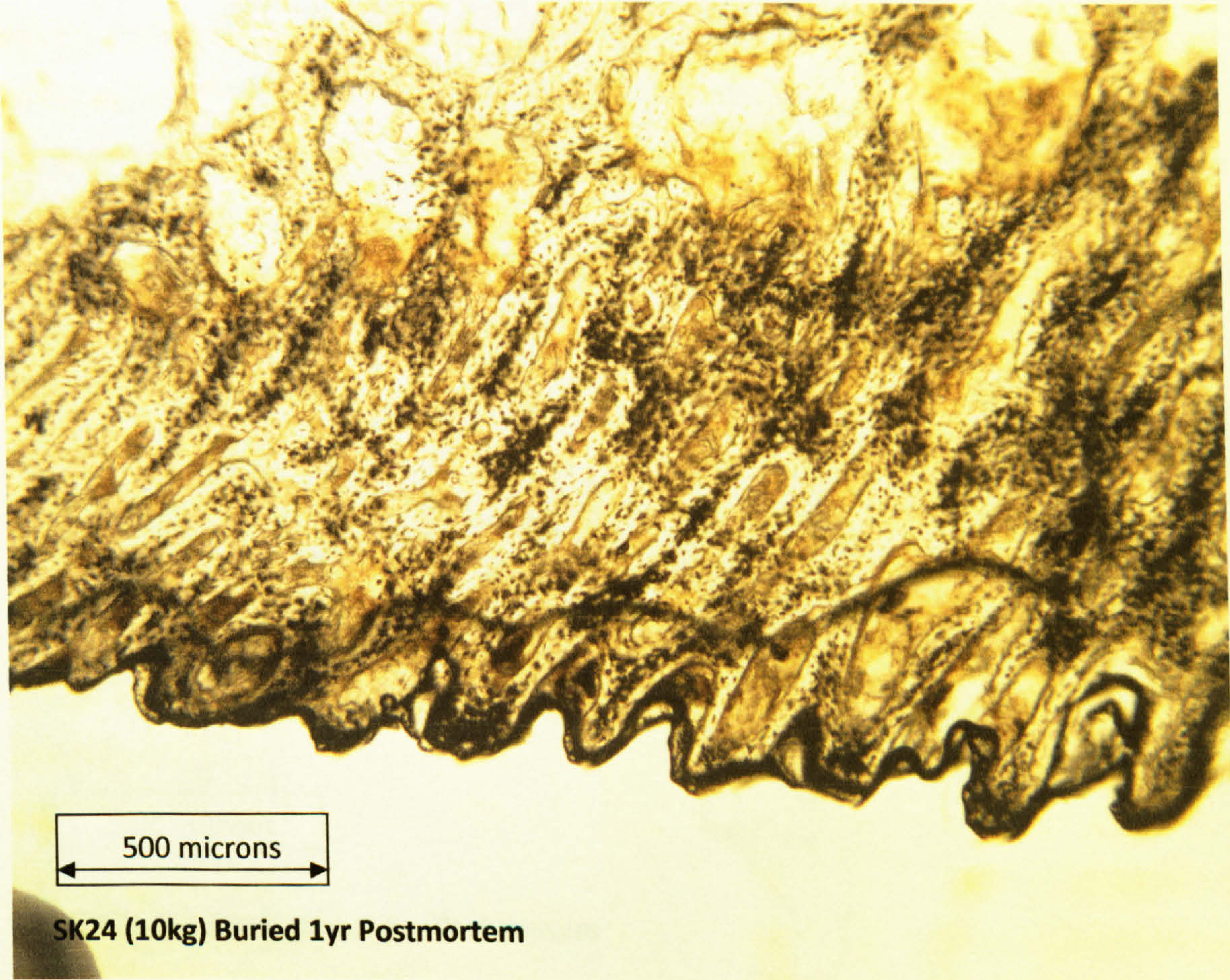


Fig 8.65 (top) Fig 8.66 (bottom)



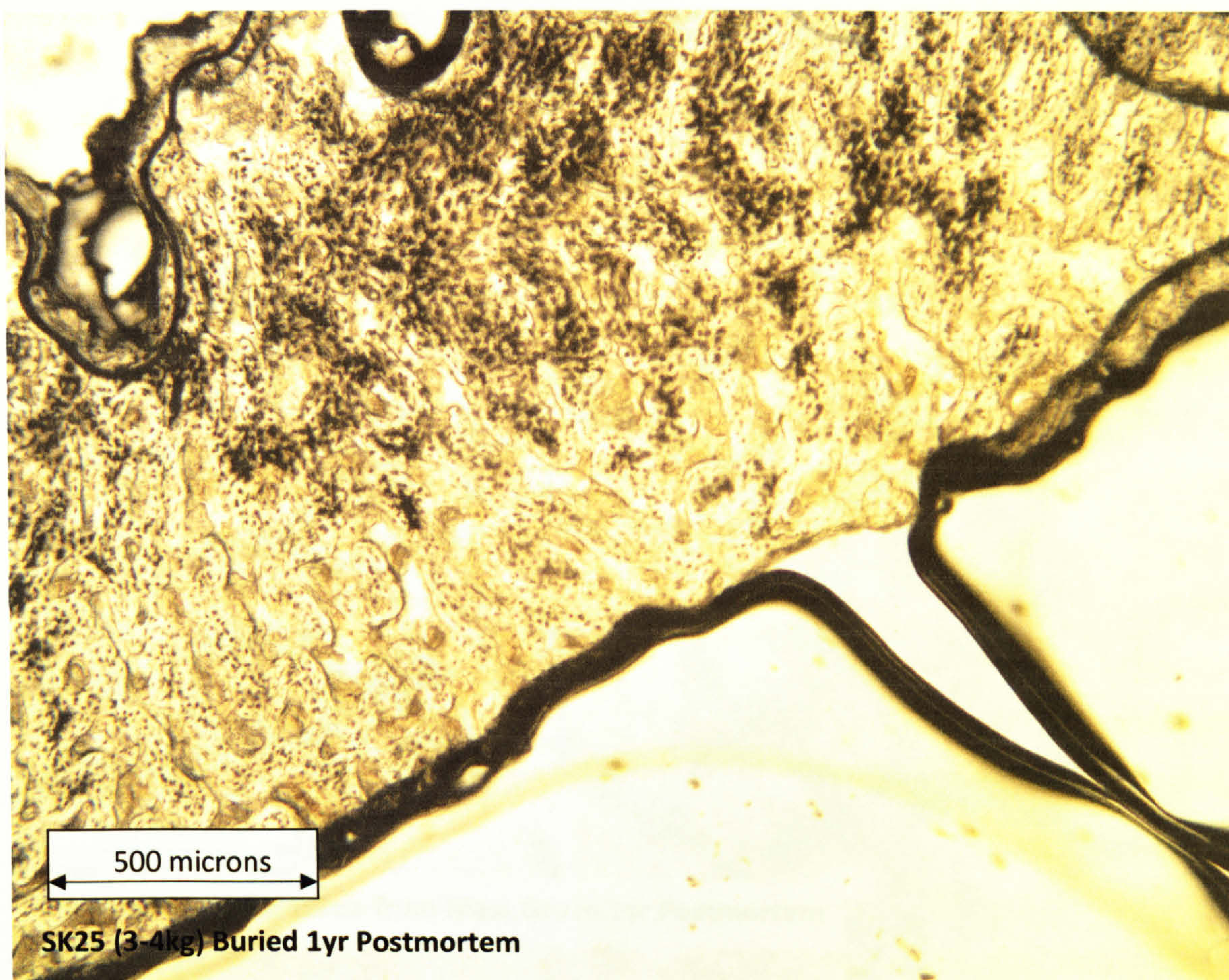
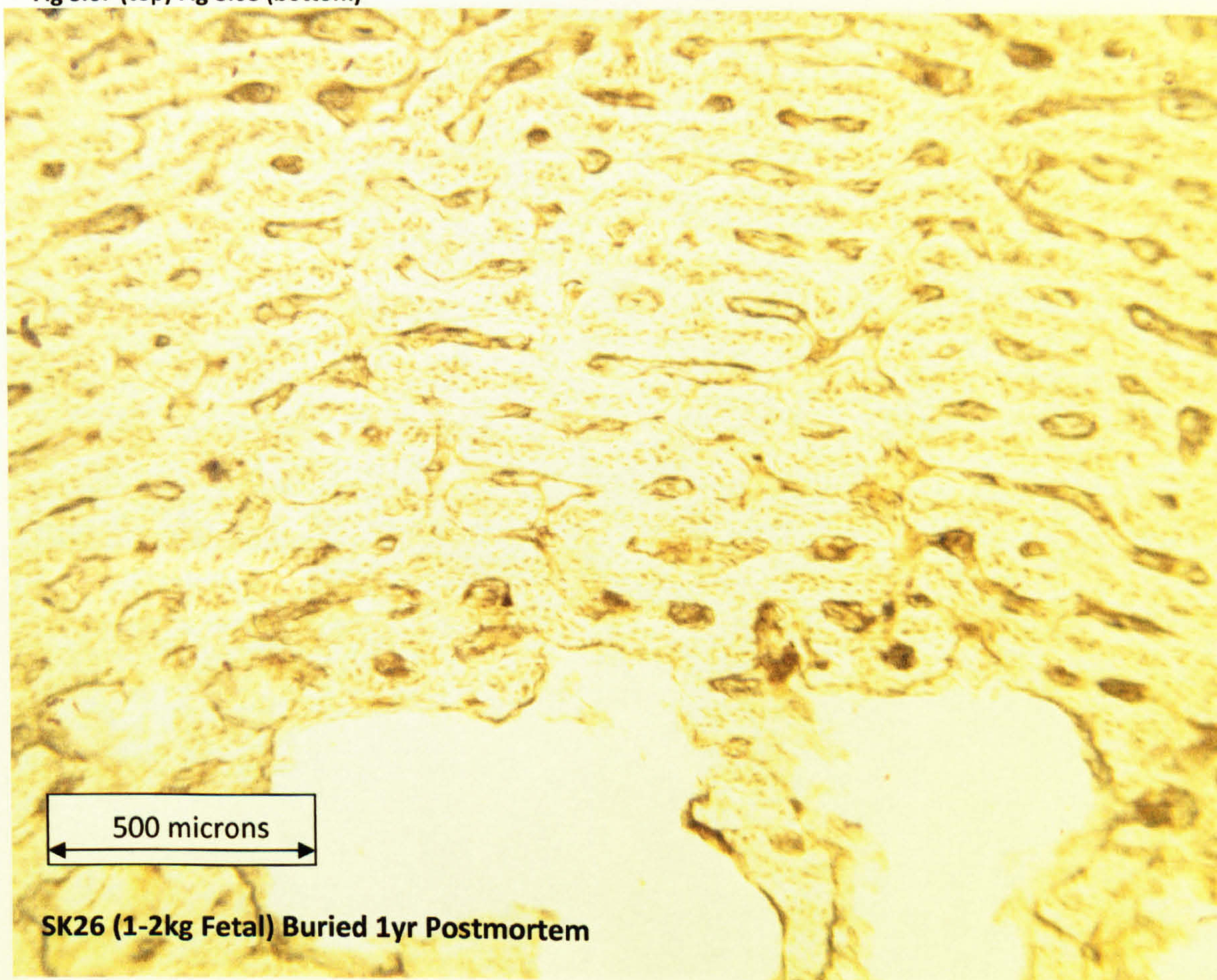


Fig 8.67 (top) Fig 8.68 (bottom)



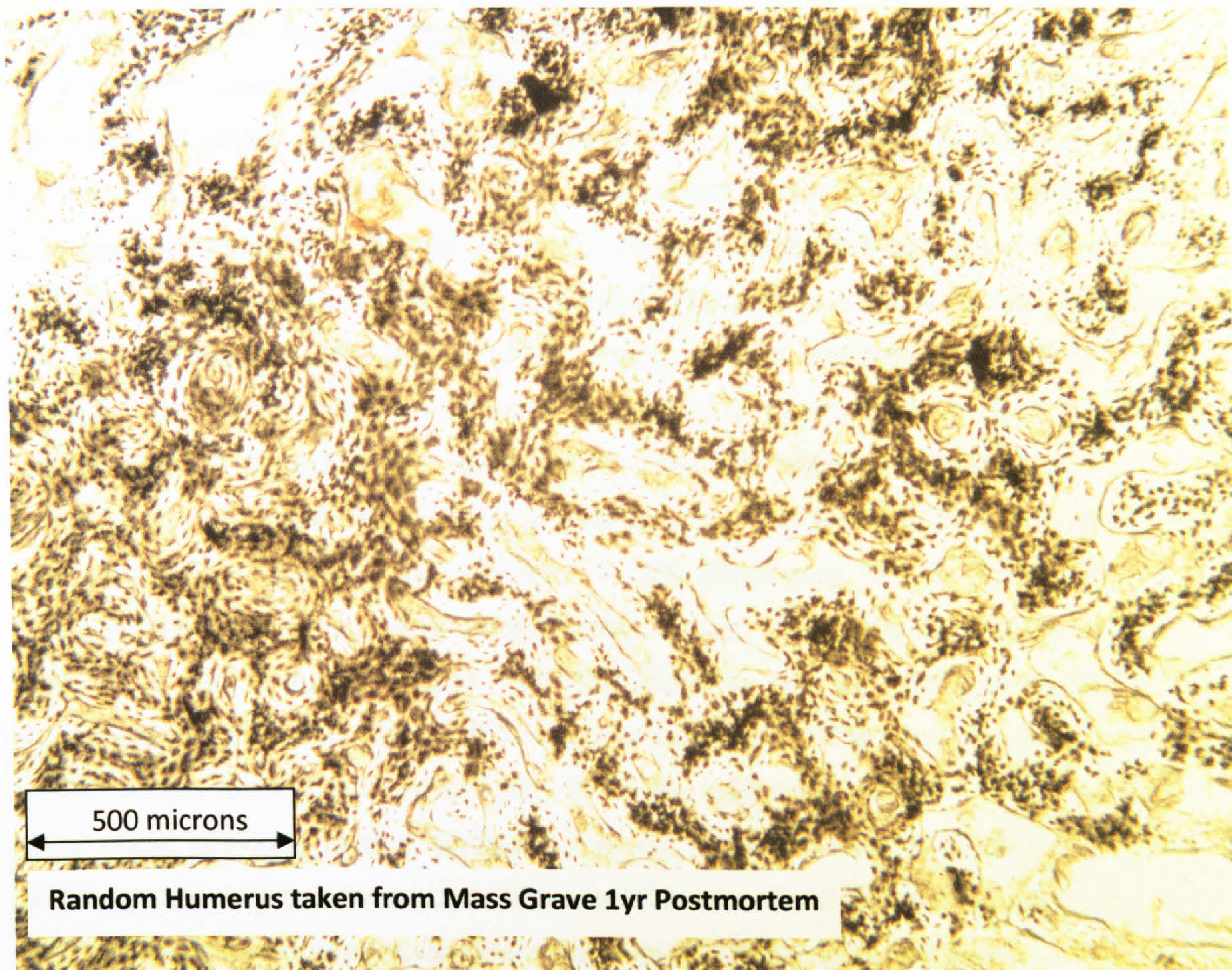
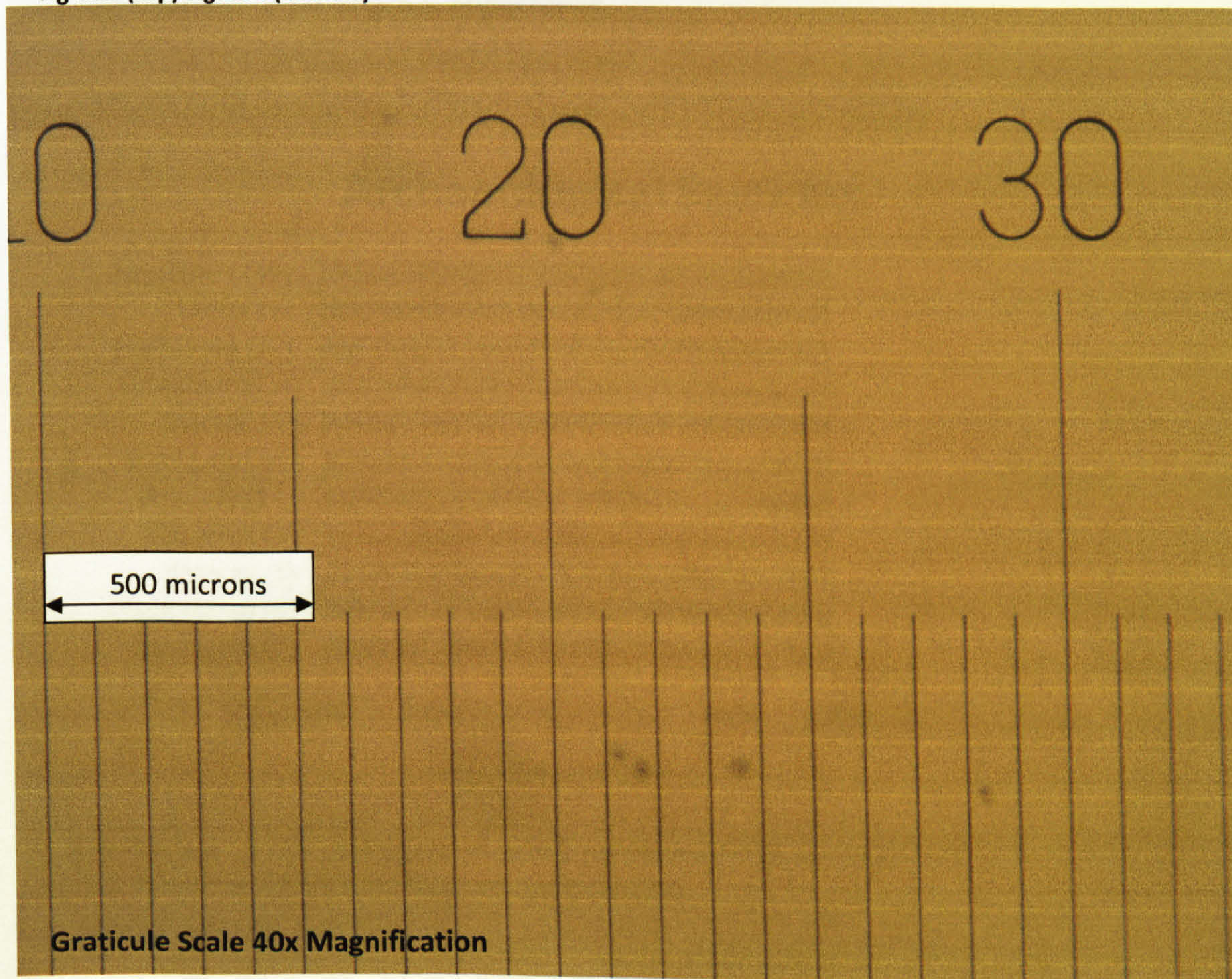


Fig 8.69 (top) Fig 8.70 (bottom)



8. 7 Results of Histology of the Archaeological Sections

In total one-hundred-forty-two human bone sections of both adults and children were observed, of which one-hundred-twenty-nine (91%) were seen to be affected by bacterial tunnelling (discrete tunnels) or generalised bacterial attack (bacterial attack type destruction without discrete tunnels). Of the eleven sites examined (table 8.12) (139 sections), one (Kilton Hill) was too degraded to visualise any bacterial damage and these were therefore classified as having general destruction. This was also applied to any other sections that could not clearly be seen. If we exclude Kilton Hill and look at the other ten sites then half of them are affected 100% by MFD. The Royal Mint site has 97.5% of sections affected (fig 8.71) and the other four all score above 70% (Bantycock 71.5%, Bolsover 71.4%, Blackgate 79% & Grantham 77%). The size of the samples is very small in some cases, and this is determined by three factors. In some cases (Kilton Hill) the remains are so poorly preserved that sectioning becomes incredibly difficult and once sampled it is clear that sectioning of further bones would be unproductive. In further cases the sections being observed were made previous to this research and are what is available for that cemetery. Finally, sectioning is a destructive process and often curators are not happy to have the bones sectioned especially where not many skeletal parts are available or where they are deemed to be of great importance.

Archaeological Sections & Percentage Tunnelling Observed

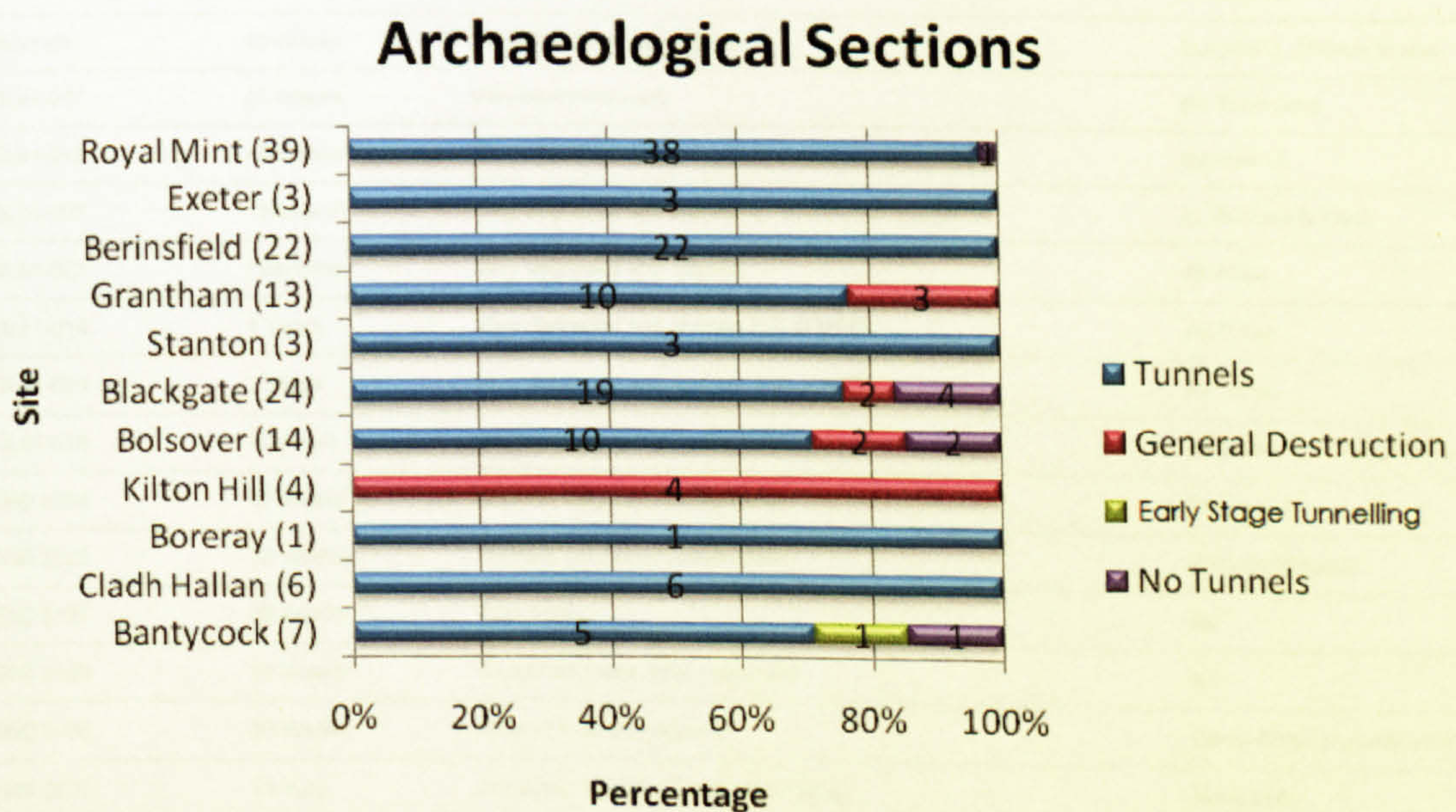


Fig 8.71 Eleven of the sites used, showing percentages of sections affected. Includes 137 sections, the other 5 from misc sites are not included.

Table of Archaeological Thin Sections and Findings (Adults and Juveniles)

Number	Age	Destruction	Tunnelling
BANT 05 SK26	Mother of SK13	Stained, very poor preservation	Tunnels Present
BANT 05 SK13	Foetus of SK26	Stained, very poor preservation	Tunnels present, budded
BANT 05 SK19	6-9 mths	Stained, very poor preservation	Tunnels present, budded
BANT 05 SK21	0-1 mth	Stained, very poor preservation	Tunnels present, budded
BANT 05 SK22	38-39wks	Better preserved than rest black dots	No
BANT 05 SK23	0-1 mth	Microcracking	Early Stage tunnelling
BANT 05 SK27	39-40 wks	Black dots	Tunnels present
CH01-SK2316	Adult	Stained dark brown	Budded, LL & Lamellate
CH01-SK2792	3 Years	Stained brown, some areas well preserved	Budded & Fungal
CH01-SK2727	10-14 Years	Stained dark brown	Budded, LL & Lamellate ??
CH01-SK2638	Adult	Endo & Ecto cranial well preserved, attack below surface	Budded, LL & Lamellate??
CH01-SK2638	Adult	Well preserved, attack below surface	Budded, LL & Lamellate??
CH01-SK2638	Adult	Well preserved, stained, some attack	Budded, LL
BRY 09-02	Adult	Some staining, darker at one side, band as seen in pigs	Yes
KH SK44	Adult	Stained, very poor preservation	Generalised destruction
KH SK86 Con 294	Adult	Stained, very poor preservation	Generalised destruction
KH SK86 Con 294	Adult	Stained, very poor preservation	Generalised destruction
KH Con 267	Adult	Stained, very poor preservation	Generalised destruction
BOL 008a	26 Weeks	Very well preserved	No Tunnelling
BOL91-056	30 weeks	Very degraded and stained	Generalised destruction
BOL91-014	33 Weeks	Very degraded and stained	All three
BOL91-014	36 Weeks	Very degraded and stained	All Three
BOL91-028	39 Weeks	Very degraded and stained	Generalised Destruction
BOL91-011	40 Weeks	Very degraded and stained	Budded LL
BOL91-017	40 Weeks	Very degraded and stained	Budded LL Difficult to see
BOL91-007	46 Weeks	Very well preserved	No Tunnelling
BOL91-012	48 Weeks	Some destruction	Budded LL
BOL91-002	12m/4yrs?	Very degraded and stained	LL Budded & Wedl
BOL91-021	18months	Very degraded and stained	All three
BOL91-018	4 Years	Very degraded and stained Full of MFD	All Three
BOL91-029	7 Years	Very degraded and stained Full of MFD	All Three
BOL91-019	40-45 yrs	Very degraded and stained	All Three
BG90 3204	40 Weeks	Stained, very poor preservation	Budded LL
BG90 3285	32 Weeks	Stained, very poor preservation	Possible Budded
BG90 3191	36 Weeks	Excellent	No
BG90 3166	39 Weeks	Darkened areas. Well preserved	No
BG90 3166	39 Weeks	Difficult to see structures	Generalised bacterial attack
BG90 3207	<1 Year	Generally well preserved, dark bands	None seen
BG90 3830	18 Months	Stained, very poor preservation	Generalised bacterial attack
BG90 3184	4-5 Years	Stained, very poor preservation	Generalised bacterial attack
BG92 3664	5-6 Years	Some well preserved, clear islands of destruction	Generalised bacterial attack
BG81	?	Poorly preserved	Generalised bacterial attack

Number	Age	Destruction	Tunnelling
BG81	?	Poorly preserved	Generalised bacterial attack
BG281	?	Poorly preserved	Generalised bacterial attack
BG3215	39 Weeks	Very degraded and stained	Budded LL
BG3277	27 Weeks	Small areas of destruction	Generalised destruction
BG3191	46 Weeks	Very well preserved	No Tunnelling
BG464	30-49 Years	No staining, full of MFD	All 3
BG442	20-29 Years	No staining, full of MFD	All 3
BG91		Stained, very poor preservation	All 3?
BG8	50+ Years	Stained, very poor preservation	All 3?
BG481	40-49 Years	Stained in places, small islands preserved bone	All 3?
BG548	45-54 Years	Poorly preserved	All 3?
BG127	Adult	Poorly preserved	All 3?
BG614	30-39 Years	Poorly preserved	All 3?
BG91-056	?	Very degraded and stained	Budded LL
Stanton	?	Poorly preserved	Generalised bacterial attack
Stanton	?	Poorly preserved	Generalised bacterial attack
Stanton	?	Poorly preserved	Generalised bacterial attack
GLR91 029	18 Months	Stained, very poor preservation	Generalised bacterial attack
Sampson		Well preserved	No
Sampson		Well preserved, bands of discolouration	No
Molar Tooth		Destruction to inner surface, rest well preserved	Budded LL Lovely specimen
Homo sapiens		Very poor preservation	Generalised bacterial attack
Homo sapiens		Very poor preservation	Generalised bacterial attack
BER127	13-16 Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER73	18-25 Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER49	45+ Years	Totally degraded, Stained dark brown	Generalised bacterial attack
BER48	7-10 Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER164	45 Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER77	30-35 Years	Totally degraded, Stained dark brown	Generalised bacterial attack
BER18	18-25 Years	Totally degraded, Stained dark brown	Generalised bacterial attack
BER134	40+ Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER164	45 Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER82	20-25 Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER126	17-20 Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER53	40+ Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER63	20-22 Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER102	17-19 Years	Totally degraded, Stained dark brown	Generalised bacterial attack
BER161	Adult	Totally degraded, Stained yellow & gray	Tunnelling visible
BER152	18-20 Years	Band of preserved bone, mesosteal	Generalised bacterial attack
BER50	38 Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER14	6-7 Years	Totally degraded, Stained dark brown	Generalised bacterial attack
BER032	45+ Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER110	40+ Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack

Number	Age	Destruction	Tunnelling
BER77	30-35 Years	Totally degraded, Stained yellow & gray	Generalised bacterial attack
BER82	Adult	Totally degraded, Stained yellow & gray	Generalised bacterial attack
EX618		Full of MFD	All 3
EX561		Full of MFD	All 3
EX635		Full of MFD	All 3
GLR91063	Adult	Full of MFD, unstained	All 3
GLR91 Test	Adult	Full of MFD, stained gray	All 3
GLR91056	Adult	Full of MFD, unstained	All 3
GLR91046	Adult	Very stained difficult to view, small islands preserved bone	Generalised attack
GLR91067	Adult	Stained dark, difficult to view	Generalised attack
GLR91070	Adult	Full of MFD, unstained	All 3
GLR91027	Adult	Full of MFD, unstained	All 3
GLR91034	Adult	Full of MFD, unstained	All 3
GLR91040	Adult	Full of MFD, unstained	All 3
GLR91022	Adult	Full of MFD, unstained	All 3
GLR91010	Adult	Full of MFD, unstained	All 3
GLR91042	Adult	Stained dark, difficult to view	Generalised attack
MIN86#23 Con#5859	35-45 Years	Stained in places, small islands of preserved bone	Generalised attack
MIN86#22 Con#5859	35-45Years	Very stained difficult to view, small islands preserved bone	Generalised attack
MIN86#15		Beautifully preserved except for periosteal edge where stained	Generalised attack
MIN86#23 Con#8210	45+ Years	Very stained difficult to view, small islands preserved bone	Generalised attack
MIN86#24 Con6545	Adult	Stained in places, large islands of preserved bone, black dots	Generalised attack
MIN86#25		Islands of destruction that are stained black dots	Generalised attack
MIN86 Con16122	45+ years	Stained in places, large islands of preserved bone, black dots	All 3
Min86#18 #10348	45+ years	Very stained difficult to view, small islands preserved bone	Generalised attack LL & Budded
MIN86 Con#10070	45+ Years	Very stained difficult to view, small islands preserved bone	Generalised attack LL & Budded
MIN86 Con#10250	5-15 Years	Very stained difficult to view, small islands preserved bone	Generalised attack
MIN86#10		Very stained, small islands preserved bone	All 3
MIN86#12 #6602	15-25 Years	Stained in places, large islands of preserved bone	All 3
MIN86#11 Con#7163	35-45 Years	Stained in places, large islands of preserved bone	All 3
MIN86#12 #8099	35-45 Years	Very stained difficult to view	All 3
MIN86#13		Destruction at periosteum and endosteum, minor changes mesosteum	All 3
MIN86#14		Stained in places, large islands of preserved bone	All 3
MIN86#16 #13666	35-45 Years	Stained in places, large islands of preserved bone	All 3
MIN86#17 #16122	45+ Years	Stained in places, large islands of preserved bone	All 3
MIN86#28 Con#6383	35-45 Years	Very stained difficult to view, small islands preserved bone	Generalized attack
MIN86#29 Con#8414	25-35 Years	Stained in places, large islands of preserved bone	Generalized attack
MIN86#30 Con#8217	35-45 Years	Stained in places, large islands of preserved bone	Generalized attack
MIN86#32aCon#11606	35-45 Years	Very stained difficult to view	Generalized attack
MIN86#326 #10213	?	Stained in places, large islands of preserved bone	Generalized attack
MIN86#33 Con#10635	45+ Years	Very stained difficult to view	Generalized attack
MIN86#34 Con#11997	?	Stained in places, large islands of preserved bone	Generalized attack
MIN86#27 Con#6583		Stained in places, large islands of preserved bone	Generalized attack

Number	Age	Destruction	Tunnelling
MIN86 Con#5728	35-45 Years	Very stained difficult to view	Generalized attack
MIN86 Con#11117	25-35 Years	Very stained difficult to view	Generalized attack
MIN86#2 Con#9849	35-45 Years	Stained in places, large islands of preserved bone	Generalized attack
MIN86#35 Con#10250	5-15 Years	Very stained difficult to view	Generalized attack
MIN86#37 Con#14421	15-25 Years	Some staining, tunnels visible	All 3
MIN86#36 Con#10240	Adult	Stained grey, difficult to view	Generalized attack
MIN86#38 Con#10801	35-45 Years	Islands of destruction	Wedl?
MIN86#1		Very stained difficult to view	Generalized attack
MIN86#36 Con#11117	25-35 Years	Stained in places, large islands of preserved bone	All 3
MIN86#4 Con#9517	35-45 Years	Destruction mainly to endosteal	Generalized attack
MIN86#6 Con#5265	25-35 Years	Stained grey, difficult to view	All 3
MIN86#9 Con#6509	35-45 Years		
Min86#20		Very stained difficult to view	Generalized attack
MIN86#21 Con8126		Well preserved	None
FCR34 02	Pig	Well preserved, microcracking	No
Sus scrofa	Pig	Well preserved, microcracking	No
Sus scrofa	Pig	Well preserved, microcracking	No
Meles meles	Badger	Well preserved, bands of destruction	Generalised bacterial attack
Meles meles	Badger	Well preserved, bands of destruction	Generalised bacterial attack
Oryctolagus Cuniculus	Rabbit	Well preserved, bands of destruction	Generalised bacterial attack
Oryctolagus Cuniculus	Rabbit	Well preserved, bands of destruction	Generalised bacterial attack
Canis familiaris	Dog	Very well preserved	No
Canis familiaris	Dog	Very well preserved	No
Canis familiaris	Dog	Very well preserved	No
Canis familiaris	Dog	Very well preserved	No
Canis lupus	Wolf	Well preserved, microcracking	No
Canis lupus	Wolf	Well preserved	No
Vulpes vulpes	Fox	Well preserved	No
Vulpes vulpes	Fox	Well preserved	No
Ovis aries	Sheep	Well preserved	No
CPC99-1000	Cattle	Destruction to Endo & Perio, some central	Generalised bacterial attack
CPC98-1032	Cattle	Destruction to entire section	Generalised bacterial attack,
CPC98-1171	Cattle	Destruction to entire section	Generalised bacterial attack
CPC98-1172	Cattle	Destruction to entire section	Generalised bacterial attack
CPC98-1047	Cattle	Destruction to Endo & Perio, some central	Generalised bacterial attack
CH C9	Cattle	Dark stained difficult to view	Generalised attack
CH C10	Cattle	Dark stained difficult to view	Generalised attack
CH C11	Cattle	Dark stained difficult to view	Generalised attack
CH C13	Cattle	Dark stained difficult to view	Generalised attack
CH C16	Cattle	Dark stained difficult to view	Generalised attack
CH 99 1291-1	Dog	Dark stained difficult to view	Generalised attack
CH S1	Sheep	Dark stained difficult to view	Generalised attack
CH S2	Sheep	Dark stained difficult to view	Generalised attack

Number	Age	Destruction	Tunnelling
CH S3	Sheep	Dark stained difficult to view	LL & Budded
CH S4	Sheep	Dark stained difficult to view	Generalised attack
CH S8	Sheep	Dark stained difficult to view	Generalised attack

Table 8.9

8.7.1 Results Histology Foetal and Newborn

When looking at the very young children the results are slightly different. If the foetal material is taken on its own (table 8.10) then only 33% are affected by tunnelling. 17% have generalised destruction and 50% are very well preserved. Of the newborns 40wks-1year 58% show tunnelling, 17% have pre-tunnelling and 25% are not affected. As with the sections for all of the cemeteries the sample sizes are small for the same reasons previously stated, plus the fact that babies and foetal material are much rarer in archaeological assemblages meaning they are not there in the first place to section and even when they are available permission to employ destructive methods is often not forthcoming.

Number	Age	Destruction	Tunnelling
Foetal			
RHSK20 Buried	Foetal	No Destruction	No
RHSK20 I Surface	Foetal	No Destruction	No
BOL008a	26 wks	Very Well Preserved	No
BG3277	27 wks	Small Areas of Destruction	No
BOL91-056	30 wks	Very Degraded & Stained	Generalised Destruction
BG90 3285	32wks	Stained, very poor preservation	Possible budded
BOL91-014	33 wks	Very Degraded & Stained	All Three
BOL91-014	36 wks	Very Degraded & Stained	All Three
BANT05SK22	38-39wks	Better preserved than rest	No
BOL91-028	39 wks	Very Degraded & Stained	Generalised Destruction
BG3215	39 wks	Very Degraded & Stained	Budded, LL
BG90 3166	39wks	Well Preserved	No
Newborn			
BANT05SK27	39-40wks	Black Dots	Tunnels
BG90 3204	40wks	Stained, very poor preservation	Budded, LL
BOL91-017	40 wks	Very Degraded & Stained	Budded, LL
BOL91-011	40wks	Very Degraded & Stained	Budded, LL
RHSK19 surface	Newborn	Destruction Endosteal	Tunnelling
RHSK19 Buried	Newborn	Very Few Black Dots	Early stage Tunnelling
BANT05SK23	0-1mth	Black Dots Microcracking	Early Stage tunnelling
BANT05 SK21	0-1 mth	Stained, Very Poor Preservation	Budded
BG3191	46 wks	Very Well Preserved	No
BOL91-007	46 wks	Very Well Preserved	No
BOL91-012	48 wks	Some Destruction	Budded, LL
BG90 3207	<1 yr	Generally Well Preserved	No

Table 8.10 Foetal and newborn sections histology results.

However, the differences are quite profound and this is probably a real discrepancy rather than one manufactured due to the small sample size. This supports the hypothesis that endogenous gut microbes are responsible for MFD rather than soil microbes.

8.8 Results of Archaeological Animal Histology

Animal remains were never meant to form a large part of this study due to the fact that they were often butchered in the past which would have removed the intestinal microbes. Therefore looking at pig bones from archaeological sites to compare with the new experimental research would be unproductive. Only a small sample of animals was therefore included in this study which included sheep, pigs, dogs and cattle. Of the thirty-two sections examined 20 (62.5%) had visible MFD, whilst twelve (37.5%) were well preserved. There was no evidence of Wedl type fungal tunnelling in any of the sections. This type of MFD has previously been suggested to be more prevalent in animal species (Jans *et al*, 2004). The extent of MFD in the animal bone sample is lower than in the archaeological human bone (91% affected) but higher than in the foetal human bone (33% MFD). If animal bones are butchered then we would expect to find very low numbers affected by MFD with a similar percentage to the foetal bone. Burial context may explain some of this discrepancy as bones may have been thrown into middens or rubbish pits that contained intestinal soft tissue and/or fresh faecal material and thereby re-inoculating the discarded bones with bacteria. Most of the sections are from unknown contexts and it would be fruitful in future research to look at animal bones where a full contextual provenance is known.

8.9 Summary

The results above appear to support the hypothesis that MFD is related exclusively to the presence of endogenous gut microbes in the early postmortem period, rather than those originating in the soil. The boxed pigs provide evidence of bacteria transmigrating from the intestines to the bone within one month postmortem and this is substantiated by the fact that the bacteria were present whether or not there was a soil covering. In the cases of pigs with no covering the bacteria must have originated from an endogenous source. At the time of the final sampling 2 years PM four of the newborn pigs had pre-tunnelling whilst none of the stillborns were affected. Results from the second set of experimental burials show that almost all of the pigs whether buried or on the surface had suffered some form of tunnelling with the exception of the two foetal pigs that were extremely well preserved.

The archaeological sections were affected by MFD with fully 91% of sections of human bone suffering from tunnelling. The foetal material had only 33% affected and the animal bones had MFD in 33% of the sections. Because in many cases the

foetal material is not affected and soil does not seem to have a direct affect on tunnelling it would seem that endogenous bacteria can be the impetus for bone tunnelling. This does not rule out soil bacteria altogether, as these could still be implicated at a later stage of the decomposition process.

8.10 Appendix: Categories and Stages of Decomposition

Modified from: Galloway,A. & W.H. Birkby, A.M. Jones, T.E. Henry, B.O. Parks. 1989.

A. FRESH

1. Fresh, no discolouration or insect activity
2. Fresh burned
3. Fresh, no discolouration, fly eggs present

B. EARLY DECOMPOSITION

1. Pink-white appearance with skin slippage and some hair loss
2. Bloating without discolouration
3. Grey to green discolouration-without bloating some flesh relatively fresh,
4. Bloating with green discolouration
5. Appearance of 1st Instar Larvae
6. Green/grey/black discolouration to most of body
7. Post bloating following rupture of the abdominal gases with discolouration going from green to dark
8. Brown to black discolouration of arms and legs, skin having leathery appearance

C. ADVANCED DECOMPOSITION

1. Decomposition of tissues producing sagging of the flesh, caving in of the abdominal cavity, often accompanied by extensive maggot activity
2. Moist decomposition in which there is bone exposure
3. Mummification with some retention of internal structures
4. Mummification of outer tissues only with internal organs lost/some still intact, through autolysis or insect activity
5. Mummification with bone exposure of less than one half the skeleton
6. Adipocere development

D. SKELETONIZATION

1. Bones with greasy substances and decomposed tissue, sometimes with body fluids still present
2. Bones with desiccated tissue or mummified tissue covering less than one half the skeleton
3. Bones largely dry but still retaining some grease
4. Dry bone

E. EXTREME DECOMPOSITION

1. Skeletonization with bleaching
2. Skeletonization with exfoliation
3. Skeletonization with metaphyseal loss with long bones and cancellous exposure of the vertebrae

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Chapter 9 Summary/Implications for Archaeology & Forensic Science/Future Directions/Conclusion

9.1 Summary

The main objective of the research was to determine the earliest point at which bacteria could become established in bone post-mortem; to evaluate how quickly tunnels become visible and to elucidate a possible culprit; exogenous versus endogenous microorganisms. Bacteria were found to be established within bone as early as one month postmortem. This data was taken from the first experimental pig carcasses that were housed in boxes and from those pigs where soil was not included. This demonstrates that bacteria are capable of migrating from the intestines to the hard tissues of the skeleton well within the early postmortem period. The bones were not examined prior to this time and hence bacteria may be able to reach the skeleton in a quicker time frame. The experimental research presented here appears to show that MFD occurs in the very early postmortem period (6-12 months in surface carcasses and 12-18 months in buried carcasses) and that it does not affect fetal skeletons, at least in the time period that was available for this study. The difference in time frame for tunnelling to occur is apparent, although the buried pigs were not sampled until one year post-mortem and therefore it is not known when the tunnelling took place. It is unfortunate that a longer period could not be covered but the cadavers will remain in-situ for the foreseeable future and can always be thin sectioned and observed at a later date. However, it should be remembered that the experimental research presented here used pigs as an analogue for humans. There are limitations to this as pigs are not and never will be human and there are inherent dissimilarities in their bone structure. Immature pig bone being of a plexiform nature has been shown to be more porous than human adult bone and this may lead to tunnelling developing faster although this is probably negligible in terms of MFD developing in the early postmortem period. Some tunnels do however demonstrate re-depositing of the mineral around the margins of the tunnel and lower mineralization of the young pig bone may make access easier. Unfortunately, this was the best possible animal model available. The pig is after all an omnivore, which was important for the species composition of the endogenous microbes and pig cadavers are easily available without the necessity for killing any animals. This is largely due to their intensive breeding and hence random death on a regular basis. Ethically, the use of pigs that have already died either due to poor farming practices or the unfortunate event of being rolled on

by their mother are preferable to the euthanasia of healthy animals. Organically raised pigs would have made a better analogue but there are inherent problems with this route. Because they are raised largely in a natural manner there are fewer deaths and this study required a large number of pigs on the same day to be delivered to site. After conversation with an organic pig farmer it was found that they have natural deaths very rarely and this averages less than 1 pig per month. Therefore if an ethical route was taken it would have taken several years and the freezing of corpses for a long period before enough pig carcasses would have been amassed and freezing may prove detrimental to the decomposition process. The only alternative to this would be unethical and expensive and would involve the large scale euthanasia of animals and subsequent purchase of the carcasses.

When looking at the surface depositions, there was a real chance that no tunnelling would be found, forensic evidence had suggested that surface deposits suffer far less from this phenomenon and the bactericidal affect of fly larvae which effectively sterilises large parts of the carcass (even within the bone) would obviously hinder this type of destruction. Drying of the corpse and exposure to sunlight as it decomposes is also inhibitive to bacterial activity. However, of the six surface pigs at one year postmortem, 1 had tunnelling, 4 had pre-tunnelling and 1 (the foetal pig) had no tunnelling. This degradation was far less advanced than in the buried pigs and is probably as a consequence of the limiting factors already stated. Some of the endogenous bacteria are obviously surviving long enough to cause some damage. It should also be noted that soil bacteria were not in direct contact with the bones as they were not buried. The tunnelling seen in the surface (possibly also the buried) bones may be attributable to the fact that during the period May-July 2007 there was an unusually high amount of rainfall (Ch 8.3) and the carcasses were very wet for long periods during the early postmortem period. This would undoubtedly have provided a favourable environment for the continuing growth of bacteria within the corpse.

Because the fetal skeletons have not suffered this fate it has to be presumed that MFD is a consequence of endogenous gut bacteria. If the soil microbes were to blame then all of the cadavers should have been affected to an equal extent, and the fact that many of the buried corpses had retained significant amounts of soft tissue (preventing access by soil microbes) tends to support this theory. Also of the six buried pigs all except the foetal pig were affected. This was buried high enough in the soil profile to be subjected to large colonies of soil bacteria and although it had

skeletonized there was no tunnelling and the bones were perfectly preserved. The archaeological sections corroborate this to a certain degree as it was found that foetal bones demonstrate MFD in 33% of cases compared to 91% of the overall population in the sections used for this research. The animal bones were less conclusive and although the percentage of MFD was lower than the human bone a significant amount (62.5%) had visible tunnelling. There may be cultural or environmental factors that account for this as there is no way of knowing where the remains came from or whether they had been butchered and none of the remains had observable butchery marks. Of course it is always difficult to reconcile what we find in cemeteries with their past histories as so many different variables act on bodies after their death that are simply uncontrollable and unknowable. A small change of rainfall in one year, or the type of soil, the bodies condition at death or postmortem processes are just a few of the issues that may affect any corpse and the subsequent way in which it then decomposes. These problems are usually seen when looking at the soft tissues of the body and in certain cases such as mummification we can visualise that something happened to that body that prevented its destruction. And in some cases we can tell that the body has been eviscerated or placed in a location known to be favourable to desiccation. The hard tissues of the skeleton are less forthcoming with their histories, once the soft tissue has decomposed we are left with nothing but bones and the story becomes more difficult to write. If the findings here are correct than maybe in the future those bones that show no signs of MFD or a retarded form when others from the same cemetery are destroyed by bacteria, then maybe we will be able to say something more meaningful about why they are so well preserved. Maybe they were mummified (i.e. Cladh Hallan Mummies) or disarticulated (Cist burials at Ingleby) or suffering from starvation or some diarrhoeal disease that left the bowel empty. This has to be a good starting point from which advances could be made quite quickly by fervent examination of these types of burial.

Microbial attack in bone presents two problems for the archaeologist. Firstly, and quite importantly, is the effect on bone integrity. Secondly, if microbial tunnelling is present in archaeological bone then we need to be looking at its source to evaluate how such bacteria impact upon the bodies that are recovered from ancient cemeteries that we subsequently analyse.

9.2 Implications for Bone Integrity Based Studies

Bone integrity is a vital component in 21st century Archaeology; more and more sophisticated techniques are being employed that are highly controlled. There is a basic need to understand the past in its fullest terms and to this end there is an endeavour to discover who the remains belonged to, where they came from, the diet that they consumed, familial relationships and the age and sex of the individuals or communities being researched.

Chemical and biomolecular analyses of archaeological bone are based on the extraction of bone collagen for studies of DNA, stable isotopes and radiocarbon dating. Some knowledge on the degradation of biomolecules in bone during the postmortem interval exists whereas data for teeth are deficient, although there appears to be a remarkable stability of collagen dental proteins over time (Dobberstein *et al*, 2008). Collagen degradation by microbial action can be detrimental to these types of research. According to Grupe (1992) bone displaying microbial invasion is less likely to yield intact biomolecules and because bacteria possess enzymes for the cleavage of all biomolecular fractions all archaeometric approaches must be aware of biases that microbial diagenetic change causes. Nielsen-Marsh (2002) adds that bones yielding well preserved biomolecules show no evidence of microbial attack. More research is required in this area to determine at what stage MFD becomes a problem for biomolecular analyses.

In a study by Kaiser *et al*, (2008) where postmortem interval indicators were being researched, it was reported that in exhumed human bone with PMI's of between 1 and 30 years, DNA was most prevalent in the outer (periosteal) part of the bone, less prevalent in the inner part (endosteal) and least prevalent in the middle of the bone (mesosteal). This would be expected as in many archaeological bone sections microbial attack is limited to the central (mesosteal) part of the bone with a clear band of preserved bone both at the endosteal surface and at the periosteal surface. However, it is then stated that although the outer section of the bone yielded the most DNA that this is due to amplification of extrinsic soil bacteria, in contrast the middle part of the bone is protected from soil bacteria and should therefore be the most useful area to extract DNA from. Two points are raised by this statement. Firstly, if the bones were buried at depth, which presumably they were as they came from a modern cemetery in Munich, then they would be protected from most soil bacteria that live much higher up in the soil profile; research shows that very few bacteria survive at depth. This may not necessarily be the case as soil and its

microbial populations are still little understood. And secondly, although soil bacteria would find it harder to penetrate the skeleton the bones are not protected from the endogenous bacteria within the body itself. The authors do not mention degradation of this type, but it is well understood and in these types of application, bones with visible MFD are often deselected for study.

Schoeninger *et al* (1989) suggest histological analysis of bone as a rapid method of eliminating those bones whose structure has been diagenetically altered. Haynes *et al* (2002) state that the relationship between histology and DNA survival may not be as strong as has been suggested and that there is still a lack of understanding associated with the mechanisms for DNA preservation. According to Burger *et al* (1999) the most important factors for DNA preservation are temperature, humidity, pH value, the geochemical properties of the soil, the amount of postmortal organic substances and the general degree of microbial infestation in the respective soil. This last point is not necessarily true as it is possible that bacterial attack in bone is caused by endogenous bacteria rather than soil microbes. Grupe and Piepenbrink (1989) were able to demonstrate that fungi contaminate bone by transmitting metals in to the bone that then becomes fixed in the mineral matrix. In soils that are heavily enriched with such contaminants they suggest that bone that appears to be affected by microorganisms should not be used for studies in to trace element analysis. Balzer *et al,* (1997) found that microbes are capable of changing bone collagen between 8 and 18mths and that this is accompanied by changes in carbon and nitrogen isotopic abundances and will hence interfere with the correct isotopic value identification of the native material.

A study of two Late-Roman populations from Spain suggests that significant diagenetic change results in elevated levels of Sr, Pb, Am and Mn. The Ca/P ratio is also altered preventing use of such bones for dietary reconstruction as well as for the investigation of the different individuals' health conditions, or of links between diet, health and skeletal indicators of growth stress (Zapata *et al*, 2006). Hiller *et al* (2004) suggest the use of small-angle X-ray scattering as a useful tool that can be used to quickly screen bone as to its preservational integrity. They quote that bone must stay on a specific diagenetic pathway for DNA to be amplifiable with the best samples having little to no mineral alteration and in addition a strong correlation between levels of nitrogen and mineral. Prior to selection for DNA analyses, Turner-Walker *et al* (2002) suggest assessing the bone for diagenetic change with HgIP as microbial assault may be so extreme as to preclude the preservation of DNA.

Garland (1987:124) asserts that chemical and biochemical techniques using the inorganic and organic composition of bone for the chronological ageing of specimens may produce spurious results if the components are sufficiently altered or absent.

If indeed endogenous gut microbes are responsible for the tunnelling that is so frequently observed, then there is a chance that any remaining microbial DNA could impact on the outcome of such studies. Similarly, Stable Isotope research that requires collagen analysis will also be affected due to the collagenolytic effect wrought upon the bone by the invading bacteria. Although it is less of a problem here as the collagen/mineral ratio does not change until 96% of the collagen has been lost. This makes it almost impossible to select for undamaged bones by analysis of the cemetery itself. Most commonly when looking at these problems the researchers have to section bones and physically select those bones that are not affected by MFD. This is good practice and should continue as there is no other way to tell a bones internal integrity at the macroscopic level.

The implications of bacterial tunnelling for modern studies that use bone collagen are apparent. Microbes are responsible for elevating or changing levels of certain biomolecules and any affected bone should be treated with caution or eliminated from such studies.

9.3 Implications for *In-Situ* Preservation

The best possible outcome would be a scenario where we could predict which of these bodies or cemeteries are likely to be affected by microbial attack without using invasive methods. This is unlikely to happen because once a body has been reduced to bones there is little evidence left as to the state of the corpse at the time of death. And this may be the only true indicator of how likely microbial attack will be in any given set of remains especially if the microbes are of bodily origin. This also rules out looking to the cemeteries themselves and helps somewhat with the decision on whether or not to preserve remains in-situ. Purely from a bacterial destruction point of view, the degradation by endogenous gut microbes will have taken place in the early postmortem period and further attack is unlikely to occur. A scenario where well preserved remains that have been in the ground for hundreds of years suddenly start being affected by MFD is highly unlikely. This is true whether the microbes are from the body or from the soil. This however is only true of

microbial attack and does not cover other areas of destruction such as corrosive soils or chemical hydrolysis.

9.4 Implications for Archaeology and Forensic Science

It is altogether obvious that for some reason not all bodies decay rapidly to a point of skeletonization and even where they do their overall condition can range from perfectly preserved to little more than a stain in the sand. Mummification is a prime example of the decay process being retarded to such a point that some bodies may be instantly recognizable even after many years of burial. In these cases it may be possible to say that bone tunnelling would be unlikely as these types of bodies provide an environment that is not conducive to bacterial growth and reproduction. In the case of infants can we decipher true cases of infanticide from natural death that occurs after a live birth? And also, should fetal remains survive preferentially in the archaeological record?

Infanticide is extremely difficult to prove. When the skeletal remains of very young babies are found in a forensic context, there is generally no way of telling when the child died. Most diagnostic tools would involve the soft tissues. Because we have seen that the fetal bones were the only ones not subjected to MFD there may be scope to use histological preservation as a guide to whether the child was sterile and therefore possibly a stillbirth or alternatively, if MFD is present, that the child had lived long enough to have developed a bacterial gut flora.

Looking at the second point raised, it has already been shown that there is scope for fetal bone to be better preserved than adult bone (Bolsover Cemetery, previously discussed) from the same cemetery and where the burials date to the same time period. However, because fetal bones are less often recovered oftentimes as a result of excavator inability to recognise such remains, but possibly also because there would have been fewer of this type of burial in the first place, it is difficult to say whether or not this is a true picture of bone survival in the very young. It is possible for bone preservation to vary across a site and this cannot be ruled out here. The effects on the soft tissue have a compounding affect on the hard tissues and it is reasonable to presume that those bodies that lack enteric bacteria for whatever reasons will possibly not suffer from diagenetic change in the form of post mortem bone tunnelling and where it does occur it will probably be limited.

9.5 Implications for Bone Preservation in the Laboratory

None of the above are ideal places for bacteria to live, they prefer warmth and moisture, access to the appropriate nutrients within the body and external conditions such as pH level, and reduction potential must all be available at the right levels. However during the experimental research carried out here, it was found that bone can quickly deteriorate when kept at ideal conditions. In five cases there has been moderate to extreme collagen loss in bone sections. Three sections (duplicates of sections) from the boxed pigs were kept but not mounted using fixative; instead they were placed on a glass slide and covered with a cover slip. These were then kept within a box in a lab. The boxed pig samples were from the first samples taken three months postmortem and were not examined again until around 18mths had passed. The slides that were fixed using Euparol had not degraded at all, but the un-mounted sections demonstrated both staining and collagen loss. The same occurred with two further sections (25kg surface 1st sample 6mths PM & Winter Burial 1st sample 1yr PM). These sections were taken in early November of 2007 and by January of 2008 there was collagen loss and staining. This rapid degradation of the collagen may be attributable to the way in which bone is treated at the point of sectioning. Thin sections of bone are cut using a Microtome; this piece of equipment consists of a diamond tipped blade that is cooled by water. The bone is therefore thoroughly drenched during the sectioning procedure. The sections are usually left only briefly to dry before a fixative is used for mounting and this must be seen as an adequate process as none of the fixed bones have degraded. However, the introduction of moisture into unfixed sections may prove detrimental. In addition to this there is a chance that environmental microbial contamination occurs at the time of sectioning. This is then compounded further by the fact that the sections were then kept at room temperature and not sterilised. Moisture, temperature and the possible addition of microbes or the reactivating of bacteria already contained within the bone may in sum act as a catalyst for microbial degradation under storage conditions. This leads to the questionability of whether or not excavated bones should be exposed to water in the post-excavation period. Bones are washed on a regular basis and this is common practice. Although bones are then left to dry either on trays or by other means some moisture will be retained for unknown periods. Having seen that collagen loss can occur in sections exposed to water (in less than 2 months) then it becomes necessary to understand that this process might be having detrimental effects on stored previously washed bones. It could be argued that skeletal remains are usually stored at lower temperatures, but it has been proven that destructive enzymes can act upon bone even under these conditions.

9.6 Future Directions

9.6.1 Bog Bodies

If indeed MFD is a product of endogenous gut microbes as this research appears to suggest then there are several ways forward. It would be of great importance to look at other bodies that had in some way been bacterially modified in the grave setting. Bog bodies we know are subjected to an antimicrobial environment that should in theory sterilise any bodies deposited there. Yet in two known cases, Lindow II & forensic remains from a muskeg bog (muskeg is a Canadian term for a bog, that often has a high concentration of large tree branches) some form of tunnelling is evident in the bone (chapter 2). Having not seen the bones it is difficult to say whether or not this is true tunnelling or some other phenomenon, but the authors themselves are well acquainted with MFD and as such it becomes more believable. It is possible that the gut microbes may be protected from the waters action for sufficient time to allow some form of modification to commence or the bodies may not have been placed in the bog immediately following death. Ideally it would be necessary to look at more bog bodies but this is unlikely to happen due to two limiting factors. Firstly, bog bodies are rare and when they are found it is unlikely that any destructive action would be taken. Unfortunately, the methods employed in this research are destructive and cannot be carried out any other way as the inside section of the bone has to be observed. Secondly, it is atypical for bog bodies to have bone surviving; rather there is often just skin and hair. Both of these facts make it unlikely that this type of research would ever be conducted.

9.6.2 Disarticulated/Excarnated Remains

Similar to this are those remains that have been subjected to destructive actions prior to burial such as disarticulated remains, especially where the remains have been disarticulated or excarnated in the very early postmortem period. This should in theory lead to bones that are largely free of microbes. Disarticulation at a point soon after death would certainly almost completely sterilise bones as the gut contents would be removed and no contamination would exist. With excarnation the outcome becomes less clear. If the remains were reduced to a mere skeleton very quickly by hot dry heat then it is possible that many of the bacteria would not survive, but in a cooler more humid setting bacteria may first proliferate within the body and migrate to the bones quickly where damage may then be caused. There is some proof that disarticulation affects bone tunnelling and this comes from two early Bronze Age cist burials at Ingleby Barwick (Ch 6), where disarticulation was

suspected due to the manner in which the bones were found. Unlike the rest of the skeletons that had been microbially altered these two displayed excellent preservation. Having examined the sections personally it would appear that disarticulation accounts for their preservation as the other burials were articulated and badly affected by MFD.

9.6.3 Mummified Remains

Mummies are another subset of remains that could prove highly definitive in MFD studies. Those remains that were unmistakably eviscerated soon after death thereby sterilising the body could shed some light on microbial tunnelling. But these are similar to the bog bodies in the sense that destructive options are not normally taken due to their cultural worth and rarity. Other mummies such as those that have mummified naturally may also be worthy of examination. Las Momias Naturales are natural mummies from Spain; they make up 2-3% of the bodies (the rest having decomposed) disinterred due to non-payment of perpetuity rights to the tomb (after five years), from crypts and niches and are known to have mummified due to two facts. Firstly, they were interred in crypts and secondly, on examination were found in general to have an empty bowel (Medina, 1993). It can only be presumed that those that became mummies had for one reason or another died with an empty bowel; this could be due to prolonged illness or diarrheal disease. Again it would be interesting to examine them further in respect of bone preservation rather than the soft tissue. More interesting are the remains from Cladh Hallan (Parker Pearson *et al*, 2005). A number of skeletons were found that from their preservational state, location and articulation pointed towards a form of mummification. One of the remains, an adult male, has a small band of microbial attack that is below the surface. Of course it would be expected that for mummification to occur, microbial activity would have to be halted. But a precedent has already been set by bog bodies that show definite MFD whilst being in an anti-microbial environment. Perhaps in certain cases bacteria are capable of migrating to the bone before the micro-environment becomes un-conducive to their survival. This could of course happen if the remains were placed in a bog for a short time or alternatively and more likely if the body was allowed to dry slowly over a longer period. Because the body would dry externally first there is time for the moist environment of the gut to carry on its usual mode of operation. The second scenario is more likely because the mode of attack is of an inside-out type that would indicate that the bacteria had got to the bone through the internal vascular system via the intestines. Either way it is

interesting to note MFD in mummified remains and demonstrates the complexity of the situation.

9.6.4 Canine Bone

Looking at the situation differently it might make sense to study canine bone. Most animal bones do not suffer from MFD because of the butchery that precedes their burial. But dogs are different because they were treated as pets and therefore they were often buried intact. The collection at Sheffield does not contain many dog sections but of the five available four were very well preserved with no MFD whilst the other was extremely affected by MFD. The four with good preservation are of modern origin and the poorly preserved specimen is from an archaeological site (Cladh Hallan). The modern specimens have no provenance and may have been obtained without the animal ever having been buried. Future studies of articulated archaeological canine bone may prove to be rewarding.

9.6.5 Juvenile Remains

When looking at foetal and juvenile bones the archaeological record is unclear as to how well they survive, and to the incidence of microbial attack. On one hand there are discrete burials of fetal material from the Bolsover collection that are near perfectly preserved. Yet from the same collection there are fetal bones that are clearly attacked. Contamination of microbial loads from other burials cannot be ruled out. The experimental research carried out here demonstrates that fetal bones should not suffer this fate, maybe enough time had not elapsed for the bodies to be affected but this is unlikely as they would have skeletonized quicker and the attack in the larger remains occurred whilst the cadavers still had considerable flesh and adipocere remaining. This would almost certainly point to a gut origin for the bacteria as the soil was not in contact with the bones. Soil bacteria are relatively immobile and it is unlikely that they had contact with the bodies. The smaller bodies had skeletonized and therefore were in contact with the soil, yet no MFD were observed. Five infants from Wijnaldum in the Netherlands (Colson *et al,* 1997) corroborate this hypothesis as they were also free of bacterial attack. When looking at a mother and her fetus who had died during pregnancy both sets of remains were equally degraded and both had extensive bone tunnelling. This is to be expected as the fetal bones would have been directly contaminated by the endogenous flora of the mother. Clearly, fetal bones appear less likely to be attacked but in some instances they are affected. It cannot therefore be a matter of a difference in bone

structure and the answer must lie firmly within the remit of bacterial action and whether or not they have access to the remains. To clarify this dilemma would entail an extensive study of fetal bones from discrete burial sites and this could be achieved by for instance looking at those remains from a site in Greece where babies and foetuses were placed in jars before being buried. This would serve two purposes; firstly, the burials are discrete and secondly they are protected from the surrounding soil at least until the jars collapse. This would provide a much more meaningful insight in to MFD phenomenon that could then be interpreted without the influence of many other burial variables.

9.6.6 Amputated Limbs

Singular bones that have been buried on their own may present another way of looking at how bacterial attack works. There are several known medical cemeteries where amputated limbs have been recovered. At first, this looks like an ideal situation to look at those buried bodies that have not been accessed by gut bacteria. If MFD were found then this would presumably have come from the soil. However, the need for medical removal of an arm or leg is probably caused by either trauma, bacterial infection or some other illness. All of these reasons will unfortunately allow bacteria to enter the bone. In the case of trauma it may be several weeks before the need to amputate becomes acute and may only take place to prevent infection affecting other parts of the body. Bacteria would already be within the bone and this may affect any MFD depending on which bacteria are present. In both of the other cases bacterial infiltration is also probable. If medical records were available that told us these factors then it might be possible to select those remains where bacterial infection was not an issue and this is unlikely to happen unless the bones came from modern amputations that had subsequently been buried. Anatomy schools would also be of interest but due to the dubious way in which bodies were often procured (grave robbing) this may also prove difficult. If the bodies had lain in the ground for any amount of time before transfer to the anatomist then there is a possibility that bacteria will already have transmigrated to the surrounding soft and hard tissues of the body. Again modern anatomy schools would be of more use but due to the constraints laid down by the Human Tissue Authority this is unlikely to happen.

9.6.7 Forensic use of Modern Human Bone

The ideal situation would involve looking at modern human bone in a forensic and controlled context. The human decomposition research facility located in America relies on donated bodies that are then left to decompose in a variety of settings. Data can then be collected that are relevant to the early postmortem period. A similar facility in this country would be of exceptional worth and although the concept is possibly achievable (as donation of bodies is a matter of consent) the ethical issues associated with such a facility would be difficult to reconcile and there is much remonstration (especially on religious grounds) against the use of such facilities in the USA. There remain problems even with this scenario as much of the research here is dependent on the bodies of the very young. Permission to use this type of material would almost certainly not be forthcoming. Other ways forward could include using bone that has been recovered forensically or by sampling of bodies that have been exhumed for re-burial. Theoretically this is possible but consent would have to be obtained for every individual; this would entail talking to the next-of-kin at a very difficult time.

9.6.8 Soil versus Skeletons

Most taphonomic studies are somewhat misdirected in their approach to MFD. This research has been the first to look solely at the differences between sterile and non-sterile bodies in a real time burial environment. Many of the other studies focus on soil processes which although insightful and interesting may be misguided if the true culprits are of a bodily source. Surely it would be more profitable to firstly eliminate the easiest suspects by looking at the bodies themselves. If answers cannot be found using this approach then a move to soil and its properties would be of use. Soil mechanics with regards to bacterial action are still little understood and in the field so many variables will affect any given body that it becomes a mammoth task to attempt to answer this question via these means. By looking at the obvious first, in terms of the abundance of archaeological material available it may be possible to illuminate this subject without having to carry out limiting field studies.

9.7 Conclusion

This has been a difficult but rewarding research theme. Ideally it would have been preferable to have the experimental research in place for a much longer period of time. Unfortunately due to the initial difficulties with DEFRA and the time constraints of the PhD itself have meant that the experiments had a limited amount of time in

which to decompose. However, the results are quite conclusive with a total lack of MFD in the foetal material; this fact really points towards the provenance of tunnelling as being of endogenous gut microbe origin. Fetal bone is surely as susceptible to bacterial attack, if not more so if it truly is more porous and less mineralized than adult bone, yet it was not found in the experimental material. Having assessed all of the evidence it would appear that endogenous gut microbes are the culprits of bacterial tunnelling of bone in the early postmortem period. Further research is necessary if this is to be stated with authority and the material needed such as bog bodies and mummified remains may shed more light on this theory. With any research protocol there is room for mistakes to be made and for false results to be recorded. It is therefore advised that further studies are undertaken looking directly at the bones themselves rather than focusing on soil science.

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