Original Article

Efficacy of Acetone and Ethanol in Dehydrating Canine Body Sheets :Before and After Resin Coating

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Abstract

The aim of this study was to compare the efficacy of ethanol and acetone in dehydration of canine body sheets before preservation in resin for a 16-week period. A 10-kg male dog which died of the respiratory failure was preserved in 10% formalin via common carotid artery at room temperature for 7 days prior to being kept in a freezer at -25^{0}C for 7 days. The body of the dog was cut into 2.5-cm-thick sheets with a band saw. The body sheets were separately dehydrated for 3 days with either acetone or ethanol. In this study, the color of muscles and fat from acetone-dehydrated sheets was more significantly distinct than those dehydrated by ethanol, while the color of the organ surface was slightly changed in both groups. The shrinkage of body sheets did not significantly differ between the two groups. After dehydration, the body sheets were subject to preservation in resin for 16 weeks. At the first day in resin, the color of organs was slightly changed. After 16 weeks, the transparency of resin was not changed and the shrinkage of body sheets was not significantly different in body sheets of both groups.

Keywords: acetone, canine body sheet, dehydration, ethanol, resin

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บทคัดย่อ

การศึกษาประสิทธิภาพของอะซิโตนและเอทานอลในการดึงน้ำออกจากแผ่นลำตัวของสุนัขก่อนและหลังเคลือบด้วยเรซิ่น

เกรียงยศ สัจจเจริญพงษ์ ภาวนา เชื้อศิริ ปกรณ์ ประจำเมือง พนิดา ไพศิริยืนยง*

วัตถุประสงค์ในการศึกษาครั้งนี้เพื่อเปรียบเทียบประสิทธิภาพการดึงน้ำ ระหว่างเอทานอลและอะซิโตนจากแผ่นลำตัวสุนัขตัดตามขวางและเก็บรักษาสภาพด้วยเรซิ่นในระยะเวลา 16 สัปดาห์ สุนัขเพศผู้จำนวน 1 ตัว น้ำหนักประมาณ 10 กก ซึ่งเสียชีวิตด้วยภาวะระบบหายใจล้มเหลว ถูกนำมารักษาสภาพโดยการฉีดน้ำยา 10% ฟอร์มาลิน ทางหลอดเลือดแดงและเก็บไว้ที่อุณหภูมิห้องเป็นเวลา 7 วัน หลังจากนั้นนำไปเก็บในตู้แช่แข็งอุณหภูมิ -25 องศาเซลเซียส เป็นเวลา 7 วัน ทำการตัดสุนัขเป็นแผ่นตามขวางที่ความหนา 2.5 ซม แบ่งแผ่นลำตัวสุนัขที่ตัดได้ เป็น 2 กลุ่ม กลุ่มที่ 1 ดึงน้ำด้วยอะซิโตน กลุ่มที่ 2 ดึงน้ำด้วยเอทานอล ทั้งสองกลุ่มใช้เวลาในการดึงน้ำ 3 วัน หลังจากนั้นนำไปเก็บรักษาสภาพในเรซิ่นเป็นระยะเวลา 16 สัปดาห์ จากการศึกษาพบว่าการเปลี่ยนแปลงสีกล้ามเนื้อและไขมันในกลุ่มอะซิโตนมีความแตกต่างอย่างชัดเจนมากกว่ากลุ่มเอทานอล แต่สีของอวัยวะเปลี่ยนแปลงเล็กน้อยทั้งสองกลุ่ม พบการหดตัวของแผ่นลำตัวเล็กน้อยในทั้งสองกลุ่มแต่ไม่แตกต่างทางสถิติ การเก็บรักษาสภาพในเรซิ่นในวันแรกมีการเปลี่ยนแปลงสีของอวัยวะเล็กน้อย แต่ตลอดระยะเวลา 16 สัปดาห์ ไม่พบความแตกต่างของสีกล้ามเนื้อ อวัยวะ และความใสระหว่างสองกลุ่ม รวมถึงการหดตัวซึ่งไม่มีความแตกต่างทางสถิติ

คำสำคัญ: อะซิโตน แผ่นลำตัวสุนัข การดึงน้ำ เอทานอล เรซิ่น

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Introduction

Currently, there are several methods to preserve whole or parts of animals for teaching and research in anatomy such as embalming in formalin solution (Bickley et al., 1987, de Boer-van Huizen et al., 1992), plastination using chemical reagents including silicone (Bickley et al., 1987, von Hagens et al., 1987, Holladay, 1988, Miklosova and Miklos, 2004) and resin (von Hagens et al., 1987, de Boer-van Huizen et al., 1992, Sora and Brugger, 2000, Latorre et al., 2004, Gao et al., 2006, Sora and Cook, 2007), and preservation in resin (Sajjarengpong et al., 1997). Each method has some distinct disadvantages; embalming in formalin solution is the risk of toxic vapor. The process of plastination requires a lot of steps: fixation, dehydration, forced impregnation in vacuum and hardening (von Hagens et al., 1987), and extremely hazardous chemicals are also needed (Holladay, 1988, Sora and Brugger, 2000). The preservation in resin may result in bubble formation when waiting for the resin to harden (Sajjarengpong et al., 1997).

Plastination has been the best method for tissue preservation (Sora and Cook, 2007) and the plastinated specimen can be kept for a long period (Miklosova and Miklos, 2004). The important step in plastination is dehydration with chemical agents such as alcohol (Henry, 1995), acetone at -25^{0}C (Bickley et al., 1987, von Hagens et al., 1987, Holladay, 1988, de Boer-van Huizen et al., 1992, Henry, 1995, Miklosova and Miklos, 2004, Sora and Cook, 2007), acetone at room temperature (Brown et al., 2002), ethanol (von Hagens et al., 1987, Henry, 1995) and methanol (Sora and Brugger, 2000, Brown et al., 2002). The comparison of dehydration using ethanol and acetone has not been extensively studied in body sheets. Sajjarengpong et al. (1997) preserved brain in resin but application in body sheets has not been investigated. Several works reported body sheets with resin by plastination (von Hagens et al., 1987, de Boer-van Huizen et al., 1992, Sora and Brugger, 2000, Latorre et al., 2004, Gao et al., 2006, Sora and Cook, 2007). Therefore, the objective of this study was to compare the efficacy of ethanol and acetone in dehydration of canine body sheets before preservation in resin for a 16-week period.

Materials and Methods

Animal: A 10-kg male dog which died from respiratory failure was donated from the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. The dog was preserved in 10% formalin via common carotid artery at room temperature for 7 days, and then stored in a freezer at -25^{0}C for 7 days.

Body sheets: A frozen dog was sliced transversely at a thickness of 2.5 cm with a band saw. The first body sheet began at the first thoracic vertebra and continued until the first caudal vertebra.

Figure 1 Dehydrated body sheet in acetone (left side) and ethanol (right side).

Dehydration: The canine body sheets were divided into two groups. The first group (n=8) was dehydrated by 100% acetone at -25^{0}C for 3 days (group A). The second group (n=8) was dehydrated by 70, 80 and 95% ethanol at room temperature for 3 days (group B). The dehydrating agents in each group were changed every 24 hour (Fig 1). The body sheets were studied by comparing the effectiveness of dehydration by looking at the color of muscle, fat and organ surfaces. The shrinkage of body sheets was measured by the height and widths of the body sheet from the muscle passing over the spinal cord (Fig 2) on the first and third day. Measured values were analyzed using *t*-test (SPSS, version 11.5) with $p<0.05$ considered to be significantly different.

Preparation and preservation in a resin block: The dehydrated body sheets were embedded in resin with a secured silicone mold. Resin and hardener in ratio of 100 ml: 1 ml were mixed and poured carefully over the body sheets and let stand to set at room temperature. Molds were carefully removed after the resin was well set. The body sheets with resin were studied in terms of the differences in the color of muscle and organ surface. The shrinkage of body sheets with resin was measured by the height of the body sheets from the muscle passing over the spinal cord (Fig 3) on the first day, 8th, 12th and 16th week respectively. Measured values were analyzed using t-test (SPSS, version 11.5). Results were considered to be significantly different when $p<0.05$. In addition, the difference in transparency of the resin blocks were observed on the first day, 8th, 12th and 16th week.

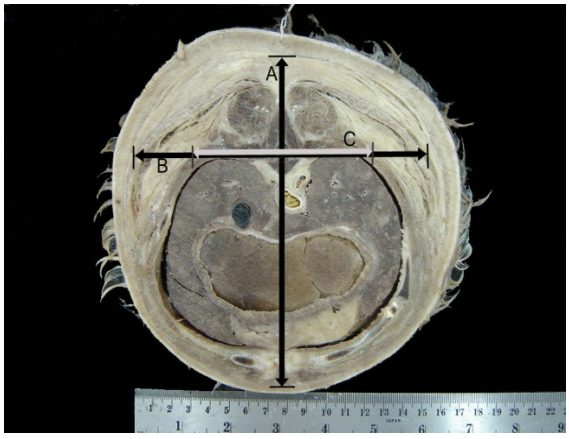

Figure 2 The height (A) and the widths (B and C) of body sheet.

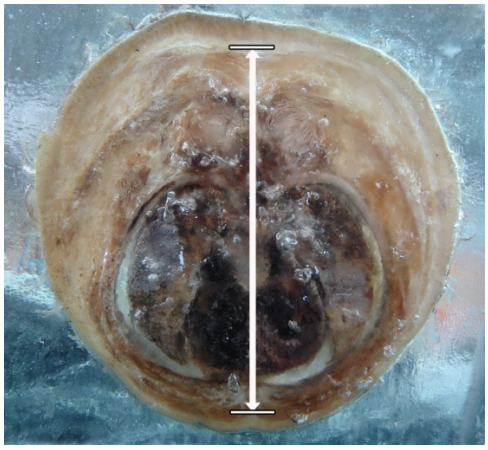

Figure 3 The length (arrow) of body sheet with resin.

Table 1 The difference of color and the shrinkage of body sheets before and after dehydration between group A and B.

Color	Group A		Group B	
	Before	After	Before	After
Muscle	Tawny	Darken	Tawny	No change
Fat	Ivory	More solid white	Ivory	No change
Organ	Brown	Slightly darken	Brown	Slightly darken
Shrinkage (cm) (N=8)				
Height (A) mean±SD	13.5±2.4	13.3±2.5	14.0±1.8	13.9±1.7
Width (B) mean±SD	13.6±3.0	13.5±3.1	13.2±2.1	12.7±2.2
Width (C) mean±SD	7.0±1.2	6.8±1.1	7.6±1.2	7.4±1.0

Table 2 The difference of color, the shrinkage of body sheets with resin and the transparency of resin blocks between group A and B during 16 weeks.

Group A	The first day	Week 8	Week 12	Week 16
Color				
Muscle	Yellowish brown	No change	No change	No change
Organ	Brown	No change	No change	No change
Shrinkage (cm) (n=8)				
Height mean±SD	12.1±1.44	12.1±1.44	12.1±1.43	12.1±1.43
Transparency	Clear	No change	No change	No change
Group B	**The first day**	**Week 8**	**Week 12**	**Week 16**
Color				
Muscle	Yellowish brown	No change	No change	No change
Organ	Brown	No change	No change	No change
Shrinkage (cm) (n=8)				
Height mean±SD	13.5±1.54	13.5±1.54	13.5±1.58	13.5±1.58
Transparency	Clear	No change	No change	No change

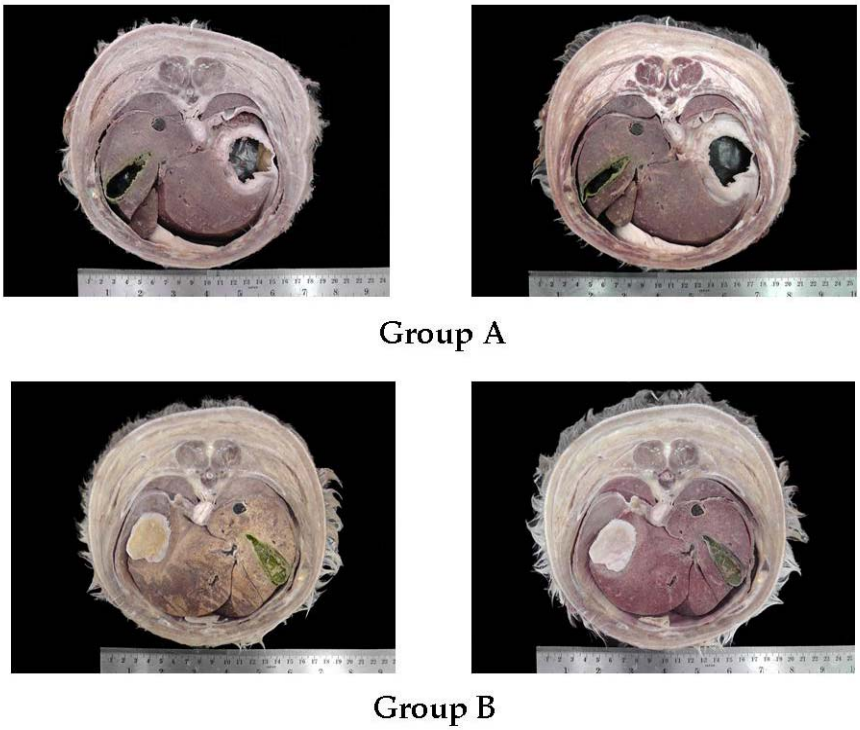

Figure 4 Body sheets of group A and B before dehydration (left side) and after dehydration (right side).

Results

The color of muscles and fat of body sheets from group A was more significantly distinct than those of group B (Table 1), while the color of the organ surface was slightly changed in both groups (Fig 4). The shrinkage of body sheets did not significantly differ between the two groups (p>0.05) (Table 1). At the first day in resin, the color of organ surface was slightly darkened. In group A, the color of muscles and organ surfaces of body sheets with resin was distinguished between two structures which was less than those in group B (Fig 5). During the 16-week period, the color of muscles and organ surface of body sheets with resin and the transparency of resin were not changed and the shrinkage of body sheets with resin was not significantly different (p>0.05) (Table 2).

Discussion

The important principle of plastination is dehydration (Holladay, 1988, Brown et al., 2002). In this study, the canine body sheets were preserved in resin and dehydrated with acetone at -25^{0}C and ethanol at room temperature. Previously, Sajjarengpong et al. (1997) studied brain in resin and dehydrated with acetone at -14^{0}C. Our study found that the color of muscles and fat could demonstrate a clear distinction in group A more than group B, while the color of the organs did not differ between the two groups. The shrinkage of body sheets was not significantly different in both groups. Acetone at -25^{0}C has been the standard method for dehydration (Brown et al., 2002, Sora and Cook, 2007) and Henry (1995) stated that acetone at -15 to -25^{0}C was the best method for dehydration. However, acetone is a

hazardous chemical agent. The authors used 70, 80 and 95% ethanol by beginning at low % ethanol as the standard method for dehydration (Henry, 1995). The period of dehydration with cold acetone should be 5 to 7 days (Brown et al., 2002), but in our study it lasted for 3 days which may affect the preservation in resin. Since the first day, while waiting for the resin to set, a number of air bubbles were found from the body sheets, similar to the study reported by Sajjarengpong et al. (1997). From their research, the air bubbles occurred fewer than those in this study because the size of the brain was smaller than that of the body sheet. It has been found that the duration of dehydration by -25^{0}C acetone was less than ethanol (Sora and Cook, 2000). However, in the present study, the duration of dehydration from the 2 groups was 3 days. The studied dog was preserved in 10% formalin via common carotid artery and kept at room temperature for 7 days. Bickley et al. (1987) reported that an appropriate percentage for preservation was 5-20% and the period for storing at room temperature should not exceed 14 days. A frozen dog was sliced transversely at a thickness of 2.5 cm, which was the maximum thickness for canine body sheet (Brown et al., 2002). In group A, the different color of muscle and fat of body sheets before embedded in resin was more apparent than after embedding whereas no changes were found in group B. Therefore, there may be a result reaction between acetone and resin. Throughout the period of 16 weeks, the color of organs and transparency of resin did not show a change that is consistent with Sajjarengpong et al. (1997). The color of resin of body sheets from the abdominal region was changed to yellow, which may be due to a lot of fat from this region molten by the reaction between fat and resin. The body sheets of the thoracic region did not show color changes. However, the transparency of resin blocks was clear in all body sheets.

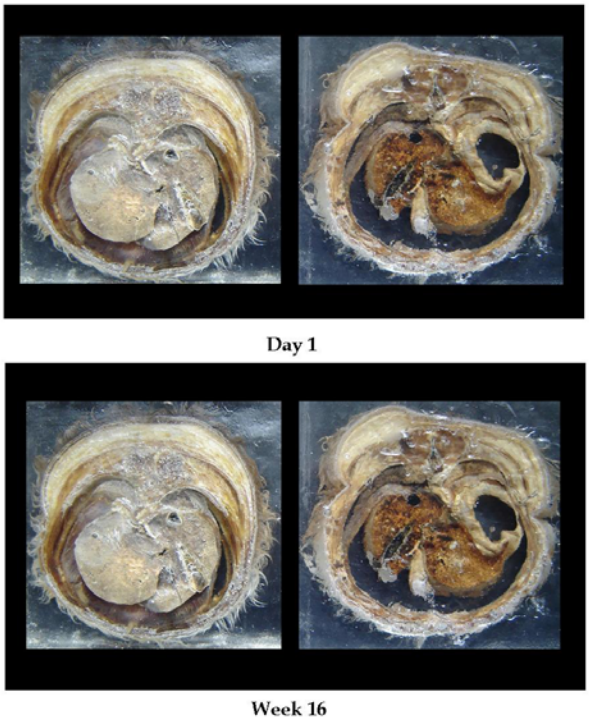

Figure 5 Body sheets with resin of group A (right side) and B (left side) on the first day and 16th week.

From our research, either ethanol or acetone could be used for dehydration. The efficacy of dehydration by acetone was more than ethanol, but ethanol was cheaper, safer and more convenient to get. For the preservation in resin, the body sheets of group A revealed the color changes of muscles and fat more than group B, while the color of the organ surface was slightly changed in both groups. The shrinkage of body sheets occurred slightly, but it did not significantly differ between the two groups. During the 16 weeks, the color of muscles and organ surface of body sheets with resin and the transparency of resin molds were not changed and the shrinkage of body sheets was not significantly different. However, the quality of body sheets with resin is not as good and beautiful as plastination sheets. If the resin has to be used, the effect of the reaction between resin and the agent for dehydration, preservation and the period of dehydration should be studied. In addition, care should be taken in terms of the prevention of air bubble formation and the reaction between resin and fat.

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