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Comparison of macerations with dermestid larvae, potassium hydroxide and sodium hypochlorite in Wistar rat crania

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Abstract

Objectives: The aim of this study was to determine the most effective maceration method to remove soft tissue without altering bone tissue conformation.

Methods: A comparison was made between maceration with insects and chemical maceration performed on heads of Wistar rats. The sample consisted of 18 biomodels, six of which were macerated by dermestid larvae and the remaining 12 divided into two groups for chemical maceration, one with potassium hydroxide and the other with sodium hypochlorite. In chemical maceration, 1%, 5% and 10% concentrations were used with varying exposure times and temperature.

Results: The ideal method for soft tissue maceration, preserving all bone components, was shown to be maceration with insects. Potassium hydroxide was effective in the removal of soft tissue. However, being a highly corrosive chemical agent, it altered the integrity of the bone tissue. Sodium hypochlorite did not meet the maceration objective.

Conclusion: This research is relevant in its contribution to discussions on appropriate maceration techniques for small bone structures.

Keywords: bone; bone tissue integrity; chemical maceration; dermestid; maceration; osteology; skull; soft tissue maceration; tissue dissolution

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Introduction

Maceration provides bone pieces on which morphological and morphometric studies can be carried out. In the fields of anatomy and morphology, it has proved to be a powerful tool to produce analysis on morphometry of bone structures, which is not only qualitative but also quantitative. For this reason, maceration is an important technique for visualizing the bone component.

Various maceration techniques provide removal of the soft tissue adhered to bone structures. The most common maceraton techniques are use of inorganic chemical agents and insects. The length of time required to carry out the different techniques vary according to the size of the specimen, the ambient temperature and the technique itself.[1] In this study, three types of maceration on Wistar rat skulls were compared with the objective of establishing the most effective method to remove soft tissue, while keeping the hard connective tissues in optimal condition: maceration using potassium hydroxide (KOH), sodium hypochlorite (NaClO) and dermestid larvae.

Materials and Methods

This research was part of a project entitled "Evaluation of the effect of obesity on craniofacial morphology in Wistar rats in the neonatal (p21), preadolescent (p38) and young adult (p77) stages", approved by the Human and Animal Ethics Committee of the Faculty of Health in the Universidad del Valle, Cali, Colombia (Internal code: 013–017). The biomodels were used in accordance with the international principle of the 3Rs, (Replacement, Reduction and Refinement), once the aforementioned research had ended.

Maceration was carried out on 18 adult rats *(Rattus norvegicus)* of the Wistar strain from the Intermediate

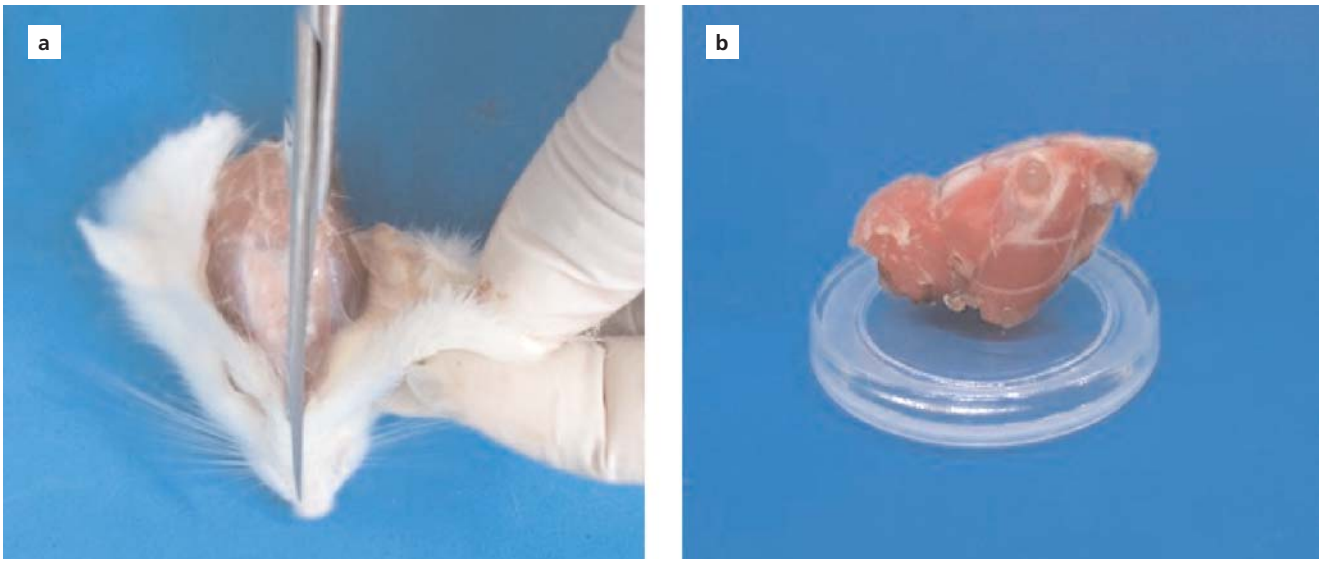

Figure 1. Wistar rat biomodel: (**a**) removal of the skin; (**b**) head removed. [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]

Laboratory of Preclinical and Animal Testing Research (LABBIO) of the Universidad del Valle in Cali, Colombia. Six of the biomodels were subjected to maceration by dermestid larvae while the remaining 12 to maceration with chemicals. The latter were euthanized with pentobarbital (390 mg/ml) and diphenylhydantoin sodium (50 mg/ml) applied intramuscularly (IM).

Subsequently, modifying the sampling protocol of Gage et al.,[2] all biomodels were cross-sectioned at the fifth cervical vertebra to obtain the head and the skin removed ventrally and dorsally towards the nose (**Figure 1**). During maceration with chemicals, written and photographic records were made during the first three days and on days 9 and 27. The heads were assigned randomly to the various maceration types. Each head was weighed on a calibrated electronic scale, Radwag brand, model WLC 2 / A2 (**Table 1**).

Maceration by Dermestid Larvae

For this study, one group of maceration was performed using dermestid larvae, which feed on dry animal material, primarily during the larval stage. The colony belonged to the Zoology Department of the Faculty of Biology at the Universidad del Valle. The beetle colony was maintained at an average temperature of 25°C with indirect natural

Table 1
Masseration type, biomodel number used (n), average weight (gr) and temperature.

Groups	Maceration	Biomodel (n)	Weight (gr)	Temperature
Group 1	Dermestid larvae	13 to 18	13.16	Room temperature
Group 2	KOH at 1%	3	15.40	Oven
	KOH at 1%	8	13.93	Room temperature
	KOH at 5%	4	12.81	Oven
	KOH at 5%	9	16.14	Room temperature
	KOH at 10%	10	13.96	Room temperature
	KOH at 10%	11	14.30	Oven
Group 3	NaClO at 1%	7	14.16	Room temperature
	NaClO at 1%	12	13.41	Oven
	NaClO at 5%	1	11.00	Oven
	NaClO at 5%	6	14.01	Room temperature
	NaClO at 10%	2	11.07	Oven
	NaClO at 10%	5	12.80	Room temperature

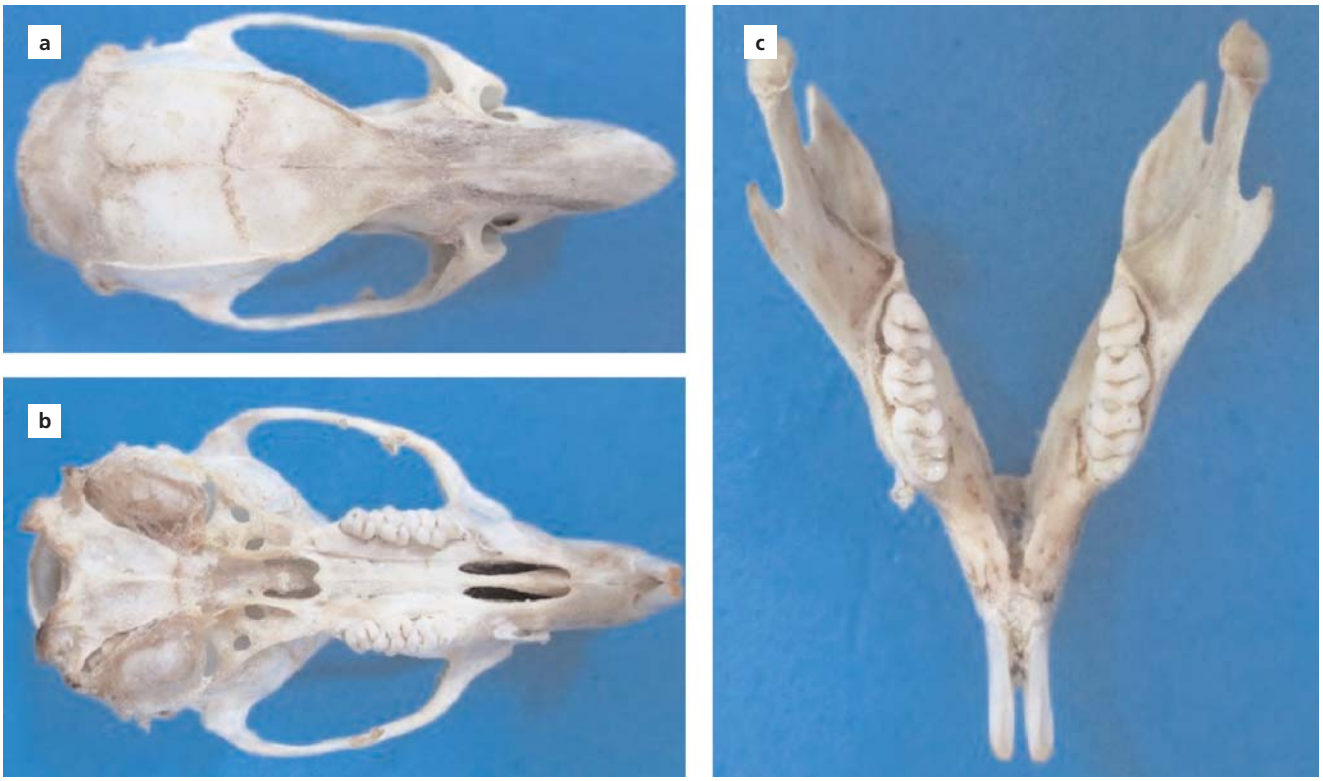

Figure 2. Maceration by dermestid larvae: (**a**) external base of cranium as viewed from above; (**b**) external base of skull as viewed from below; (**c**) jaw. [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]

light. For this maceration, the biomodel was euthanized with inhaled isoflurane; the technique used on the other 12 biomodels could not be performed in this case as exposure to chemicals intramuscularly in the specimen results in rejection of the tissue by the dermestid colony. Once the heads were obtained and the skin removed as detailed in the chemical macerations, the tongue, eyes and brain were removed to facilitate handling in the colony. The heads were put in an oven (Thermo Fisher Scientific Inc, Waltham, MA, USA; model 6528) for drying the soft tissue at a temperature of 56°C for 12 hours before being taken to the colony where it remained for eight days.

Maceration with KOH

Six KOH solutions were prepared in glass containers, two for each concentration. The solutions were 1% (1 g of KOH/100 ml of distilled water), 5% (5 g of KOH/100 ml of distilled water) and 10% (10 g of KOH/100 ml of distilled water). Six heads were selected at random and divided into two equal groups: Group A: Heads were subjected to heat in an oven (Precision Scientific Co, Tamil Nadu, India, model 16), at a temperature that ranged between 37°C and 45.5°C; Group B: Heads were kept at room temperature which ranged between 21.5°C and 25.4°C. The distribution of concentrations for each group is shown in **Table 1**.

Maceration with NaClO

Six NaClO solutions were prepared in plastic containers, two for each concentration. The solutions were 1% (7.7 ml of NaClO/92.3 ml of distilled water), 5% (38.4 ml of NaClO/61.6 ml of distilled water) and 10% (76.9 ml of NaClO/23.1 ml of distilled water). Six heads were selected at random and divided into two equal groups: Group A: Heads were subjected to heat in an oven (Precision Scientific Co, Tamil Nadu, India, model 16), at a temperature that ranged between 37°C and 45.5°C; Group B: Heads were kept at room temperature which ranged between 21.5°C and 25.4°C. The distribution of concentrations for each group is shown in **Table 1**.

Results

Soft tissue maceration was achieved in two of the three methods evaluated. The dermestid larvae completely macerated the soft tissue, while preserving the bone tissue intact (**Figure 2**). KOH eliminated the majority of soft tissue. However, it disarticulated some bones and caused

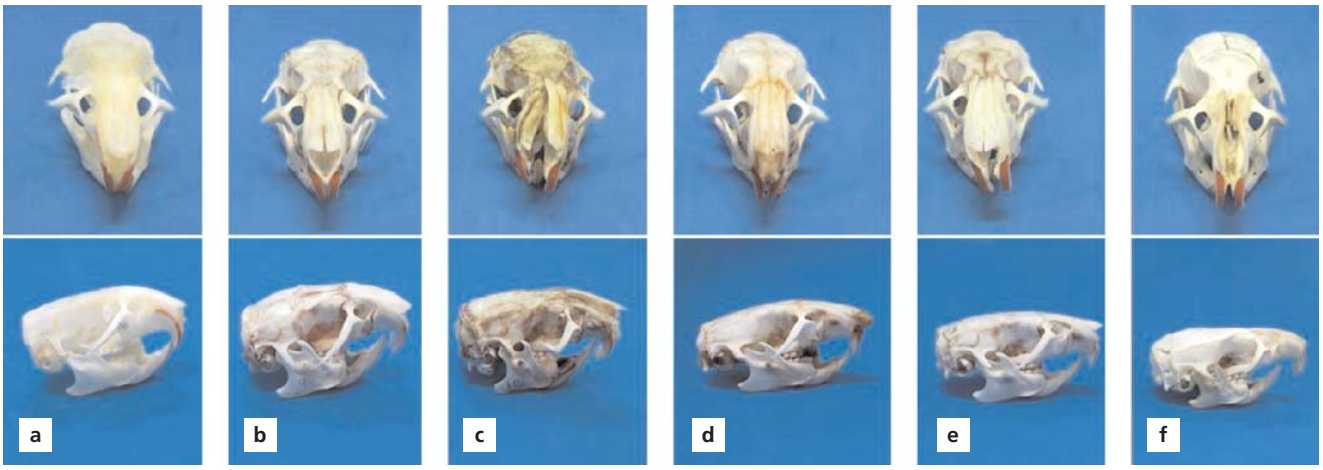

Figure 3. Results of maceration with (**a**) 1% KOH at room temperature; (**b**) 5% KOH at room temperature; (**c**) 10% KOH at room temperature; (**d**) 1% KOH at oven temperature; (**e**) 5% KOH at oven temperature; (**f**) 10% KOH at oven temperature. [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]

some bone loss in others (**Figure 3**). NaClO did not meet the objective of macerating the soft tissue (**Figure 4**).

Discussion

The experiments showed that maceration by dermestid larvae was the most effective method as the hard tissue did not present any type of alteration and the soft tissue was removed in its entirety, preserving the bone piece in excellent condition. However, it is essential that the biomodel has not been exposed to any chemical agent or substance that emanates odor, so that it can be processed by these larvae. Taking into account the weight of the biomodel, the time required to obtain the desired final result was eight days.

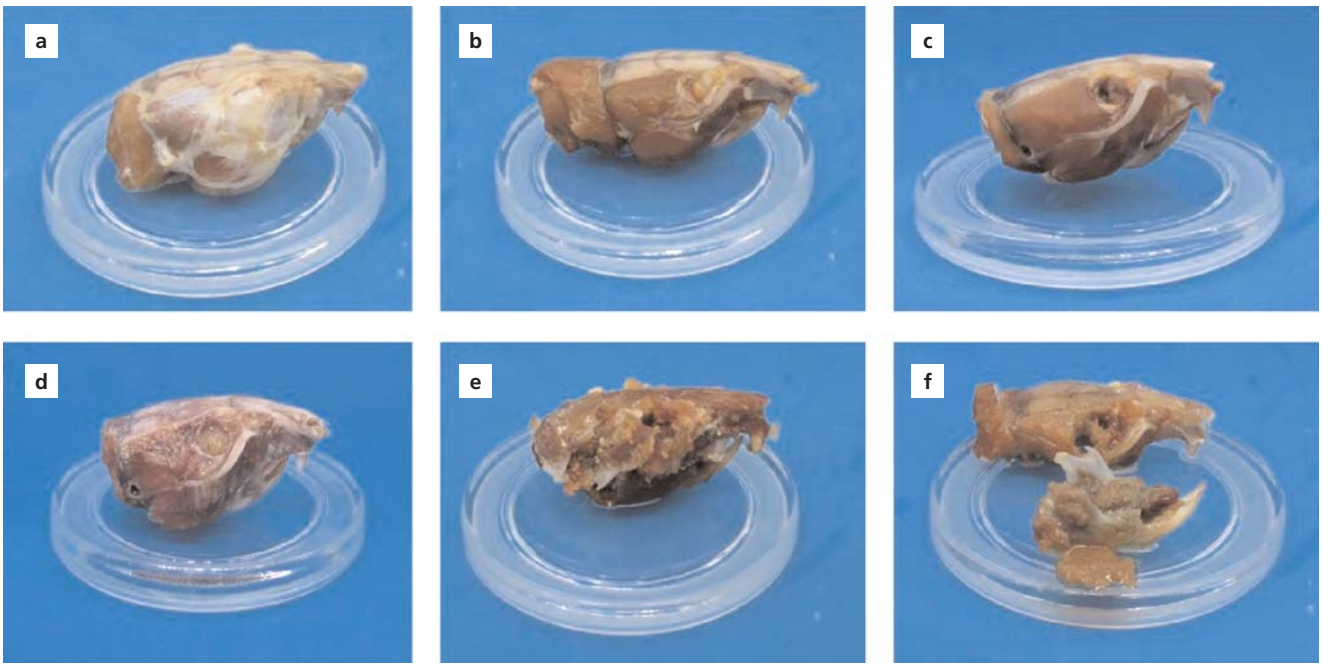

Figure 4. Results of maceration with NaClO: (**a**) 1% at room temperature; (**b**) 5% at room temperature; (**c**) 10% at room temperature; (**d**) 1% at oven temperature; (**e**) 5% at oven temperature; (**f**) 10% at oven temperature. [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]

Authors such as Ajayi et al.,[3] reported that dermestid larvae are useful as a technique to clean bones, especially for parts that are difficult to dissect. Ator et al.,[4] indicated that the dermestid beetles in larval stage (those used in this experiment) have high appetite and achieve excellent maceration of small pieces.

Yet Couse et al.[5] and King et al.[6] stated that in order to implement maceration by dermestids, the laboratory requires a colony, something that in itself involves significant maintenance. Given that forensic science is not typically profitable, the additional costs associated with maintaining a colony could be a limiting factor for employing this type of maceration. This disadvantage is minor, however, when compared to the risk of escape for such beetles, due to the potentially destructive capacity of the species. In addition, the overexposure of a piece can also be harmful, since the beetles can begin to feed on the mineralized tissue, making daily monitoring necessary. King et al.[6] showed that the duration of the procedure ranged from weeks to months. This period of time can be further extended due to the beetles' preference for dry tissues over fresh.

The maceration by 1% KOH and 5% KOH at room temperature completely eliminated the soft tissue and required a time of 27 days and 45 hours, respectively. However, 1% KOH caused the loss of the right and left zygomatic bone. A whiter coloration was observed when compared to the rest, indicating a possible lower bone density and, therefore, greater fragility of the bone tissue. The skull submerged in 5% KOH showed disarticulation of the nasal bones, incisors and the scaly portion of the left temporal bone. In addition, the loss of the right and left zygomatic bone was observed.

Miller and Tarpley[7] obtained effective macerations with 3% KOH concentrations at room temperature with continuous agitation during the cycle, unlike this study in which the samples were not stirred. The same authors reported that increasing the concentration of KOH led to the loss of samples, while eliminating the agitation or decreasing the concentration of KOH produced an incomplete maceration.

The maceration by 10% KOH at room temperature in a period of 18 hours failed to completely remove the soft tissue. Remnants were observed in the inner part of the mandibular branches and around the petrous portion of the left temporal bone. Likewise, it generated disarticulation of the nasal bones and left incisor, loss of the right and left zygomatic bone and of the petrous portion of the right temporal bone. A darker coloration was obtained when compared with the rest of the pieces.

Gibb[1] reported that the size and physical characteristics of the sample, the concentration of the chemical agent and the temperature at which the procedure is carried out should be taken into account in macerations with KOH. The process performed at night and at room temperature is generally adequate for most specimens; samples that resisted heat required longer periods at higher temperatures. However, Renaud et al.[8] and Gelfand et al.[9,10] advised caution when handling the chemical, because it is highly corrosive and therefore constitutes a disadvantage for an inexperienced operator, although an advantage for the technique.

Maceration with 1% and 5% KOH in the oven was carried out for five and four hours, respectively. The 1% KOH preserved soft tissue around the petrous portion of the temporal bone and lingual part of the molars and preserved the nasal cartilage but generated loss of the right and left zygomatic bone. The 5% KOH preserved soft tissue in the lingual part of the molars and eliminated the right and left zygomatic bone. Unlike the previous concentrations, 10% KOH required two hours and 30 minutes to completely eliminate the soft tissue. Loss of the right and left zygomatic bones and disarticulation of the interparietal bone, nasal and mandibular symphysis were observed. In his book, Boyde[11] explained that small bones could be treated with strong solutions of KOH at 50°C, which eliminated all soft tissues and the matrix at night. However, with the use of the oven in our experiment, the same effect was achieved in a few hours.

King and Birch[6] showed that maceration with chemicals is fast, depending on the size of the specimens. However, chemical products can be difficult to control, because prolonged exposure can demineralize the bone, leading to fragility and loss of bone integrity. Renaud et al.[8] used glass containers, observing an incomplete maceration process, similar to that found in our experiment where all macerations with KOH presented hard tissue damage.

The maceration with NaClO in different concentrations and temperatures in a period of 27 days did not meet the maceration criteria, and soft, adherent, dehydrated tissue of firm consistency was observed. Dutta and Saunders[12] used concentrations of 1.36% and 4.65% which, after 60 minutes of exposure to the substance, did not show dissolution of the soft tissue. However, Fuente et al.[13] used NaClO for nine hours at a concentration of 35% and immersed specimens in boiling water, obtaining positive results. Mann and Berryman[14] used concentrations of 3% to 6% of NaClO and concluded that it is a fast, safe and effective method for exposing bone tissue.

Steadman et al.[15] stated that NaClO is an easy-to-use, accessible and economical chemical maceration method that allows rapid bone cleaning. They used 10%

NaClO plus 3% hydrogen peroxide and recorded that the procedures that lacked heat were substantially slower than the techniques in which hot or water brought to boiling point was used. Furthermore, the pieces gave off stronger smells and did not necessarily produce better quality bone tissue. They recommended concentrations that did not exceed 10%. Hildebrand[16] in his book proposed NaClO in concentrations of 1% to 2% at 50°C for five days for small pieces.

Conclusion

The results of this study showed that maceration by insects is an ideal method for soft tissue maceration, preserving all bone components. Maceration by KOH was an effective, but aggressive method for the bone tissue. Conducting further studies in which the chemical agent is agitated during this type of maceration is recommended. It was not possible to achieve a successful maceration with NaClO; immersing the pieces in boiling water first was another recommendation.

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