

Method of Producing Flat Skin and Skeleton Specimens of Laboratory Mice for Exhibition at a University Museum

Kohei KAWAKAMI¹⁾, Kazuhiro EGE²⁾ and Takaya YAMADA¹⁾

¹⁾*Department of Experimental Animals, Interdisciplinary Center for Science Research, Organization for Research, Shimane University, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan*

²⁾*Shimane University Museum, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan*

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We manufactured flat skin and skeleton specimens of laboratory mice in response to a request from Shimane University Museum. Since diminutive species such as rodents have very small bones, previous methods for making specimens of them involve tedious procedures and require too much labor and time. In this study, we established an easier approach by using sodium hypochlorite and denture cleanser. Our method is not only very simple and thus saves labor and time, but can also keep many bones articulated throughout the making of specimens. This paper describes the series of procedures involved, such as the removal of flesh from a sample, dissolution and removal of fat using sterilizing medical liquid, decoloration using hydrogen peroxide, and shaping and drying. Our method should also be available for the making of hard bone skeleton specimens of vertebrate animals.

Key words: mice, flat skin, skeleton specimen

INTRODUCTION

Since the late 1990s, many universities in Japan have built museums to use their academic resources effectively. One of the characteristics of university museums is that they display resources that the museum or the university owns [1, 2]. Shimane University Museum defines the specimens and other

resources owned by Shimane University as the university's tangible intellectual property, and collects, organizes, preserves and analyzes them. They are then exhibited with the aim of making an educational contribution, as well as for public enlightenment, promotion of information transmission and contributing to the local community. Shimane University Museum exhibits specimens and other materials from various fields that Shimane University has collected since the Meiji period, which enables us to conduct a crossover between browsing and research. In addition, it ensures that various specimens at Shimane University and the university's historical documents are not scattered and lost.

The experimental animal section of our laboratory received a specimen request from Shimane University Museum. Mice, which are the animals used most in laboratory experiments, have many strains. Among them, we used mice with different colors and produced specimens of their flat skins and skeletons for donation to our university museum. Some natural history museums are known to make skeleton specimens and tanned skins of the animals that they are storing by themselves; some reports and books about making such skeleton specimens and tanning skins have also been published. The methods proposed so far are as follows: a method of feeding them to insects [3], a method of making them decay through water and soil [4], a method of boiling them [5], the papain method [6], the tashinaze method [7], the sodium hypochlorite method [8] and a method of using denture cleanser [9]. The problems of the feeding method and the decaying method are the required level of labor and the cost of keeping insects, as well as the bad smell caused by them. The boiling method is effective for large animals, but not for small ones, because it causes the joints to sepa-

Correspondence: Kohei Kawakami Ph.D.

Department of Experimental Animals, Interdisciplinary Center for Science Research, Organization for Research, Shimane University, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan

Tel: +81-853-20-2363

Fax: +81-853-20-2360

E-mail: kkawaka@med.shimane-u.ac.jp

rate and collapses the frame structures, which then increases the risk of damaging bones via the heat. The problem of the papain method, which uses a medicine called papain, is that this medicine is both expensive and difficult to handle due to its highly toxic nature. In addition, the tashinaze method uses a proteolytic enzyme called tashinaze, the resolution of which is inefficient in a medicated bath unless the samples are boiled in advance, and the use of a medicated bath also takes more than 15 hours.

Against this background, in this paper, we introduce a method of using both sodium hypochlorite and denture cleanser for the preparation of flat skin and skeleton specimens of mice within a comparatively short time and with virtually no disarticulation.

MATERIALS AND METHODS

Animals

Twelve-week-old male mice of ICR, C57BL/6J, C3H/HeN, DBA/2J, KK-Ay/Ta and BALB/c-nu/nu (two of each kind; CLEA Japan, Inc., Tokyo, Japan) were used. The mice were kept under constant room temperature ($23 \pm 2^\circ\text{C}$) and humidity ($55 \pm 10\%$) and a 12-h light cycle (lights on at 07:00 and off at 19:00). The mice were individually housed in TPX flat-bottomed cages ($W260 \times D330 \times H170$ mm; Japan CLEA Inc., Tokyo, Japan) with wood chips on the floor. All mice were fed plain commercial solid feed (NMF; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*. Animal care and experimental procedures were approved by the Animal Research Committee of Shimane University and conducted according to the Regulations for Animal Experimentation at Shimane University.

Medicines and test tools

A mixed preservative was prepared for mold-proofing, which contained powdery naphthalene, powdery boric acid and powdery alum at a ratio of 1:1:1. In addition, 2% sodium hypochlorite (Purelox; OYALOX Co., Ltd., Tokyo, Japan), denture cleanser that was commercially available (structural components were sodium bicarbonate, sodium percarbonate, potassium persulfate, citric acid, sodium carbonate

and others) (Polydent; Glaxo SmithKline, K.K., Tokyo, Japan) and 3% hydrogen peroxide (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were also prepared. As for the test tools, postmortem scissors, tweezers, micro-tweezers and a bat were used for dissection, and an interdental brush was used for dissolution. We also used rulers and Vernier calipers for measurement, a Styrofoam lamina, setting pins, brass wire, glue and superglue for specimen shaping.

Measurements

All mice were sacrificed under general anesthesia (pentobarbital 100 mg/kg *i.p.*). Body weights were measured at 12 weeks of age (HF-3000; AND Co., Ltd., Tokyo, Japan). Table 1 shows body weights and sizes of the mice. The meanings of the abbreviations used in Table 1 are as follows: HB (head and body length): the length from the tip of the nose to the caudal tip, that is, the length calculated by subtracting the tail length from the entire body length; TL (tail length): the length from the caudal root to the tip, not including the hairs; TR: the tail rate (TL/HB); E (ear): the length of the auricle, that is, the width from the bottom to the tip; FFsu (forefoot with no nail): the longest length from the root of the hand to a fingertip (not including nail); FFcu (forefoot with nail): the length from the root of the hand to the point of the nail; HFsu (hindfoot with no nail): the length from the heel to a fingertip (not including a nail); HFcu (hindfoot with nail): the length from the heel to the point of the nail; and WH: the width of the hand.

Method of producing flat skin

1. Open up only the skin along the line linking the anus to both hindleg necks (Fig. 1-A).
2. Pull up the caudal vertebrae without severing the tail (Fig. 1-B).
3. Cut the bones of both ankles so that the tips of the foot stay attached to the skin (Fig. 1-C).
4. Denude the skin towards the head while inverting it (Fig. 1-D).
5. Denude the forefeet up to the anterior ankles and cut the bones of the anterior ankle (leaving the fingers attached to the skin).
6. Cut the root part of the ears at the position of a thin pipe shape.
7. Separate the inside of the eyelids and the labia from the head.
8. Cut the cartilage of the nose and finish inverting the skin (Fig. 1-E).
- 9.

Table 1. Body weights and sizes of 12 weeks male mice of six strains

Strain	IDN	BW (g)	HB (mm)	TL (mm)	TR (%)	E (mm)	FFsu (mm)	FFcu (mm)	HFsu (mm)	HFcu (mm)	WH (mm)
ICR	1	35.6	205	105.4	51.4	14.8	8.2	8.7	19.3	20.2	4.5
	2	33.4	196	102.7	52.4	14.4	8.5	8.6	19.8	20.3	5.1
C57BL/6J	3	29.3	183	100.0	54.6	13.1	7.8	8.1	19.7	20.1	5.3
	4	28.1	180	95.7	53.2	13.8	7.7	8.1	20.0	20.2	5.2
C3H/HeN	5	23.0	179	86.0	48.0	13.3	9.4	9.7	17.4	18.3	5.3
	6	24.0	178	86.0	48.3	14.3	7.7	8.3	17.6	18.7	5.0
DBA/2J	7	27.0	179	84.0	46.9	14.2	8.1	8.3	17.3	18.3	5.1
	8	24.0	174	86.0	49.4	13.8	8.8	9.0	17.4	18.4	5.7
KK-AJ/Ta	9	49.2	195	96.6	49.5	14.4	8.4	9.4	18.1	19.2	5.0
	10	48.8	198	98.8	49.9	15.0	8.5	9.4	18.1	19.1	5.5
BALB/cA-nu/nu	11	29.6	185	100.0	54.1	16.1	10.2	10.4	18.8	20.2	4.4
	12	30.1	188	100.0	53.2	15.9	9.4	10.0	17.6	18.3	5.2

BW: body weight, HB: head and body length, TL: tail length, TR: the tail rate (TL/HB), E: the length of auricle, FFsu: forefoot with no nail, FFcu: forefoot with nail, HFsu: hindfoot with no nail, HFcu: hindfoot with nail, WH: the width of the hand.

Apply preservative thinly by sprinkling and rubbing it on the back side (inside) of the skin (Fig. 1-F). 10. Put the nose part of the fur on a paper pattern's tip and cover the pattern with the skin (Fig. 1-G). 11. Add data and place the flat skin in position for pressing between paper towels or papers like botanical specimens, with the right hand palm upward and the left hand palm downward, and dry the skin (for approximately two weeks). 12. Complete the flat skin specimens of mice with different colors (Fig. 1-H).

Method of producing skeleton specimens

1. Remove the internal organs of a mouse that has already been denuded when making a specimen of its flat skin (Fig. 2-A). 2. Remove femoral regions, lumbar part and muscle around the scapula with tweezers and postmortem scissors. 3. Put the sample in a 100 mL glass bottle and pour in 2% sodium hypochlorite until it is immersed in it (Fig. 2-B). Conduct the operation in a draft of air within 30-40 minutes so as not to be poisoned by the chlorine generated in the process, while confirming the state of parts such as the rib cartilage and the carpal bone, among others. Immerse soft tissues such as muscle or nerves for a comparatively long time. 4. Next, cleanse with denture cleanser for approximately 4-6 hours (Fig. 2-C). 5. Bleach and

deodorize with 3% hydrogen peroxide (immersion for 2-12 hours). 6. Insert brass wire into the spinal canal and determine the spinal curvature. Dry the sample on a Styrofoam board and fix with setting pins (Fig. 2-D). Dry the separated cranial bone at a location away from the main body. 7. Shape the sample and place it in a low-humidity environment for the rapid drying of soft tissue. 8. After drying, remove the main body off the Styrofoam board and glue the cranial bone and the body together with superglue (Fig. 2-E).

Making captions

In terms of information on the specimen, we recorded the specimen number (registration number), the generic name, the scientific name, the Japanese name and the measurement data. In terms of collection information, we recorded the gatherer's name, the site of collection, the collection date and the purchase source (Fig. 2-F).

RESULTS AND DISCUSSION

Art museums are institutions for education and research through "collecting materials, preserving them and exhibiting them" [1]. They are places where people consider and learn about culture, history and nature through objects (exhibited materials).

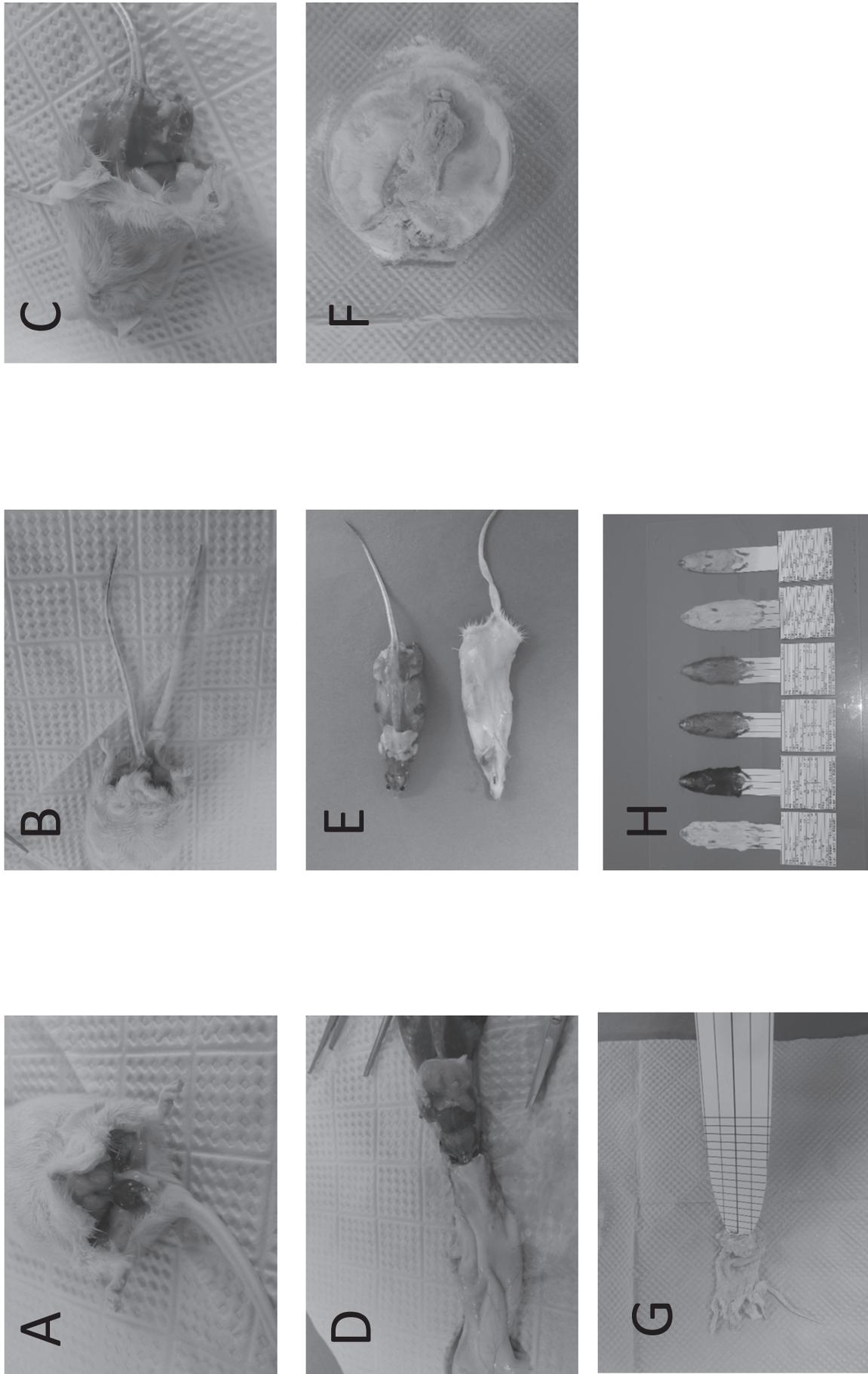


Fig. 1. Procedure of producing flat skin of mice
 A: Skin incision, B: Pull up the tail vertebrae, C: Leave both ankles on the skin side, D: Denude the skin towards the head while inverting it, E: Invert the skin, F: Apply preservative, G: Cover the pattern with the skin, H: Completion



Fig. 2. Procedure of producing skeleton specimens of mice
A: Remove the internal organs, B: Put the sample in 2% sodium hypochlorite, C: Cleanse with denture cleanser, D: Shape and dry the sample, E: Completion, F: Making captions

Museums store various materials, including ancient documents, archeological documents, works of art, materials related to natural history, animal specimens and plant specimens. These are treasures that we have inherited from earlier generations. Under the Museum Law of Japan, museums are supposed to "collect, preserve and display materials, promoting education and necessary accompanying research, and conducting academic research on the materials" [2]. The experimental animal section of our laboratory received a specimen request for experimental animals from Shimane University Museum. We took advantage of this opportunity to promote and prepare tangible specimens for the museum in order to familiarize students and citizens with experimental animals. The main mission of the university museum is to keep specimens and documents for educational and research purposes at the university and to disclose information, such as releasing the results of significant research on the specimens and documents to the local community in an effective manner.

Since diminutive species such as rodents have very small bones, previous methods for making specimens of them involve tedious procedures and require excessive labor and time [3, 4, 5]. In this study, we were able to complete an ideal skeleton specimen by using, for dissolution, a dilute liquid of Purelox (2% sodium hypochlorite), which we normally use as antiseptic in the rearing room of our animal experiment institution, and by cleansing mildly with denture cleanser. Soft tissue, such as muscles and nerves, which was retained at numerous sites, was immersed in 2% sodium hypochlorite for about 30 minutes. However, some sites of soft tissue changed less with a dissolution time of about 5 minutes. This was because of hard tissue such as bone that was located at sites covering these small parts of soft tissue. This method can maintain the articulation of numerous bones throughout the making of specimens. The process involves a number of steps, including the decomposition of components of food debris and bacteria in commercially available denture cleanser. The denture cleanser dissolved protein moieties gently regardless of the processing time. It has a reaction speed that is slower than that of the 2% sodium hypochlorite reaction. Femur and metacarpal bones were not dismantled by adding

this process. For the bleaching and deodorization of bone, 3% hydrogen peroxide was used. However, immersion for too long a period of time can damage bone. The duration of immersion was thus adjusted by observing the appearance.

When we produce specimens, it is necessary to devise specific techniques according to the type of specimen, size and condition. In addition, certain findings related to this issue are made during such work. The method described in this paper could be applied not just to skeleton specimens of rodents, but also to hard bone skeleton specimens of vertebrate animals.

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