Int. J. Morphol.,
***34(4)*:1442-1454, 2016.**

Walter Thiel's Embalming Method. Review of Solutions and Applications in Different Fields of Biomedical Research

Método de Embalsamamiento de Walther Thiel. Revisión de las Soluciones y sus Aplicaciones en Diferentes Campos de Investigación Biomédica

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OTTONE, N. E.; VARGAS, C. A.; FUENTES, R. & DEL SOL, M. Walter Thiel's embalming method. Review of solutions and applications in different fields of biomedical research. *Int. J. Morphol., 34(4)*:1442-1454, 2016.

SUMMARY: Walter Thiel developed the method that enables preservation of the body with natural colors in 1992. It consists in the application of an intravascular injection formula, and maintaining the corps submerged for a determinate period of time in the immersion solution in the pool. After immersion, it is possible to maintain the corps in a hermetically sealed container, thus avoiding dehydration outside the pool. The aim of this work was to review the Thiel method, searching all scientific articles describing this technique from its development point of view, and application in anatomy and morphology teaching, as well as in clinical and surgical practice. Most of these studies were carried out in Europe. We used PubMed, Ebsco and Embase databases with the terms "Thiel cadaver", "Thiel embalming", "Thiel embalming method" and we searched for papers that cited Thiel`s work. In comparison with methods commonly used with high concentrations of formaldehyde, this method lacks the emanation of noxious or irritating gases; gives the corps important passive joint mobility without stiffness; maintaining color, flexibility and tissue plasticity at a level equivalent to that of a living body. Furthermore, it allows vascular repletion at the capillary level. All this makes for great advantage over the formalin-fixed and fresh material. Its multiple uses are applicable in anatomy teaching and research; teaching for undergraduates (prosection and dissection) and for training in surgical techniques for graduates and specialists (laparoscopies, arthroscopies, endoscopies).

KEY WORDS: Embalming; Dissection; Walter Thiel; Surgical training; Review.

INTRODUCTION

The aim to preserve the body has existed since ancient times. This wish was in response to questions of a religious nature and the need to allow the deceased the passage to eternal life. With the beginning of human body studies and although religious rites remained in place, the need arose to preserve cadavers for prolonged periods of time. Several centuries passed until the discovery of formaldehyde (18th century), which has been the fixative par excellence until today. However, its toxicity and its use which results in increased cadaver stiffness and tissue darkening makes dissection and the practice of surgical techniques on the cadaver difficult. Therefore, in addition to the need to preserve bodies with characteristics that approximate the fresh cadaver as much as possible, considerable research has been devoted to search for the right solution to reduce and/or substitute the use of formaldehyde.

Among these solutions is the one created in 1992 by anatomist Walter Thiel. His fixation method involves preserving the body with natural colors through intravascular injection of a solution and subsequent immersion in a second solution for a determined amount of time, allowing the cadaver to be stored in a sealed container, outside the tank, without preservation fluid. This method of handling the cadaver is more efficient and convenient, and lacks the toxic or irritating gases due to minimum formaldehyde concentrations used in the formula.

The Thiel embalming method uses, among other components, 4-chloro-3-methylphenol as well as various salts for fixation, boric acid for disinfection, and ethylene glycol for the preservation of tissue plasticity (Thiel, 1992; 2002) (Table I).

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Table I. Basic composition of injection and immersion solutions described by W. Thiel in 1992.

Solution A	***Injection Solution***	***Immersion Solution***
Boric acid 3 g	Solution A 14300 ml	Ethylene glycol 10 ml
Ethylene glycol 30 ml	Solution B 500 ml	Formaldehyde 2 ml
Ammonium nitrate 20 g	Formaldehyde 300 ml	Solution B 2 ml
Potassium nitrate 5 g	Sodium sulfate 700 g	Boric acid 3 g
Hot water 100 ml		Ammonium nitrate 10 g
Solution B		Potassium nitrate 10 g
Ethylene glycol 10 ml		Sodium sulfate 7 g
4-chloro-3-methylphenol 1 ml		Hot water 100 ml

The cadavers are perfused via the femoral or carotid artery with an intravascular solution containing 14300 mL of solution A, plus 500 mL of solution B and the addition of 700 g of sodium sulfite as well as 300 mL formalin (Table I) for a body weighing 80 kg.

The original study described perfusion of the lungs with additional solutions using a tracheal tube, the intestine using a gastric tube and the brain through the superior sagittal sinus. These procedures seem to be unnecessary in cadavers used for surgical training courses (Balta *et al*., 2015a). Cadavers are stored for approximately 6 months in the immersion solution that contains 3 % (w/v) boric acid, 10 % (v/v) (mono-) ethylene glycol, 10 % (v/v) ammonium nitrate, 5 % (w/v) potassium nitrate, 7% (w/v) sodium sulfite, 2 % (v/v) formalin and 2 % (v/v) of solution B. After that, the cadavers are kept in zipper polyethylene bags. The cadavers can be used for months or years (Thiel, 1992, 2002; Balta *et al*., 2015b). The formalin content of the fixative is drastically reduced, with a final concentration of 0.8 % of the total formula, and thus the odor is reduced (Balta *et al*., 2015b).

The result of the application of the Thiel formula is a non-irritating and almost odorless product, preserving the color, flexibility and plasticity very similar to the living body with ample joint mobility that maintains tissue elasticity, apt for training surgical techniques and invasive clinical procedures.

The aim of this work was to review the Thiel embalming method, identifying authors who developed the original technique, those who modified it, and the studies showing the various uses of the body embalmed with this method in relation to teaching anatomy, morphology, and development of clinical and surgical abilities and skills. New anesthetic techniques, imaging and so forth are also examined, noting advantages and drawbacks in the application of this method compared to other fixation and preservation techniques.

METHODOLOGY

A review of the Thiel method was realized, searching in "Pubmed", "Ebsco", "Embase" where the following terms were used: "Thiel embalming," "Thiel embalming method", "Thiel cadaver" also searching all articles that have cited Thiel`s work. This search, conducted between March 21 and April 4, 2016, yielded 79 articles (Table II), and we added one article not included in these databases.

Table II. Search of Thiel´s technique articles in PubMed, EBSCO and Embase databases.

Database	**PubMed**			**EBSCO**			**Embase**		
Terms	Thiel embalming method	Thiel cadaver	Thiel embalming	Thiel embalming method	Thiel cadaver	Thiel embalming	Thiel embalming method	Thiel cadaver	Thiel embalming
n	**42**	**107**	**43**	**11**	**23**	**20**	**78**	**214**	**77**
Total		192			54			369	
Total found articles					**615**				
Repeated		42			11			78	
No Thiel		64			3			135	
Final selection of Thiel´s		43			20			77	
Total					**140**				
Repeated ones between					61				
Final selection					**79**				

The search for scientific articles related to the development of Thiel´s technique and its countless applications was carried out using PubMed, EBSCO and Embase databases. Applying the terms "Thiel embalming," "Thiel embalming method" and "Thiel cadaver" 192, 54 and 371 articles from each database were found for a total of 617 articles. From this last number, repeated articles and those that did not correspond to Thiel´s technique were identified and eliminated and thus it was reduced to 43 PubMed articles, 20 EBSCO articles and 79 Embase articles. A total of 142 articles were gathered. Finally, we compared these 142 articles and those that repeated were removed, reaching the final number of 81 articles (Table II).

In the search carried out of databases mentioned, and after selecting a total of 82 articles related to the Thiel embalming method, these were classified according to subject matter: anesthesia 10 articles, anatomic dissection 10 articles, method explanation 6 articles, animal models 4 articles, education 5 articles, surgical techniques (plastic, laparoscopic, gastric band surgery) 22, anatomic techniques 8, imaging 8, biomechanics 4, original article by Thiel 2, study of the middle ear 1.

DESCRIPTION OF THE THIEL METHOD AND ITS VARIANTS

In 2011 a survey was conducted in 311 anatomy centers worldwide, which established that only 11 % of centers regularly use the Thiel embalming method, and all were European. It confirmed further, that formalin was still the most frequently used fixative for embalming (Benkhadra *et al*., 2011a).

In South America, the Thiel method has been developed in Argentina, by the Dissection Team of the Anatomy Chair, at the Faculty of Medicine at the Universidad de Buenos Aires (Bertone *et al*., 2011).

As described initially (Table I), the Thiel method is basically comprised of two types of solutions: intravascular injection and immersion. In this regard, since the appearance of the Thiel method in 1992, many authors have reproduced the original technique (Groscurth *et al*., 2001; Wolff etal., 2008; Hölzle *et al*., 2012; Hunter *et al*., 2014; Cabello *et al*., 2015; Willaert *et al*., 2016) and in more recent studies, they have made modifications to the technique: in compound concentrations, in their combination and in the creation of solutions (Kerckaert *et al*., 2008; Bertone *et al*.; Eisma *et al*., 2013b; Hayashi *et al*., 2014; Hammer *et al*., 2015a) (Tables III and IV). The cadavers are perfused using the internal saphenous vein (Thiel, 1992; Groscurth *et al*.) and in case of difficulty finding it, the femoral or the common carotid artery can be used (Bertone *et al*.; Hammer *et al*., 2015a).

Thiel also describes another series of solutions to inject into the body: intrathecal, intratracheal and intrarectal (Thiel, 1992, 2002; Groscurth; Wolff *et al*., 2008; Hunter *et al*.; Cabello *et al*.). Nevertheless, not many authors have used them (Bertone *et al*.; Hammer *et al*., 2015a). Thiel (1992) indicates that every solution has to be prepared and used immediately, but this is not necessary with the main solution (solution A).

Table III. Injection solution by Walther Thiel and other authors (*1. Groscurth, 2001; Wolff *et al*., 2008; Hunter *et al*., 2014; Cabello *et al*., 2014). ** These authors adapted the proportion of Thiel´s formula according to the number of cadavers.

Solutions	SOLUTION A (for n)						SOLUTION B (for n)			INJECTION SOLUTION (for one cadaver)				
Authors	**Boric acid**	**Ethylene glycol**	**Ammonium nitrate**	**Potassium nitrate**	**Hot water**	**Total (aprox.)**	**Ethylene glycol**	**4-chloro-3-methylphenol**	**Total (aprox.)**	**Solution A**	**Solution B**	**Formaldehyde**	**Sodium sulfate**	**Total (aprox.)**
Thiel (1992; 2002)*1	3 g	30 ml	20 g	5 g	100 ml	-	10 ml	1 g	-	14300 ml	500 ml	300 ml	700 g	15800 ml
Bertone et al. (2011) (n: 1)	320 g	3200 ml	2100 g	530 g	1055 ml	1375 ml	3000 ml	300 g	3000 ml	1370 ml	3000 ml	300 ml	700 g	17000 ml
Kerckaert et al. (2008) (n: 6)	1900 g	19000 ml	12600 g	3200 g	63300 ml	75900 ml	18200 ml	1800 g	20000 ml	14300 ml	500 ml	300 ml	700 g	15800 ml
Eisma et al. (2013)	250 g (a)	2,5 L (a)	1,7 kg (a)	420 g (a)	6800 ml (a)	13000 ml (a)	10000 ml	1000 g	-	-	500 ml (a)	2100 ml (a)	-	-
	80 g (v)	780 ml (v)	520 g (v)	130 g (v)	1450 ml (v)	5500 ml (v)	(a;v)	(a;v)			190 ml (v)	1500 ml (v)		
Hammer et al. (2015) (n: 2)	300 g	3500 ml	2000 g	500 g	-	-	-	300 g	-	-	-	300mL	700 g	-

Table IV. Immersion solution (for n) by Walther Thiel and other authors (*1. Groscurth, 2001; Wolff *et al*., 2008; Hunter *et al*., 2014; Cabello *et al*., 2014). ** These authors adapted the proportion of Thiel´s formula according to the number of cadavers.

Authors	Ethylene glycol	Formaldehyde	Solution B	Boric acid	Ammonium nitrate	Potassium nitrate	Sodium sulfate	Hot water
Thiel (1992)*1	10 ml	2 ml	2 ml	3 g	10 g	10 g	7 g	100 ml
Bertone *et al*. (2011) (n: 1)**	1200 ml	2400 ml	2400 ml	3600 g	12000 g	6000 g	8300 g	120000 ml
Kerckaert *et al*. (2008) (n: 6)**	71900 ml	14400 ml	14400 ml	21600 g	71900 g	36000 g	50000 g	720000 ml
Eisma *et al*. (2013)**	150000 ml	125000 ml	30000 ml	45000 g	150000 g	75000 g	105000 g	1250000 ml
Hammer *et al*. (2015) (n: 2)**	22000 ml	4000 ml	190 g	6000 mg	20000 g	10000 g	20000 g	228000 ml

With respect to variations of Thiel´s technique, some authors explained the reason to apply these modifications. Hammer *et al*. (2015a), modified the injection site, injecting the femoral artery instead of the external iliac artery, saving work time in the cadaver. Eisma *et al*. (2013b), use lower formalin concentrations as opposed to the standard formalin concentration (8.9 % vs 37.5%), reducing the solution toxicity.

Another variation reported was the time the cadaver was kept in the immersion solution. In Thiel's original technique (1992), the immersion time is six months. However, Bertone *et al*. kept the body submerged for one month; Hammer *et al*. (2015a) and Eisma *et al*. (2013b) for two months; Healy *et al*. (2015) for two to three months; and Kerckaert *et al*. described the immersion of cadavers between four and six weeks.

None of the authors, who adapt the Thiel´s original solutions, compared the results with Thiel's original technique.

Despite variations in the application of Thiel's original method (Kerckaert *et al*.; Bertone *et al*.; Eisma *et al*., 2013b; Hayashi *et al*., 2014; Hammer *et al*., 2015a) the results in terms of the characteristics of the cadavers were similar in all studies; the cadavers were well maintained and had good color preservation.

The advantages of the Thiel method over embalming with formaldehyde were found to be, limited exposure to pathogens, less use of inhalable toxic products, a significant reduction in formaldehyde use. In relation to tissues, the color and texture were similar to the living body, maintaining the integrity of the three vessel layers (Odobescu *et al*., 2014). In terms of joint mobility, the Thiel-embalmed cadaver presented joint flexibility and muscle elasticity (Hayashi *et al*., 2014; Bertone *et al*.). Compared to fresh cadavers, although they also allow joint mobility to be studied efficiently with real coloration and minimal modifications to the tissue forms, the time of use is reduced, with rapid deterioration and advancement of the decaying process in a few hours, with the ensuing high risk of infections in the individuals handling these cadavers (Hayashi *et al*., 2016; Bertone *et al*.).

Moreover, Thiel-embalmed cadavers can be injected with various types of dyes, resins, natural latex, among others, that enable even the smallest vascular branches (smaller than 1 mm) to be identified, with the opportunity to visualize the entire vascularization (Hubmer *et al*., 2004; Bertone *et al*.; Healey *et al*., 2015).

The Thiel-embalmed cadavers are preserved for more than a year after removal from the embalming tank (Healey *et al*., 2015). Bertone *et al*. managed a correct preservation of the cadaver for three years with regular administration of the immersion fluid for hydration, just as Eisma *et al*. (2013a) who also reported the various surgical techniques to which cadavers were subject during the training of specialists. After the three years mentioned, the cadavers were disposed of by cremation. By contrast, Balta *et al*. (2015a) carried out a historical review and comparison of embalming techniques, suggesting that further research is needed on the chemical secreted prior to cremation of the Thiel-embalmed cadavers. Janczyk *et al*. (2011), mention that some chemicals used in the Thiel method are toxic and highly flammable, explosive and extremely dangerous for health and the environment.

The Thiel soft-fix embalming method for preserving cadavers with "natural colors" achieves well preserved organs and tissues, with natural color, consistency, flexibility, plasticity and transparency based on three processes: fixation, disinfection (bactericide) and preservation with solutions that contain 4-chloro-3-methywhich phenol acting as a fixative, boric acid which acts as disinfectant and ethylene glycol for the preservation of tissue plasticity, as the basic components (Thiel, 1992; Groscurth *et al*.; Thiel, 2002; Kerckaert *et al*.; Wolff *et al*., 2008; Bertone *et al*.;

Hölzle *et al*.; Eisma *et al*., 2013b; Hayashi *et al*., 2014; Hammer *et al*., 2015a; Healey *et al*., 2015; Hunter *et al*.; Cabello *et al*.; Willaert *et al*., 2016). Sodium sulfate and formalin are added just prior to perfusion. The final formaldehyde concentration is less than 0.8 %. Formaldehyde contributes antiseptic properties (Hammer *et al*., 2015a). The salts used (ammonium nitrate, potassium nitrate, sodium sulfate) absorb the water in the tissues, the nitrates they contain give the muscles a more reddish color through the action of the nitrosomyoglobin that is formed from the muscles themselves (Janczyk *et al*.; Hammer *et al*., 2015a). Thiel (1992) established that ethylene glycol is responsible for the haptic properties of the tissues. It is a fact that the preservation odor with this method is faint; it is also effective in disinfection and does not release noxious substances into the environment (Verstraete *et al*., 2015). Thiel confirmed the disinfection efficacy of the technique by bacteriologic tests and none of the cadavers showed mold (Thiel 1992). Kerckaert *et al*., cited the same.

Thiel (1992, 2002) described a special solution for brain fixation: tap water 40 mL, (mono) ethylene glycol 10 mL, Isopropyl alcohol 40 mL and Formalin 10 mL. The application of this solution is done with a lumbar puncture needle trough the lamina cribosa of the ethmoid bone, to get to the subarachnoid space in direction to the Bregma to get the anterior horn of the lateral ventricle and finally to get to the corpus callosum cistern. With this procedure, the solution will reach brain and spinal cord. Thiel described that a peristaltic pump may be used for this perfusion that allows a constant flow of the fixation solution (0,17 - 19 mL/min). The softness of the brain conservation was fixed by the addition of high concentrations of formalin.

Kerckaert *et al*. said that in future studies they will apply Thiel´s techniques for fixation of the central nervous system.

Eljamel *et al*. (2014) did some imaging studies of Thiel embalmed cadavers with MRI, revealing a good imaging with clear differentiation of white and gray matters, that suggested that it was a very good preservation of the brain.

Benkadra *et al*. (2011a) and Bertone *et al*. obtained a bad brain preservation with Thiel´s method. Probably, because they did not use Thiel´s specific brain solution.

To prove what Thiel said about the good flexibility and plasticity preservation in organs and tissues, several authors studied the biomechanical properties. Liao *et al*. (2015) studied the elastic properties of the ankle tendons and ligaments to evaluate the deformation rates. The results of this study of biomechanical traction demonstrated that the elasticity of Thiel-embalmed tendons and ligaments increases with the rate of deformation.

Verstraete *et al*. analyzed the elastic modulus, concluding that Young's modulus of human tissue is significantly altered in Thiel-embalmed tendons, a key determinant for the biomechanical properties, observing an increase in stiffness compared to fresh frozen samples.

Referencing studies by Liao *et al*. and Verstraete *et al*., Hammer *et al*. (2015b) wrote a letter to the editor mentioning that, to allow a more detailed interpretation of the results, it would be useful to know how material slippage and the stiffness of the testing machine were determined. They also established the need to add a summary of the Thiel fixative composition, in view of the existence of some variations, recommending that greater care be taken when determining that "Thiel-embalmed tissues are sufficiently similar to fresh tissues and suitable for many procedures and applications" (Liao *et al*.) on the basis of three donors, since, as they say very well, "Many other factors influence the final effect of Thiel embalming" (Verstraete *et al*.).

For their part, Joy *et al*. (2015) made a comparative quantitative evaluation with Young's modulus of the properties of Thiel-preserved tissues using shear wave elastography. The result was that the elasticity of the different tissues was similar in Thiel soft-embalmed cadavers and living humans. The elasticity was predicted by the embalming time and the tissue type, but not by the age at death or gender. In addition, there was a decrease in the mechanical efficiency of the bone after six months of immersion in the Thiel solution (Unger *et al*., 2010).

Another study researched the effects of Thiel embalming on the deep flexor tendons of fingers on cadavers versus fresh frozen cadavers and with rat tail tendon fascicles, because this is a well-established biomechanical model of a tendon. However, the results showed differences in the tendons preserved with the Thiel method in collagen fiber networks, likely due to the partial denaturation caused by boric acid. The tendons of Thiel-embalmed cadavers do not realistically represent the biomechanical characteristics of fresh frozen tendons (Fessel *et al*., 2011). Wilke *et al*. (2011) compared the biomechanical properties of the spine between fresh and Thiel-embalmed calf cadavers, concluding that the Thiel fixation maintains the non-linear load deformation but increases the range of motion. Therefore, they prefer fresh spinal columns for quantitative biomechanical testing since these maintain physiological conditions. Nevertheless, for preliminary orientation tests, the use of Thiel-preserved cadavers is recommended.

In other studies, preserved cadaver heads using the Thiel method have been used to analyze the mechanics of the middle ear in humans, with bone conduction, for clinical

Table V. Hayashi *et al*. (2014) (n: 2)

SOLUTION A		SOLUTION B		SOLUTION C		
4-chloro-3-methylphenol	Ethylene glycol	Ammonium nitrate	Hot water	Boric acid	Potassium nitrate	Hot water
66 g	660 ml	2500 g	4000 ml	370 g	620 g	5000 ml
RESERVE SOLUTION						
Solution A	Solution B	Solution C	Propylene glycol	Hot water		
660 ml	4000 ml	5000 ml	3700 ml	3300 ml		
FINAL SOLUTION						
Reserve solution	Sodium sulfate	20% Formaldehyde	Morpholine	Ethanol	Total	
16660 ml	800 g	600 ml	300 ml	1300 ml	18860 ml	

use in diagnosis and for the manufacture of headsets. Thiel embalming has the advantage of durability, which makes it possible to conduct experiments for long periods of time with reproducible results (Guignard *et al*., 2013).

In reference to disinfection ability, Thiel (1992) described that for cadaver conservation, the possibility of infection of conserved material must be considered. That is why he determined the bacteriological effects of conservation solutions through qualitative experiments from suspension culture, according to DGHM (Deutsche Gesellschaft für Hygiene und Mikrobiologie) standards to prove and assess the disinfectant effect of formula components. The utilized bacteria were: Staphylococcus aureus, Pseudomonas aeruginosa and Mycobacterium tuberculosum. Thiel´s conservation solutions were compared to a solution of phenol 5 % and a solution of formalin 5%. Notably, the infusion solution of the cadavers 1989 and viscera solution 1989 did not show major differences. On the other hand, phenol solution 5 % on Pseudomona effect, was very similar to viscera solution 1989, and did not differ either with infusion solution 1989. All germs were eliminated after five minutes of being diluted at 75 %. With formalin solution, results with or without serum were much worse. In its non-diluted state it required 60 minutes to eliminate Staphylococcus and Mycobacterium and when diluted at 50 % it took 6 hours. Nevertheless, viscera solution diluted at 50 % killed all bacteria after 5 minutes. Even with immersion solution 1986, which is not as strong, the results were far better in its non-diluted state than with formalin solution at 5 %. This clearly shows that Thiel´s conservation solutions have strong disinfection powers, however it would be incorrect to state that cadavers remain germ-free after long periods of conservation. Mugrowsky (1935, cited by Thiel, 1992), was able to demonstrate that after conserving cadavers in formalin and phenol, numerous aerobic and anaerobic bacteria survived.

It was determined that bacterial infections in dissection rooms did not have importance, but doubts remain whether AIDS can be excluded from these conclusions. In regards to this topic, at Graz University where the composition and effect of both new and old conservation solutions were studied, they noted in 1988: "According to experimental and practical information, these measures are enough to safely kill the HIV".

Since it was considered sufficient to stop the bacteria increase to prevent the bacterial decomposition effect in the cadaver, blood cultures in Staphylococcus and Pseudomonas under the inactivation substance CSL + 3 % Tween 80 + 3 % Saponin + 0,1 % Cystein + 0,1 % Histidin were made. For cadaver infusion dissolution diluted at 7.5 % in Staphylococcus an increase was not detected. Neither was an increase detected in 3 out of 4 samples of Pseudomonas. The viscera dissolution 1989 did not show either *Pseudomonas* or *Staphylococcus* increase (Thiel, 1992).

The blood culture with "phenol" dissolution at 5 % showed similar results, while the results with formalin dissolution at 5 % show effects on both bacteria, down to a dissolution of 2.5 %. This shows that formalin dissolution at 5 % has better inhibition results in comparison with phenol dissolution at 5 % but with worse disinfection results (Thiel, 1992).

Normally, in this injection procedure, conservation liquid equivalent to 1⁄4 of the cadaver's weight is added; considering that it is distributed evenly, a concentration of 20 % of conservation liquid should be detected. Just 1/3 of this concentration is enough to stop the bacteria increase, so after the injection solution, the cadaver should be safe from bacterial decomposition effects (Thiel, 1992).

The immersion solution without diluting it has a high safety factor to obtain a great cadaver conservation. With the immersion solution diluted at 90 % Staphylococcus aureus is killed in 10 minutes, *Pseudomonas aeruginosa* is eliminated in 5 minutes and Mycobacterium tuberculosum in 15 minutes (Thiel, 1992).

The drawbacks of the Thiel method were primarily the high costs of the required materials and assembly. In addition, it did not allow identification of the thrombogenic potential in the practice of vascular sutures (Odobescu *et al*., 2014). The preparations had to be preserved in hermetically sealed bags between 0° and 6 °C to avoid mummification (Healey *et al*., 2015). Bertone *et al*. also kept the cadavers in hermetically sealed bags, but at room temperature (20º C), although constantly controlling the dehydration of the body by regularly submerging it in the immersion tank. The appearance of blisters and blemishes can occur when exposed to the air for prolonged periods. Occasionally, due to causes unknown, the organs can display a gelatinous consistency (Benkhadra *et al*., 2011a).

Histological and morphological studies have been conducted comparing muscle fibers and tendons in fresh frozen cadavers embalmed with the Thiel method and with formalin, trying to identify anomalies in the tissue that explain the mobility of Thiel-embalmed cadavers. Staining was also used to visualize collagen and keratin. The collagen structure that forms the base of the muscle structure was preserved. The muscle fibers appeared as if they had been cut or ground, but were contained in a collagen sheath that stays intact, thereby preserving the general shape of the muscle. It is considered that boric acid is responsible for the damage observed (Benkhadra *et al*., 2011b). There is also less preservation of the histological characteristics, which would make using these cadavers for this type of research difficult (Hayashi *et al*., 2016).

Various articles described Thiel´s method applications. Thiel-embalmed cadavers have been used to carry out morphometric studies of anatomical muscle variations (Pichler *et al*., 2005; 2007), insertions (Grechenig *et al*., 2000), perforating vessels (Kappler *et al*., 2005), that have clinical implications in reconstructive surgery, repairs and reinforcement of tendons, and prophylaxis. Thanks to the Thiel technique, vessel diameters are very well preserved (Anderhuber, 2005). Anatomical studies have been conducted to implement new, standardized, non-invasive techniques to find certain anatomical structures in order to reduce the rate of injury or paralysis during osteosynthesis surgery (Lim *et al*., 2012). Hubmer *et al*. carried out dissection work and an analysis of the use of the term "recurrent" in the recurrent branch of the posterior interosseous artery of the distal part of the forearm.

Some anatomy centers have indicated that the cadavers are not suitable for dissections lasting several hours. Bertone *et al*., however, stated that when cadavers are well hydrated with the immersion solution, they can be subjected to daily sessions of at least 5 hours of dissection without difficulties.

The lumbar sympathetic trunk has been investigated for the identification of anatomical reference points through topographical studies correlated with images to locate its block point (Grechenig *et al*.). In addition, the posterior branch of the spinal nerve has been studied in relation to epidural anesthesia procedure (Saito *et al*., 2006). Feigl *et al*. (2011; 2013a) studied spondylopathies, their influence on the retroperitoneal spaces in the lumbar sympathetic trunk and their impact on the distribution of local anesthesia.

Several authors have determined the minimum volume of anesthetic product for application in three procedures: local anesthesia during breast surgery (Guay & Grabs, 2011), ultrasound-guided application (Desroches *et al*., 2013), and the stellate ganglion block (Feigl *et al*., 2007a).

Studies have been done on a supraomohyoid plexus block to avoid complications or collateral effects on other nerves (Feigl *et al*., 2006) and the spread of local anesthetic to block peripheral nerves (Munirama *et al*., 2012). For their part, Feigl *et al*. (2013b), identified a simple orientation mark to carry out the vertical obturator nerve block. In 2014, Feigl *et al*., evaluated the anatomical distribution of the nerves and its relation to the lumbar plexus to avoid the occurrence of interference complications during the block. Benkhadra *et al*. (2012), explained the possible failures during the ultrasound-guided infraclavicular nerve block. In addition, comparative studies have been done between Thield-embalmed and fresh cadavers for training in the new ultrasound-guided anesthetic technique in the cervical region (Benkhadra *et al*., 2009).

De Crop *et al*. (2012), studied the correlation between the physical and clinical image of the thorax x-ray in Thiel-embalmed cadavers using insufflation of the lungs while the image was being taken to simulate the pulmonary anatomy view on the thorax x-ray.

Sikora *et al*. (2015) used samples of the anterior wall of the vagina from patients who had had a hysterectomy, which were preserved in one of four ways: i) in cold, ii) fresh without fixation, iii) with formaldehyde, and iv) with Thiel solution, to demonstrate the potential of multiphoton microscopy in the study and 3D analysis of the extracellular matrix of the pelvic organs, in which they detected collagen and elastin, for later comparison with standard histological methods. The authors found no differences in collagen and elastin volumes when comparing the samples from the four groups.

Karakitsios *et al*. (2014a; 2014b) conducted studies to estimate the value of proton resonance frequency (PRF), as a preclinical model, for treatment with MR-guided focused ultrasound in human and bovine liver and porcine muscle. The PRF coefficient values measured in the Thiel-embalmed tissue were higher than for the fresh tissue; these results can be explained by the chemical composition of Thiel's fluid and electrical conductivity. Another study reported that the PRF in the focused ultrasound machine must be calibrated in order to apply it in Thiel-embalmed tissues. Gueorguieva *et al*. (2014) identified signal and contrast loss in clinical MRI sequence images due to high conductivity of fluids from the Thiel method.

Using elastography, changes in the muscle module section with, and without the tissues that cover it (skin and epimysium) were examined. The short muscle module decreased significantly by 50 % after the skin was eliminated (Yoshitake *et al*., 2016). Ando *et al*. (2014) analyzed the validity of estimating fascicle length in the vastus lateralis and intermedius of the quadriceps by ecography, concluding that quality of echographic images of Thiel-embalmed cadavers is equivalent to the quality of images obtained from living human beings.

Eljamel *et al*. (2014) suggest that long-term use of these cadavers (up to 15 months) makes the Thiel embalmed cadaver an excellent model for any other computer-assisted image-guided, minimally-invasive neurosurgery for training, such as DBS insertion. They consider that Thiel embalmed cadavers can be used for developing and testing various stereotactic functional neurosurgical procedures

Kerckaert *et al*. reported the results of the creation of the Endogent center, a center for anatomy and invasive techniques at the University of Ghent, Belgium, publishing their work experiences and uses of the Thiel embalming technique. The group that founded the center received specialist training at the University of Fribourg, Switzerland to learn this technique, which maintains the flexibility, plasticity and color of the cadavers and organs like a living person, with movable joints, the only difference being the absence of blood flow. They used the Thiel method for its subsequent use in education and surgical specialization courses for medical professionals in various techniques and procedures.

Expert anesthetists executed ventilation maneuvers with mask and tracheal intubation by direct laryngoscopy using Thiel-embalmed cadavers and mannequins, concluding that the Thiel-embalmed cadavers were more suitable for the ventilation with mask and provided a more realistic atmosphere for the laryngoscopy and tracheal intubation than the mannequins (Szucs *et al*., 2016). In addition, they have been used as a training method for regional anesthesia, as they present almost ideal conditions for the practice of this procedure (Feigl *et al*., 2007a; 2007b).

Results were published of a survey administered to undergraduate and graduate anatomy students on the experience of working with Thiel- and formalin-embalmed cadavers (Balta *et al*., 2015a). In the results students found that the Thiel solution was less irritating than formalin, raising concerns about the preservation of the nervous system and the difference in appearance of Thiel-embalmed cadavers and the images in the texts. Thus, the authors proposed that new books and manuals on dissection be produced. They demonstrated that surgical training has been successful in almost all areas using Thiel-embalmed cadavers. The appearance similar to the living body increases the risk of emotional and psychological impact on students after their first dissection session. It is possible to avoid this by preparing the students before their first encounter with Thiel-embalmed cadavers. According to Bertone *et al*., at no time has this been proven beyond the normal reaction of certain students in front of a cadaver, a situation that also occurred with formalin-embalmed cadavers.

One article presented the courses given at the University of Switzerland for the management of the new Thiel soft-fix embalming technique and construction of models that can be used for training in the microsurgery of paranasal sinuses, rhinoplasty and facial plastic surgery, surgery of the temporal bone (Groscurth *et al*.; Feigl *et al*., 2008), neurosurgery of the middle and posterior cranial fossa, endoscopic surgery of the abdomen and pelvis, interventional neuroradiology, and other techniques. Through these changes, knowledge of the macroscopic anatomy is improved as an essential topic for surgical training in this specialization (Groscurth *et al*.). An analysis of the usefulness of cadavers in anatomy education takes into account the importance of cadavers, considered "silent teachers", as they teach things that books or 3D software cannot, such as the anatomical variability between individuals, the effect of diseases or lifestyle on the body and the different textures and behavior of tissues (Eisma & Wilkinson, 2014).

The use of conventional embalming techniques with formalin presents an important limitation in terms of tissue texture (stiffer), odor, durability, as well as the impossibility of creating the pneumoperitoneum in formalin-embalmed cadavers, and thus the difficulty of undertaking endoscopic surgical techniques (Healey *et al*., 2015; Feigl *et al*., 2013b; Liao *et al*.). In this review, a large amount of literature was found that proposes the use of the Thiel-embalmed cadaver as a simulator to replicate surgical techniques and the training of surgeons. This is due to the preservation of real characteristics and the easy manipulation that such cadavers present (Bertone *et al*.; Eisma & Wilkinson; Healey *et al*., 2015). This way, the use of Thiel-embalmed cadavers has extended to various areas of surgery, particularly urological surgery (Healey *et al*., 2015), thyroidectomies (Eisma *et al*., 2011), cricothyroidotomies (Benkhadra *et al*., 2008), liver surgery simulations (Eisma *et al*., 2013a), arthroplasty (Windisch *et al*., 2001; Kamei *et al*., 2013), artery sutures (Odobescu *et al*., 2014; 2015), peripheral nerve repair (Matzi *et al*., 2015), flap surgeries (Hassan *et al*., 2014a; Wolff *et al*., 2014), neurosurgery (Schwalenberg *et al*., 2010), anastomosis techniques (Hassan *et al*., 2014b) and their comparison with animal models. Skills development in the area of angiology and laparoscopy are an additional advantage, several studies indicating the possibility of producing a pneumoperitoneum in the patient with the aim of accurately recreating abdominal laparoscopic surgery (Giger *et al*., 2008; Slieker *et al*., 2012) at kidney level (Prasad Rai *et al*., 2012; Ubee *et al*., 2014; Rai *et al*., 2015). Thus, it has been proven that the preservation of anatomical characteristics in Thiel-embalmed cadavers is adequate and allows for the realistic recreation of surgery.

Studies with Thiel-embalmed cadaver faces were conducted for cosmetic purposes, determining that the filling of the premaxillary space is an opportunity to make discrete corrections of the philtrum, in addition to having a better filling for superficial injections (Pilsl *et al*., 2014). Okada *et al*. (2012) reported using Thiel-embalmed cadavers for preoperative assessments and confirmed their usefulness, especially for the prevention of complications, or in the evaluation of surgical approaches. They concluded that Thiel-embalmed cadavers have many advantages over cadavers preserved with other methods and that they would also be useful for the development of new surgical implements or for the evaluation of a surgeon's abilities.

Holzle *et al*. (2012) used Thiel's technique to preserve cadavers destined for education and training courses in oral surgery and implantology through the positioning of dental implants in cadavers. This is the only study that applied Thiel's solution for such surgical training. The differences with respect to the animal models lie in certain differences in the morphology of the bone and teeth compared to the human, and the absence of resistance in the virtual models when performing the procedure. The advantages of animal and virtual models, on the other hand, lie in their reusability, which is not the case with a human cadaver.

Thiel's technique has also been applied to animals, mainly to produce animal models for the training of various surgical techniques. Willaert *et al*. (2014) created an animal model to achieve reperfusion of the porcine pulmonary vascular system using an injector pump, by means of which they introduced paraffin perliquidum into the pig's vascular system. They were able to determine that the paraffin perliquidum was suitable to reproduce reperfusion of the porcine pulmonary vascular system; however, found that the small vessels were broken in the process. In another study, Willaert *et al*. (2015) developed a model similar to the previous one to reproduce renal circulation. Later, Willaert *et al*. (2016) also produced another animal model for porcine vascular reperfusion that adequately reproduced the blood flow, reaching the vessels of the small intestine, providing real coloration of the serosa and mucosa as well as the remaining organs, achieving a more realistic reperfusion of the kidney with preservation of the anatomy. These researchers used methylene blue to identify the scope of the artificially produced reperfusion, and were able to prove that Thiel's technique is a low-viscosity solution, that passes easily through the capillary walls. Similarly, among some of the limitations raised by this study was the accumulation of the incorporated liquid, making it difficult to visualize the anatomical structures and their reperfusion.

Hassan *et al*. (2015) made a comparative study of surgical simulations for the repair of zone II flexor tendons in Thiel-embalmed human and porcine cadavers. They also compared them to formalin-embalmed cadavers. They showed the benefits of Thiel-embalmed cadavers in terms of better tissue flexibility, which allowed a correct surgical repair of the tendon, preserving the anatomy compared to formalin-embalmed cadavers and porcine models, with the latter being the most frequently used in surgical technique classes due to their greater availability. Thus, the superior usefulness of Thiel-embalmed cadavers for surgical practice was demonstrated.

CONCLUSIONS

Walter Thiel's method of fixation and preservation of cadavers with "natural colors" is based on three processes: fixation, disinfection, preservation with solutions that contain 4-chloro-3-methylphenol, potassium nitrate, ammonium nitrate, sodium sulfite, boric acid and monoethylene glycol as basic components. Thiel´s original and complete technique is complex in both its preparation and application in cadavers, nevertheless, this technique enables a prolonged preservation, maintaining the color, texture, plasticity and flexibility, similar to a fresh specimen. While using this method, storage does not require the use of tanks except for rehydration. In addition, it allows the injection of vessels and canaliculi up to the thinnest branches.

All these characteristics that Thiel-embalmed cadavers present, make possible their use with great advantage over formalin-embalmed cadavers, and even over fresh material due to the simplicity of their handling, reduced toxicity and prolonged duration. Their multiple uses are applicable in morphological research for the purpose of undergraduate and graduate teaching, in addition to training in specialized surgical techniques.

When analyzing the literature, the advantage of working directly on a human body is noteworthy, exceeding the possibilities of doing so on animal or virtual models which, though they aid in developing skills, do not have the same value of incorporating knowledge, as has the practice on a real human model.

Walter Thiel's technique of preserving the body in natural colors is a valuable contribution to the field of cadaver preservation and a breakthrough in the elimination of formaldehyde as the only fixative and preservative method, presenting a number of advantages that render it the first choice, when implementing a technique that is useful in education as well as research and surgical training.

OTTONE, N. E.; VARGAS, C. A.; FUENTES, R. & DEL SOL, M. Método de embalsamamiento de Walther Thiel. Revisión de las soluciones y sus aplicaciones en diferentes campos de investigación biomédica. *Int. J. Morphol., 34(4)*:1442-1454, 2016.

RESUMEN: En 1992, Walter Thiel desarrolló el método que permite la preservación del cuerpo con colores naturales. Consiste en la aplicación de una fórmula de inyección intravascular y el mantenimiento del cuerpo sumergido en pileta, en una solución de inmersión específica, durante un período determinado de tiempo. Después de la inmersión, es posible mantener el cuerpo en un recipiente herméticamente sellado, evitando así la pérdida del líquido fijador, fuera de la pileta. El objetivo de este trabajo fue revisar el método de Thiel, buscando todos los artículos científicos que describen esta técnica desde el punto de vista de su desarrollo, y su aplicación en la enseñanza de la anatomía y morfología, así como en la práctica clínica y quirúrgica. La mayoría de estos estudios se realizaron en Europa. Utilizamos las bases de datos PubMed, Ebsco y Embase con los términos "Thiel cadaver", "Thiel embalsamamiento", "método de embalsamamiento de Thiel" y se buscaron los documentos que citan el trabajo de Thiel. En comparación con los métodos comúnmente utilizados con altas concentraciones de formaldehído, este método carece de emanación de gases nocivos o irritantes; Da al cuerpo una movilidad articular pasiva importante sin rigidez; Manteniendo el color, la flexibilidad y la plasticidad del tejido a un nivel equivalente al de un cuerpo vivo. Además, permite la repleción vascular a nivel capilar. Todo esto hace una gran ventaja sobre el material fijado con formalina y fresco. Sus usos múltiples son aplicables en la enseñanza y la investigación de la anatomía; (prosección y disección) y para la formación en técnicas quirúrgicas para graduados y especialistas (laparoscopias, artroscopias, endoscopias).

PALABRAS CLAVE: Embalsamamiento; Disección; Walter Thiel; Entrenamiento quirúrgico; Revisión.

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Received: 16-08-2016
Accepted: 22-09-2016