Thiel Embalming

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Published in:
Clinical Anatomy

DOI:
10.1002/ca.23491

Publication date:
2020

Document Version
Peer reviewed version

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Journal:	*Clinical Anatomy*
Manuscript ID	CA-18-0309.R2
Wiley - Manuscript type:	Original Communications
Keywords:	anatomy
Additional Keywords:	Thiel embalming, anatomical teaching, histology, skeletal muscle, tendon

Author Accepted Manuscript version of McDougall, S, Soames, R & Felts, P 2019, 'Thiel Embalming: Quantifying histological changes in skeletal muscle and tendon and investigating the role of boric acid', Clinical Anatomy. https://doi.org/10.1002/ca.23491

1) Title and Abstract

Thiel Embalming: Quantifying histological changes in skeletal muscle and tendon and investigating the role of boric acid

Introduction: Cadaver preservation methods impact their utilisation in anatomical research and teaching. Thiel-embalmed cadavers show flexibility, however the cause remains poorly understood. This study aimed to i) describe qualitative and quantitative histological differences between Thiel-embalmed and formalin-fixed skeletal muscle and tendon tissue; ii) investigate whether boric acid in Thiel solution is solely responsible for modification of tissues, and iii) explore whether the modifications observed could potentially explain the mechanisms underpinning the flexibility of Thiel cadavers. **Materials and Methods:** Skeletal muscle and tendon samples were harvested from mice preserved using formalin, Thiel solution, or modified-Thiel solution (without Boric Acid). Using standard H&E and Gomori's trichrome histological methods, tissues were examined to determine whether differences were apparent between the preservative treatments. **Results:** Differences were present between the Thiel and formalin-fixed tissues; formalin-fixed samples remained substantially more intact while Thiel-embalmed samples showed fibre fragmentation and lack of nuclei. The mean cell diameter of Thiel-embalmed muscle (24.4 µm) was significantly smaller ($P<0.005$) than formalin-fixed muscle (40.7 µm). There was significantly greater ($P<0.005$) fragmentation in Thiel-embalmed muscle (631.5 per $1mm^2$) compared to formalin-fixed muscle (75.4 per $1mm^2$). Samples embalmed using modified-Thiel showed a severe lack of integrity within internal tissue structure. **Conclusions:** This suggests that Thiel solution significantly alters tissue structure at cellular level, with quantitative data demonstrating

measurable differences between Thiel and formalin-fixed specimens. While the precise mechanism for these alterations remains unknown, it is shown that boric acid is not the only component of Thiel responsible for degradation of internal tissue structure.

Keywords: Thiel embalming; anatomical teaching; histology; skeletal muscle; tendon

2) Text

Introduction

Teaching, research and understanding of anatomy is facilitated by the preservation and subsequent use of cadavers. The method of cadaver preservation impacts on how it can be utilised within the context of anatomical teaching and research. Traditionally, anatomy departments have used formalin embalming solutions. However, the resulting unrealistic texture, inflexibility and colour, combined with the deleterious effects of formalin on human health, have encouraged the search for alternative methods of preservation (Coleman and Kogan, 1998; Messmer et al., 2010; Hammer et al., 2011; Hammer et al., 2012). One alternative method is Thiel embalming (Thiel, 1992; Thiel, 2002). While Thiel embalming solution still contains a number of potentially hazardous ingredients including formalin, boric acid, chlorocresol and morpholine, these are found in very low concentrations, making the use of Thiel cadavers during dissection safer than traditional formalin cadavers (Eisma et al., 2010).

Cadavers preserved using the Thiel method demonstrate texture, flexibility and colour that is more similar to *in vivo* conditions for many of the body organs and tissues while articulated joints are freely movable (Thiel, 1992; Jaung et al., 2011). However, preservation of some organs, e.g. the brain, is problematic often resulting in additional fixation being required. Despite Thiel preserved cadavers being used in an increasing number of surgical and anatomical investigations (Benkhadra et al., 2009; Boryor et al., 2010; Eisma et al., 2010; McLeod et al., 2010; Fessel et al., 2011; Prasad Rai et al., 2012; Ernesto Ottone, 2016; Verstraete et al., 2016; Lone et al., 2017; Zwirner et al. 2019) a distinct paucity of information remains pertaining to how and why it produces cadavers with more life-like qualities.

Few studies have specifically investigated the microscopic effects of Thiel embalming on tissue, however Benkhadra et al. (2011), Hammer et al. (2015) and Martynuik et al. (2014) have reported qualitative signs of histological degradation in tissue samples embalmed using Thiel solution. Benkhadra et al. (2011) examined skeletal muscle and tendon samples from Thiel preserved human cadavers, Hammer et al. (2015) examined skeletal muscle from Thiel cadavers and Martynuik et al. (2014) examined porcine Thiel embalmed skeletal muscle and tendon. All three reported modifications to the tissue samples including fragmentation, acellularisation and loss of integrity, with the skeletal muscle being affected more than tendon. To date, no studies have attempted to establish whether these histological differences between Thiel-embalmed and formalin-fixed tissues are quantifiably significant.

Additionally, while the cross-linking effect of formalin-fixation on tissue is widely accepted, the components responsible for the modification of Thiel-embalmed tissue

remain poorly understood. Benkhadra et al. (2011) attempted to explain this modification by highlighting the corrosive effect of acid on proteins. They suggested that the boric acid component of Thiel solution may be denaturing the muscle proteins, resulting in the breakdown of muscle fibre structure. However, while the degradative effects of acids on proteins are well documented, boric acid may not hold the sole responsibility for modifying the Thiel embalmed tissue - there are additional components of the Thiel solution which are likely to have an effect on tissue structure and these require further investigation. Both the Thiel perfusion and submersion solutions contain high concentrations of chemical salts. Salts are known to alter tissue structure, a phenomenon encouraged and harnessed in the meat technology industry in order to influence 'sensory' characteristics of meat such as appearance and texture (Desmond, 2006; Henney et al., 2010). It is therefore prudent to consider whether these chemical salts are playing an important role in modifying the structure of Thiel-embalmed tissues. Comparing a modified Thiel solution, with the Boric Acid removed, to the standard Thiel solution and using formalin as a control will help to determine whether it is the only component causing any modifications observed, or whether further consideration needs to be given to the role other components, such as the chemical salts, are playing in modifying Thiel-embalmed tissue. By better understanding which components of Thiel are responsible for the modifications observed in skeletal muscle and tendon, it may be possible to begin to better understand the mechanisms underpinning the modifications and their potential role in creating the flexibility found in Thiel cadavers.

The ability to understand the flexibility of Thiel-embalmed cadavers is important in the context of anatomical research and surgical training as this may impact on the types of procedures that can be reliably undertaken using the cadavers. Changes in the soft tissues surrounding bones, including skeletal muscle and tendon, are largely responsible for the flexibility, or inflexibility, of cadaver joints (Jaung et al., 2011), therefore the use of histology as a starting point to describe the gross structural changes occurring in skeletal muscle and tendon tissue may give an indication as to why this flexibility occurs in Thiel-embalmed cadavers.

The aims of this study were to use histology as a starting point to: i) describe differences between Thiel-embalmed and formalin-fixed skeletal muscle and tendon tissue both qualitatively and quantitatively; ii) investigate whether boric acid is solely responsible for the modifications seen in the tissue structure of Thiel-embalmed skeletal muscle and tendon tissue; and iii) ascertain whether the modifications observed could potentially indicate the mechanisms underpinning the flexibility of Thiel cadavers.

Materials and Methods

Due to the fact that part of the study involved the removal of the boric acid component of Thiel embalming solution and it could not be guaranteed that this modified solution would embalm effectively, mice were used as an animal model for human skeletal muscle and tendon tissue. Given that mice are routinely used as animal models for human skeletal muscle and tendon, and a pilot study showed there were no observable

differences between the effects of Thiel embalming on mouse tissue compared to human tissue, it was deemed acceptable to use them as an animal model in this context.

All mice used in the study were sacrificed by CO_2 inhalation and immediately preserved. Mice were embalmed in one of three ways; intra-cardiac perfusion of formalin, intra-cardiac perfusion of Thiel solution (Table 1) followed by 2 weeks in Thiel submersion solution; or intra-cardiac perfusion of modified Thiel (Table 2) followed by 2 weeks in modified Thiel submersion solution. Given the small size of the animals used, we feel that perfusion followed by two weeks in submersion fluid was ample to achieve full fixation. After this time the mice were then processed through tissue harvesting and histological examination. The number of mice used in each embalming treatment group, as well as the tissues harvested and histological processing carried out is listed in Table 3. Tissue samples were discarded if they suffered structural damage during harvesting or processing. Samples were selected to allow comparisons to be made within individual animals, as well as between different animals.

The tissues were processed for histological examination using standard techniques. Briefly, tissues were dehydrated through increasing concentrations of ethanol, passed through xylene and embedded in paraffin wax. A Leica microtome was used to section the samples at 6 µm before they were stained using haematoxylin and eosin (H & E), examined and photographed using a light microscope (Leitz Orthoplan).

Further transverse muscle sections were taken at 4 µm from the formalin and Thiel-embalmed samples and stained using Gomori's trichrome to visualise changes between muscle, collagen and nuclei which stain red, green/blue and black respectively.

The qualitative parameters used to determine differences between the preservative treatments on the basis of visible histomorphology included overall cell structure, cell organisation and integrity, the presence or absence of nuclei, the appearance of skeletal muscle striations in the muscle samples, visibility of connective tissue within the skeletal muscle samples and collagen fibre alignment in the tendon samples. Similar parameters were assessed in a total of eight additional control Thiel-embalmed samples (4 skeletal muscle and 4 tendon) that were either 'washed' overnight in ethanol/water prior to staining or were post-fixed in formalin. This was a posthoc investigation to ensure that any fragmentation or lack of staining occurring in samples was not the result of salts embedded in the sample, or the tissue being inherently too fragile for processing.

Quantitative data were gathered by measuring the diameter of muscle fibres on transverse sections using ImageJ software (Schneider et al., 2012) which calculates the minimum Feret's diameter of each fibre. The minimum Feret's diameter is the 'minimum distance of parallel tangents at opposing borders of the muscle fibre' (Briguet et al., 2004) and is used to minimise the impact of issues such as orientation of the cutting angle during sample sectioning when measuring the diameter of skeletal muscle fibres.

In addition, the number of visible breakages within longitudinally sectioned muscle fibres was recorded, using the counter tool in ImageJ, in order to quantify fragmentation of the muscle tissue. Finally, the presence of any areas of disruption within longitudinally sectioned tendon fibres was recorded using Image J to quantify disruption of the tendon tissue.

Statistical Analysis

The statistical analyses of the comparison between the formalin and Thiel-embalmed sample groups, for the mean diameter of skeletal muscle fibres, the mean occurrence of muscle fibre fragmentation, and the mean disruption of the tendon fibres was carried out using a Mann-Whitney test for two independent samples for non-parametric data. All analyses were carried out using IBM SPSS Statistics 21 software package.

The study was approved by the relevant institutional review board.

Results:

Post-hoc Thiel samples 'washed' overnight in ethanol/water, or post-fixed in formalin, prior to staining, did not show any observable difference in staining or fragmentation compared to those samples included in the main study. This suggested that any changes observed were a result of the Thiel embalming process itself rather than histological processing.

Qualitative changes: Formalin vs. Thiel vs. Modified Thiel

Skeletal Muscle:

On visual inspection all muscle samples maintained their gross structure, however when examined histologically all Thiel-embalmed and modified-Thiel-embalmed muscle samples showed considerable differences compared to the formalin-fixed samples: Figure 1 shows typical attributes of the different tissue treatments in longitudinal

sections. Changes in tissue structure were substantial enough to be obvious at low magnifications. All Thiel-embalmed muscle samples, including the modified-Thiel samples, were affected in a number of ways, including a loss of nuclear staining and a loss of definitive striations in some cells, considerable fibre fragmentation and a loss of cell structure integrity. These changes appeared to be more severe in the modified-Thiel-embalmed muscle samples compared to the standard Thiel samples. These histological changes were observed in each of the three muscles sampled (vastus lateralis, pectoralis major and extensor digitorum longus), demonstrating that the changes were not limited to any specific muscle or to one individual animal. These observations were not present in the formalin-fixed muscle tissues, where the fibres and their nuclei retained their histological integrity.

Gomori-trichrome staining of the Thiel-embalmed transverse muscle sections, showed relatively intact collagen present within the endomysium, perimysium and epimysium of the muscle samples (Fig. 2), suggesting that perhaps collagen is stabilising the gross structure of the tissues.

Tendon

On visual inspection all tendon samples appeared to maintain integrity of gross structure. However, on histological examination, the Thiel-embalmed and modified-Thiel-embalmed tendon samples showed minor differences compared to the formalin-fixed tendon samples (Fig. 3), with the changes becoming more apparent at higher magnifications. The Thiel-embalmed and modified-Thiel-embalmed samples, while very

similar to each other in appearance, showed less uniformity in the collagen bundles, with a slightly disrupted appearance and more breakages when compared to the formalin samples. Despite these modifications to the microscopic structure the fibres remained aligned. The histological modifications were observed in both tendons examined (calcaneal and extensor digitorum longus tendons), in each of the animals, demonstrating that they were not specific to individual tendons or individual animals.

These observations were not present in the formalin-fixed tendon tissues, where the fibres retained their histological integrity.

Quantitative changes: Formalin vs. Standard Thiel

Skeletal Muscle

The mean diameter of Thiel-embalmed muscle fibre samples was significantly smaller ($P<0.005$) at 24.4 µm (n=10, SD=±6.5) than that of formalin-fixed samples at 40.7 µm (n=10, SD = ±4.4), using a Mann-Whitney test.

The mean occurrence of fragmentation within Thiel-embalmed samples was significantly greater ($P<0.005$) at 631.5 per 1 mm^2 (n=10, SD = ±245.36) compared to 75.4 per 1 mm^2 (n=10, SD = ±49.07) in formalin-fixed samples, using a Mann-Whitney test.

Tendon

There was a significant increase ($P<0.05$) in the average amount of disruption in Thiel embalmed tendon sample images at 60 per 1mm^2 (n=10, SD = ±30) compared to the

formalin embalmed tendon sample images at 27.5 per 1mm^2 (n=10, SD = ±15), using a Mann-Whitney test.

Despite significant differences in the amount of disruption observed in Thiel and formalin tendon samples, the total disruption in Thiel embalmed tendon is very low compared to the disruptive fragmentation seen in Thiel embalmed muscle (631.5 per 1mm^2 in muscle compared to 60 per 1mm^2 in tendon).

Discussion:

It is clear that the two methods of preservation used in the current study are operating via different process: traditional formalin fixation produces crosslinking which preserves the tissue by preventing degradation, while Professor Thiel himself described his method as a process similar to 'pickling' (Thiel, 1992), a process which uses high concentrations of salts to preserve foods via anaerobic fermentation (Barrett, 2003). It is important to compare and contrast the effects of the two different preservation processes for a number of reasons. Comparing Thiel and formalin specimens serves as a control; there are fewer changes in structural integrity noted in formalin-fixed specimens, demonstrating that histological processing is not the likely cause of the degradation seen in Thiel-embalmed tissue, but rather that the degradation is inherently linked to the Thiel process itself. Perhaps even more importantly, if anatomy departments are to use soft-fix embalming methods such as Thiel, then they need to understand the effect this has on tissue structure, how it differs from the traditional formalin fixation used and what effect this may have on future anatomical and clinical research projects.

The present study has shown, both qualitatively and quantitatively, that the internal structure of skeletal muscle and tendon preserved using the Thiel method show significant changes. This supports the observations of both Benkhadra et al. (2011) and Martyniuk et al. (2014) who demonstrated similar degradative changes in skeletal muscle and tendon, as well as Hammer et al. (2015), who described Thiel-embalmed skeletal muscle cells as being 'dissolved' and having a 'washed-out' appearance, with a lack of nuclei and degraded structural borders. The quantitative data of the current study demonstrate that these tissue structure changes result in significant measurable difference between the Thiel and formalin-fixed specimens.

While changes in tissue characteristics of Thiel-embalmed samples have previously been described in a number of investigations (Benkhadra et al., 2011; Fessel et al., 2011; Wilke et al., 2011; Martyniuk et al., 2014; Hammer et al., 2015; Verstraete et al., 2015; Venne et al., 2018; Zwirner et al., 2019), only Benkhadra et al. (2011) have attempted to specifically explain which of the components of Thiel solution are responsible for the changes observed. They suggest that, given the denaturing effects of acids on proteins, boric acid must be responsible for the degradation of proteins involved in maintaining the integrity of skeletal muscle and tendon structure. This study sought to determine whether boric acid was the sole component responsible for tissue structure degradation by comparing tissue changes in a modified Thiel solution which contained no boric acid to those observed in the standard Thiel solution, with formalin-fixed samples serving as a control. The results of this study showed that the internal structure of skeletal muscle samples, from the modified-Thiel solution, underwent a severe change in internal

structure even though boric acid was excluded from the embalming solution, suggesting that other components also play a role in driving these changes.

As previously discussed, Thiel solution contains high concentrations of chemical salts in both the perfusion and submersion solutions (Thiel, 1992). The chemical salts in Thiel solution are considered to be responsible for fixation (Kerckaert et al. 2008) and indeed various salts have been used as part of the embalming process for thousands of years (Coleman and Kogan, 1998; Bremner, 2014). However, these salts may also affect tissue structure by solubilisation, a phenomenon encouraged and harnessed in the meat technology industry in order to influence the 'sensory' characteristics of meat such as appearance, texture and smell, (Desmond, 2006; Henney et al., 2010), although the process by which this occurs is complex and not completely understood. Links have previously been made between salt-based embalming processes and the similar process occurring during salting or curing of meat (Coleman and Kogan, 1998). It is, therefore, proposed that the chemical salts found in Thiel solution also play a major role, alongside boric acid, in denaturing the skeletal muscle myofibrillar proteins, leading to changes in fibre structure.

While the internal structure of Thiel and modified-Thiel samples appears to be affected, the external gross structure remains essentially intact. This could be explained by a lack of degradation of collagen in the structural membrane of the tissue samples. As shown using the Gomori trichrome stain, the collagen in the Thiel-embalmed muscle samples remains relatively stable and intact, despite the degradation and fragmentation of the muscle fibres. Preservation of collagen would also explain why tendon shows less disruption than skeletal muscle. It may also be the case that the proteins degraded

within the muscle samples play a role in helping to maintain the overall gross structure of the muscle samples. Indeed, Wolff et al. (2008) suggest that protein denaturation occurs due to the presence of high salt levels in Thiel solution, leading to precipitation and homogenisation of the tissue which, together with additional precipitation and linkage caused by the Thiel solution, helps to maintain the texture of the tissues. This is similar to a process often termed the 'salting out effect' in the meat technology industry where salts help to solubilise meat proteins which then form an exudate which acts as a sticky 'cement' between meat pieces (Smith, 2001, Hutton, 2002). It is perhaps likely that a combination of these two processes is helping to maintain the external tissue structure while the internal structure is degraded.

The flexibility of cadavers preserved using Thiel solution compared to formalin-fixed cadavers is one of the main advantages of this method as it allows more movement and facilitates training in surgical procedures (Giger et al., 2008; Kerckaert et al., 2008; Eisma et al., 2010; Prasad Rai et al., 2012; Yiasemidou et al., 2017); however very little is known about how this flexibility arises. This becomes important in understanding the role Thiel cadavers can play in education, research and surgical training. As the flexibility of cadavers is largely related to the composition of soft tissues surrounding joints (Jaung et al., 2011) it is highly likely that Thiel cadaver flexibility is, at least partially, a result of the change in tissue structure integrity, particularly that of skeletal muscle tissue. The widespread fragmentation observed in the Thiel fibres results in a lack of longitudinal integrity, meaning that there will be very little resistance offered to any stretch applied to the tissue. It is hypothesised that the mechanism underpinning the flexibility is likely a complex interplay between the degradation of the internal structure of skeletal muscle,

relative preservation of collagen integrity, as well as perhaps a role for protein exudate 'stickiness'.

Study Limitations and Suggestions for Future Work

There are a number of limitations with this study, which should be addressed in future work. Firstly, as previously discussed, due to modification of the Thiel solution as part of the study, it was deemed inappropriate at this stage to use human donor material, however the study would ideally be carried out using human cadaveric material.

This histological study alone cannot provide a definitive answer as to what is causing the flexibility observed in Thiel cadavers. However it is part of a wider study including both biomechanical and protein analysis investigations, which may provide a more robust hypothesis as to the mechanisms underpinning flexibility of Thiel cadavers. The results of these studies highlight the potential role of collagen in maintaining overall gross structure while the internal muscle structure is degraded. It is recommended that future work would involve a more detailed analysis of collagen structure to determine whether the collagen is indeed remaining relatively intact and providing the overall support for the gross tissue structure. This may be aided by examining the histological structure of additional body tissues embalmed using Thiel solution; those with relatively higher collagen content such as skin and ligaments, compared to those with relatively lower collagen content such as kidney and spleen.

A further recommendation would be to specifically investigate the role of the other components in the Thiel solution, in order to understand whether they are playing a key

role in the degradation of the skeletal muscle structure, as seems to be indicated by the results of this study. While it is hypothesised that it is the chemical salts playing a major role in the degradation of internal tissue structure, it would be prudent to investigate this further, while perhaps also investigating the effects of other Thiel solution components such as the ethylene-glycol which is believed to be responsible for the plasticity or 'soft haptics' of Thiel cadavers (Hammer et al, 2015). While it is likely that there is a complex interplay between the components of the Thiel solution and their effects on tissue, it would be useful to better understand the effects of each component on tissue structure.

Understanding how Thiel differs from formalin in its effect on cadaveric tissue, which components are responsible for the changes seen and what the overall effects of these changes are, is very important in determining which anatomical and surgical training procedures cadavers and their tissues can be used for. Not only will this allow more robust and reliable data to be gathered from any associated studies, but it will also permit departments to plan their use of cadavers accordingly – ensuring that cadavers are used to their full potential within an anatomical and surgical teaching and research setting.

Concluding remarks

From the results of this study, it is concluded that there are observable and measureable differences between tissues embalmed using Thiel solution and formalin. These differences are not solely due to the effects of boric acid on the tissue proteins and it is suggested that perhaps the chemical salts present in the Thiel solution are playing a role

in the degradation of the internal tissue structure. The changes observed, with degradation and fragmentation of the muscle fibre cells, while the surrounding collagen remains relatively intact, are very likely to be influencing the flexibility of Thiel cadavers as there is not the same impediment to flexibility that occurs when the tissues are 'hardened' during formalin fixation via crosslinking.

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2) Footnotes

There is no conflict of interest to declare.

3) Figure Legends

Figure 1: Comparison of H and E stained longitudinal mouse skeletal muscle samples preserved using Thiel (A, B, C and D), modified-Thiel (E and F) and formalin (G and H) shown at increasing magnification (A, C, E and G x4, B, D, F and H x40). A, B, E, F, G and H represent samples of vastus lateralis muscle, C and D represent pectoralis major muscle.

Figure 2: Comparison of Gomori trichrome stained transverse mouse vastus lateralis skeletal muscle samples fixed with Thiel's solution (A and B) or formalin (C and D), shown at increasing magnification (A and C x4, B and D x10).

Figure 3: Comparison of H and E stained mouse calcaneal tendon samples preserved using Thiel (A, B, C and D), modified-Thiel (E and F) and formalin (G and H) shown at increasing magnification (A, C, E and G x10, B, D, F and H x40). A, B, E, F, G and H represent samples of calcaneal tendon, C and D represent samples taken from extensor digitorum longus muscle/tendon complex.

4) Tables

Table 1 – Thiel solution recipe as used at Institution

Table 2 – Modified Thiel solution recipe – without boric acid

Table 3 – Sample Data

***Please see attached files**

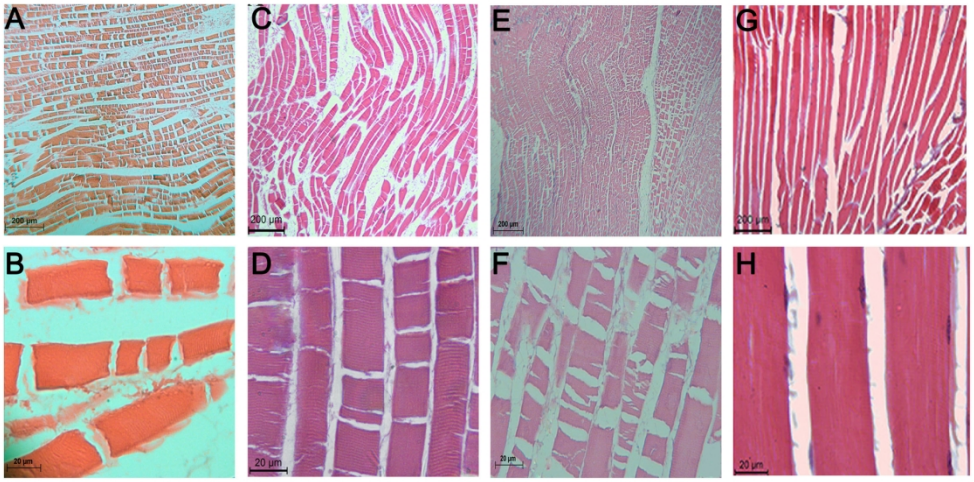

Figure 1: Comparison of H and E stained longitudinal mouse skeletal muscle samples preserved using Thiel (A, B, C and D), modified-Thiel (E and F) and formalin (G and H) shown at increasing magnification (A, C, E and G x4, B, D, F and H x40). A, B, E, F, G and H represent samples of vastus lateralis muscle, C and D represent pectoralis major muscle.

160x81mm (300 x 300 DPI)

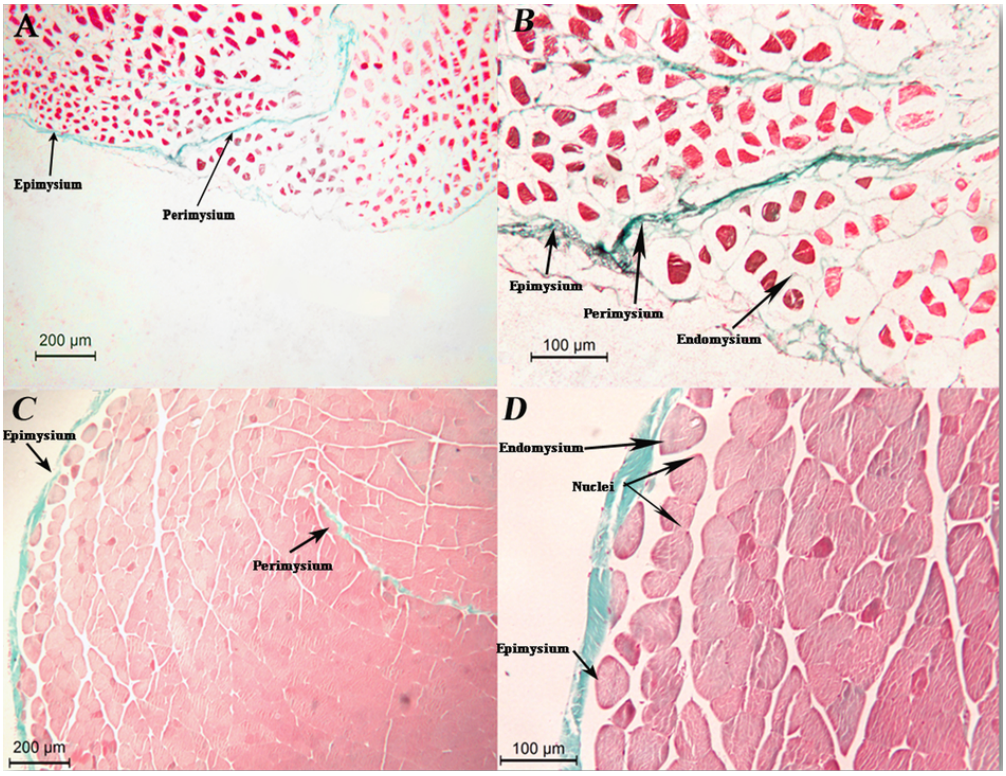

Figure 2: Comparison of Gomori trichrome stained transverse mouse vastus lateralis skeletal muscle samples fixed with Thiel's solution (A and B) or formalin (C and D), shown at increasing magnification (A and C x4, B and D x10).

80x61mm (300 x 300 DPI)

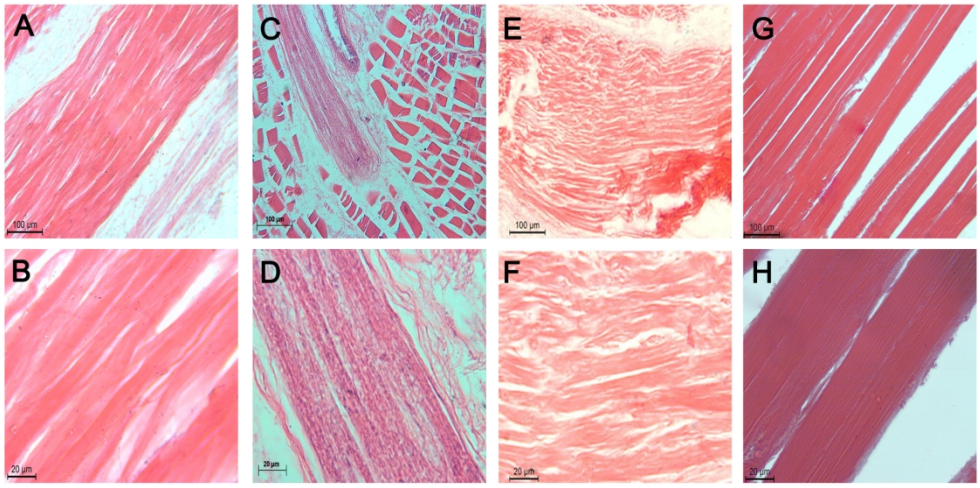

Figure 3: Comparison of H and E stained mouse calcaneal tendon samples preserved using Thiel (A, B, C and D), modified-Thiel (E and F) and formalin (G and H) shown at increasing magnification (A, C, E and G x10, B, D, F and H x40). A, B, E, F, G and H represent samples of calcaneal tendon, C and D represent samples taken from extensor digitorum longus muscle/tendon complex.

160x80mm (300 x 300 DPI)

Table 1 – Thiel solution recipe as used at Institution

Thiel Arterial Solution: (As used at Institution)	1 litre	**Thiel Submersion Solution:** (As used at Institution)	1.5 litre
Water	590ml	**Water**	1200ml
Boric acid	18g	**Boric acid**	41.25g
Propylene glycol	175ml	**Propylene glycol**	135ml
Ammonium nitrate	118g	**Ammonium nitrate**	135g
Potassium nitrate	30g	**Potassium nitrate**	67.5g
Stock II	35ml	**Stock chlorocresol solution**	28ml
Sodium sulphite	50g	**Sodium sulphite**	94g
Formalin (37%)	35ml	**Formalin (37%)**	28ml
Morpholine	10ml		
Alcohol	70ml		

Table 2 – Modified Thiel solution recipe – without boric acid

Thiel Arterial Solution: (As used at Institution)	1 litre	**Thiel Submersion Solution:** (As used at Institution)	1.5 litre
Water	608ml	**Water**	1241ml
Propylene glycol	175ml	**Propylene glycol**	135ml
Ammonium nitrate	118g	**Ammonium nitrate**	135g
Potassium nitrate	30g	**Potassium nitrate**	67.5g
Stock II	35ml	**Stock chlorocresol solution**	28ml
Sodium sulphite	50g	**Sodium sulphite**	94g
Formalin (37%)	35ml	**Formalin (37%)**	28ml
Morpholine	10ml		
Alcohol	70ml		

Table 3 – Tissue sample data

Treatment	No. of mice used	Samples Harvested (number in brackets)	Histological Processing & Analysis
Formalin Control - (8.9% formalin solution with phenol)	12	- vastus lateralis (23) - pectoralis major (6) - extensor digitorum longus muscle/tendon complex (22) - calcaneal tendon (24)	- H&E - Gomori's trichrome - Qualitative Analysis - Quantitative Analysis
Thiel	12	- vastus lateralis (24) - pectoralis major (5) - extensor digitorum longus muscle/tendon complex (20) - calcaneal tendon (22)	- H&E - Gomori's trichrome - Qualitative Analysis - Quantitative Analysis
Modified Thiel without boric acid	3	- vastus lateralis (4) - pectoralis major (2) - extensor digitorum longus muscle/tendon complex (3) - calcaneal tendon (4)	- H&E - Qualitative Analysis