



UNIVERSITI PUTRA MALAYSIA

***DETERMINATION OF POST-MORTEM INTERVAL (PMI) VIA
IMMUNOHISTOCHEMICAL LOCALIZATION AND EXPRESSION OF
BIOGENIC AMINE (CADAVERINE)***

YEOW MEI JUAN

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**DETERMINATION OF POST-MORTEM INTERVAL (PMI) VIA
IMMUNOHISTOCHEMICAL LOCALIZATION AND EXPRESSION OF BIOGENIC
AMINE (CADAVERINE)**

YEOW MEI JUAN

A project paper submitted to the
Faculty of Veterinary Medicine, University Putra Malaysia
In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
University Putra Malaysia
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CERTIFICATION

It hereby certified that I have read this project paper entitled “Determination of Post-mortem Interval (PMI) via Immunohistochemical Localization and Expression of Biogenic Amine (Cadaverine)”, by Yeow Mei Juan and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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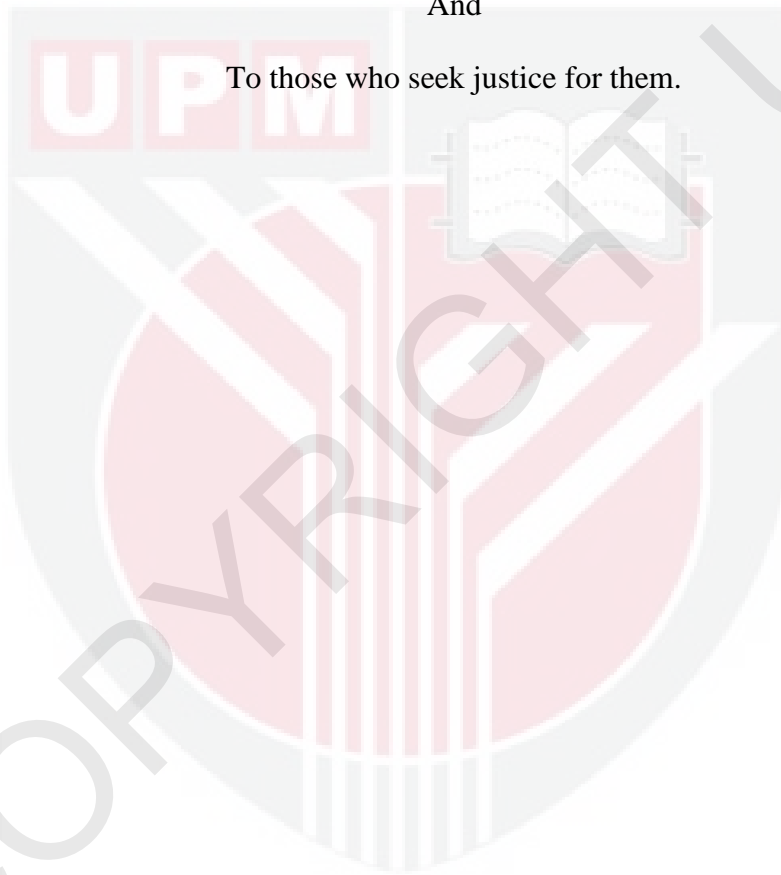
DEDICATION

This project is dedicated to my beloved family for being my tower of strength;

To all the animals that have suffered injustice;

And

To those who seek justice for them.



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First and foremost, I would like to thank my supervisor, Prof. Dr. Noordin Mohamed Mustapha for giving me this opportunity to conduct this project. It was indeed one of the most unforgettable and interesting experiences throughout my DVM years.

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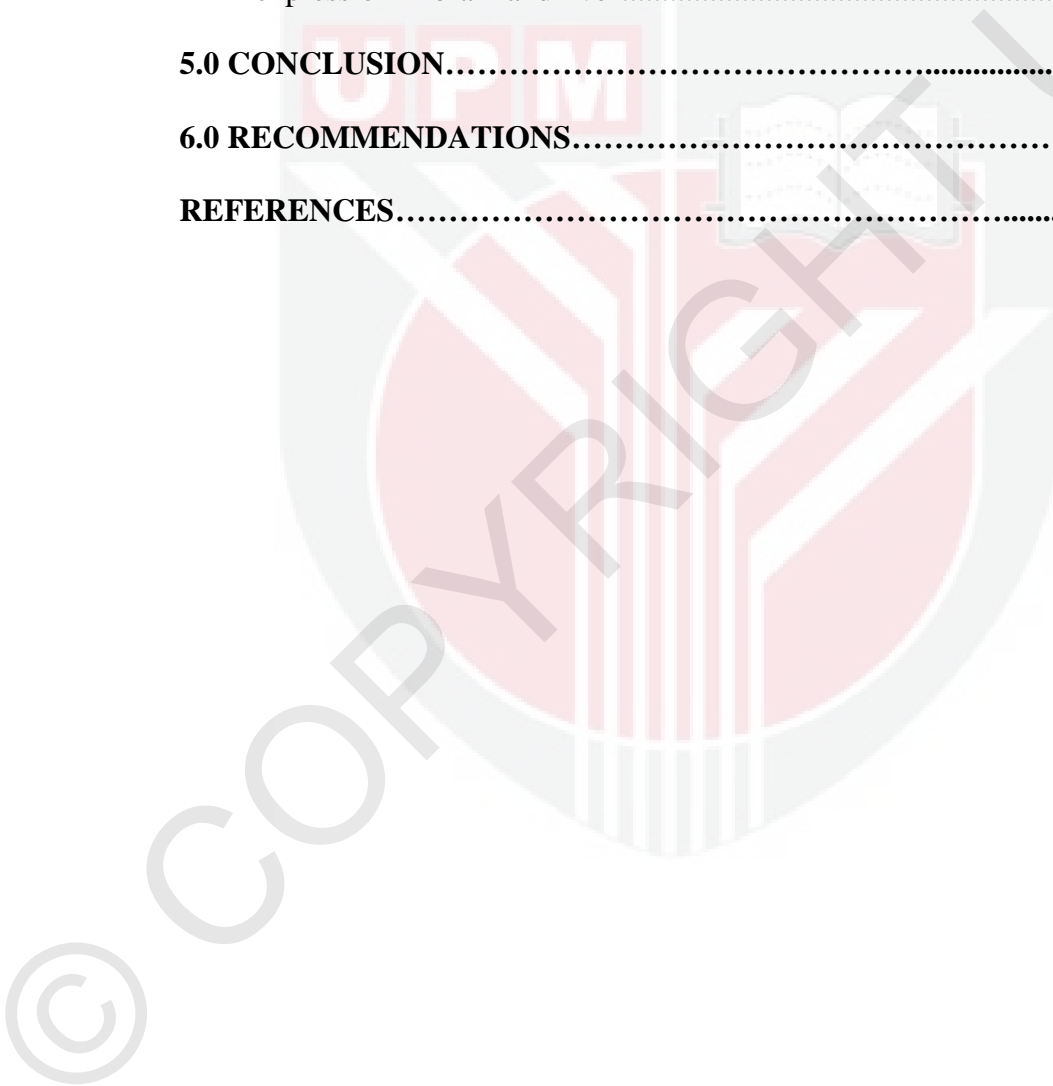
Last but not least, I would like to thank myself for the perseverance, the courage and the undying spirit to strive despite all possible challenges. Never forget to stay humble, positive and firm in everything you pursue. Have faith, and always stay strong!

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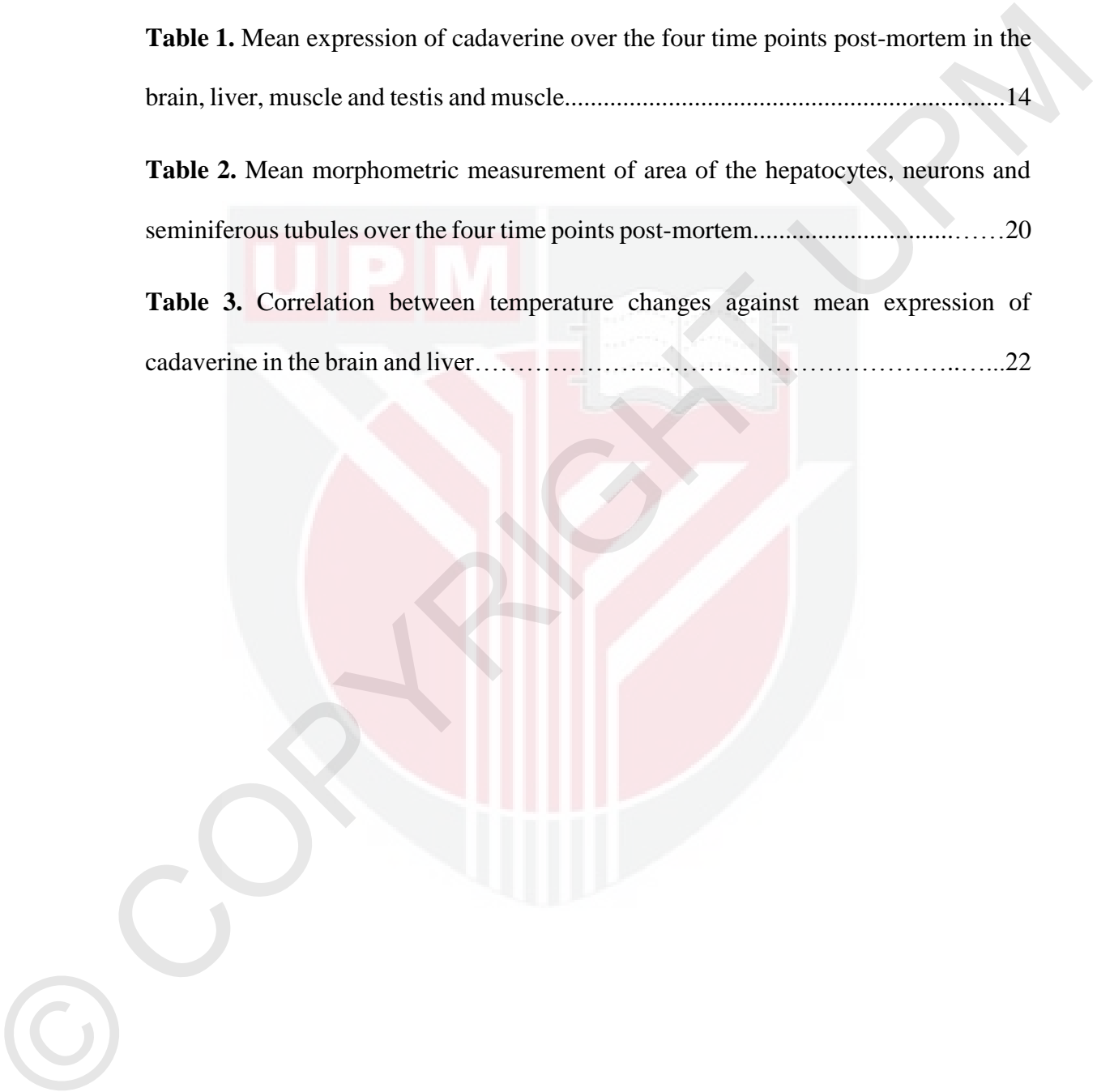
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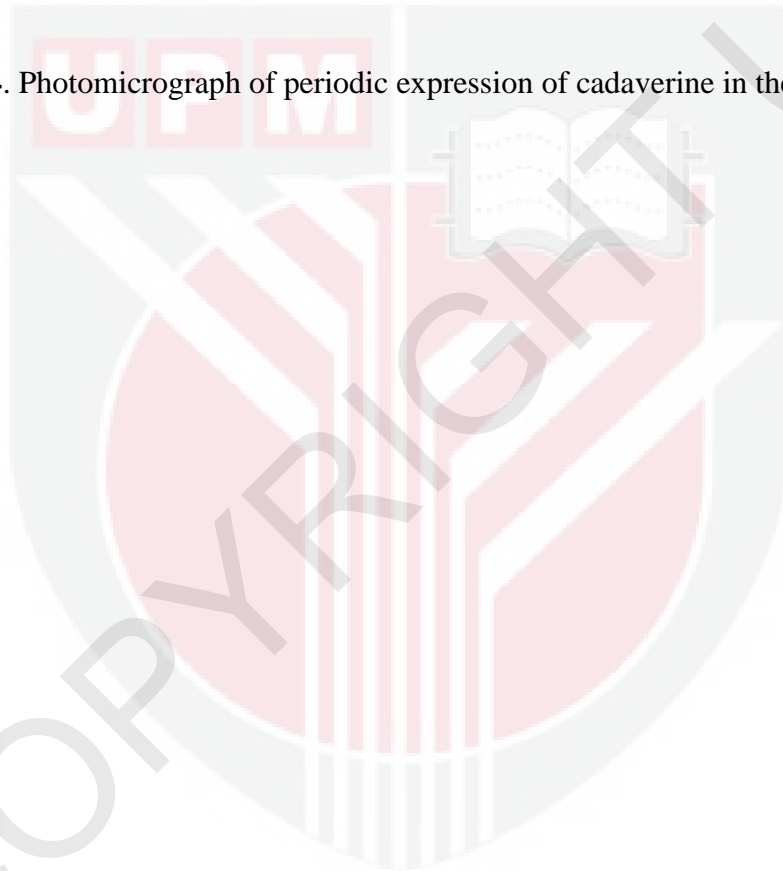
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LIST OF ABBREVIATIONS

| | |
|-----|-----------------------------|
| % | percent |
| °C | degree Celcius |
| μl | microliter |
| BSA | bovine serum albumin |
| cm | centimeter |
| DPX | dibutyl phthalate xylene |
| H&E | Hematoxylin and Eosin |
| IHC | immunohistochemical |
| kg | kilogramme |
| mL | mililiter |
| mM | milimolar |
| PBS | phosphate buffered solution |
| PMI | post-mortem interval |

ABSTRAK

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar
untuk memenuhi sebahagian daripada keperluan kursus

VPD 4999 - Projek Tahun Akhir

**PENGANGGARAN TEMPOH KEMATIAN MELALUI PEWARNAAN
IMUNOHISTOKIMIA DAN KEPEKATAN AMINA BIOGENIK**

(KADAVERINA)

Oleh

Yeow Mei Juan

2017

Penyelia: Prof. Dr. Noordin Mohamed Mustapha

Kekurangan pengetahuan mengenai sela masa pasca kematian (PMI) masih wujud disebalik penyelidikan meluas termasuklah amina biogenik (kadaverina) belum pernah dinilai sebagai petunjuk PMI. Maka, kajian ini bertujuan untuk mencadangkan parameter baru untuk penganggaran PMI melalui imunohistokimia dan taburan kepekatan kadaverina. Sampel otak, hati, otot dan testis yang diperolehi daripada tiga ekor anjing pada jam ke-0, ke-12, ke-18, dan ke-24 pasca eutanasia diawet dalam formalin 10% dan diproses untuk histologi dan imunohistokimia kadaverina (IHC). Sampel pewarnaan H&E juga dijalankan dan didedahkan kepada analisis morfometri. Kepekatan setanding kadaverina didapati pada jam ke-18 dan ke-24 pasca kematian adalah lebih tinggi daripada yang lain ($p < 0.01$). Kepekatan kadaverina hepatic melebihi tisu-tisu lain, kecuali kepada otak ($p < 0.01$). Walau bagaimanapun, kesan

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bergantungan kepada masa didapati pada semua organ diuji ($p < 0.01$). Walau bagaimanapun, kesan bergantungan kepada masa didapati pada semua organ diuji ($p < 0.01$). Ukuran morfometri hepatosit, neuron dan tubul seminiferous berbeza secara ketara ($p < 0.01$). Kesimpulannya, ternyata bahawa ungkapan kadaverina boleh berfungsi sebagai penganggar PMI. Walau bagaimanapun, kajian yang sama perlu tertakluk kepada amina biogenik yang berbeza dengan kuantifikasi tepat.

Kata kunci: Amina biogenik, imunohistokimia, kadaverina, sela masa pasca kematian

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ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary

Medicine in partial requirement for the course

VPD 4999 – Final Year Project

**DETERMINATION OF POST- MORTEM INTERVAL (PMI) VIA
IMMUNOHISTOCHEMICAL LOCALIZATION AND EXPRESSION OF
BIOGENIC AMINE (CADAVERINE)**

by

Yeow Mei Juan

2017

Supervised by: Prof. Dr. Noordin Mohamed Mustapha

A dearth of knowledge still exists despite extensive research being conducted on a reliable post mortem interval (PMI) indicator. Biogenic amine has never been attempted as an indicator of PMI and is evaluated in this study based on immunologically expressed cadaverine. Samples of the brain, liver, muscle and testis obtained from three dogs at 0, 12th, 18th, and 24th hour post-euthanasia were immediately fixed in 10% formalin and routinely processed for histology (Haematoxylin and Eosin) and cadaverine immunohistochemistry (IHC). The H&E-stained samples were also subjected to morphometric analysis. Comparable cadaverine expressions found at 18th and 24th hour post-mortem were higher than 0 and 12th hour ($p < 0.01$). Hepatic cadaverine expression surpassed other tissues, except

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of the brain ($p < 0.01$). However, a time-dependent effect was found on all organs tested ($p < 0.01$). The morphometry of hepatocytes, neurons and seminiferous tubules were significantly different ($p < 0.01$). In conclusion, it appears that cadaverine expression may serve as a PMI indicator. However, similar studies should be subjected to different biogenic amines along with its exact quantification.

Key words: Biogenic amine, cadaverine, immunohistochemistry, post-mortem interval



1.0 INTRODUCTION

The study of the time since death, or the post-mortem interval (PMI) has been one of the most popular fields of research in human forensic medicine. However, in a veterinary context, published data on the estimation of the PMI is scarce, especially studies on companion animals and domestic livestock (Erlandsson and Munro, 2007). Furthermore, most of the data were from finding under temperate conditions which may be incompatible to those found in the tropics. Accurate estimation of the time of death is crucial to the investigation of death via eliminating possible suspects or events, especially in cases of alleged offences related to neglect or abuse of companion animals, deaths of high number of animals, or animal deaths during transportation. Besides, in accordance with the Malaysian Government Act 999 (2009) and Animal Welfare Act (2015), the establishment of a legal framework which primarily aims for animal welfare warrants the need of a veterinary forensic knowledge. Hence, a reliable medico-legal death time estimation would allow evidence-based prosecutions to be carried out.

Despite some improvements which have been made for the past 30 years, according to Swift (2010), the existence of any single, reliable and accurate method in estimating the time since death during the early PMI remains debatable. Munro and Munro (2012) stated two basic approaches to the estimation of time of death: (1) the measurement of change that takes place at a known rate, and (2) the comparison of the occurrence of events known to have taken place at a specific time with the time of death. A list of techniques adopted in veterinary forensic investigations for the

estimation of time of death was stated by Munro and Munro (2012), namely temperature-based methods, post-mortem chemistry, electrical stimulation of muscle and nerves, gross appearance of body (rigor mortis, decomposition, shape, colour and luminosity of the eye etc.), histopathology and electron microscopy, radiology, DNA and RNA analyses, entomology and environmental and associated evidence. However, the lack of validation of many of these methods due to failure in demonstrating quantitative measurement and inclusion of a mathematical description causes the results open to challenge which may lead to ambiguity in legal cases.

Besides, it is worth noting that veterinary pathologists often estimate the time since death based on their experience of gross post-mortem changes such as autolysis, livor mortis, rigor mortis and putrefaction. However, the vast range of species covered and the variety of circumstances in which they are found renders these estimations to be questionable. Similarly, experienced pathologists in the field of human forensic pathology frequently underestimate PMI (James and Knight, 1965).

Forensic entomology can be of considerable value in a veterinary context, although the identification of the types and stages of maggots and beetles requires a prerequisite of knowledge on insect fauna (Anderson and Huitson, 2004). However, it is deemed necessary for veterinary pathologists to acquire appropriate skills and knowledge in the collection of entomological evidence.

On the other hand, various studies on the pattern of temperature drop (algor mortis) in estimating PMI showed important findings. These include the thumb rule of post-mortem rectal temperature fall of 1.5 °F per hour (Baccino *et al.*, 1996) and the

development of nomogram based on a single rectal measurement (Henssge, 1988), whereby the latter is currently the most widely accepted and practical method on time of death estimation. Body weight, varying degrees of ambience, the effect of wind, surface conductance and irradiation were among the confounding factors which have to be taken into consideration when applying temperature-based methods. A study by Abdulazeez and Noordin (2010) showed the rate of cooling under tropical conditions was less consistent than in temperate climates, also the absence of lag phase in the cooling curve which was derived from temperature measurements of organs in dogs as compared to human (Erlandsson, 2003; Abdulazeez and Noordin, 2010; Okene, 2010).

Thus, careful consideration and attention are required during the application of temperature-based methods in different regions, also during extrapolation between species. Researches on other methods in the determination of post-mortem interval, such as post-mortem chemistry, microscopic and ultrastructural changes and post-mortem radiology, however may serve as means of support and refinement, or as alternatives for further investigation and independent validation which are of relevance to forensic veterinary pathology (Munro and Munro, 2012).

Three main sources in which investigators could derive additional information during the determination of PMI would be evidences i. obtained from the carcass, ii. associated with the environment and iii. anamnestic which is based on ante-mortem movements or daily activities of an individual (Swift, 2010). Studies on methods extolling the vast number of identifiable changes which may occur during the PMI

should therefore be constantly conducted, in order to attribute a temporal value for the time since death.



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