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DO NOT DISPOSE OF HISTORIC FLUID COLLECTIONS: EVALUATING RESEARCH POTENTIAL AND RANGE OF USE

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Abstract.—The use of specific preservative solutions by museum professionals to maintain fluid-preserved specimens has fluctuated over the years with advances in chemistry. The determining factors for the original choice of solution closely correlate with the historical parameters and original usage of the collections. Consequently, for any given collection, changes and substitutions over time in the types of preservative fluids used have likely occurred. The present comparative analysis of the state of brain preservation, carried out at macroscopic, microscopic, and molecular levels, allowed us to evaluate the effect of the different treatments applied over time to fluid-preserved collections. Our results confirm that the duration of formaldehyde exposure of the tissues clearly has an effect on their long-term preservation. Despite the controversies associated with the quality or use of some historic fixatives, modern analytical methods such as medical imagery reveal the preservation quality in historic specimens and their potential for future research use. However, the choice of fixatives and storage fluids to preserve the specimens is of critical importance because today's choices will influence the use of the specimen for advanced analytical methods in the future.

Key words.—comparative analysis, ethanol, fluid-preservation, formaldehyde, historic collections, macroscopic, mammalian brain, microscopic, molecular, Owen's liquor

INTRODUCTION

Since the 18th century, “spirit of wine” (ethyl alcohol), among other substances, had been the preferred fluid preservative. The less expensive formaldehyde largely replaced ethyl alcohol as a fixative at the end of the 19th century (Parker and Floyd 1895, Down 1989, Herbin 2013). A diluted mixture of formaldehyde and water, widely known under the brand name *formalin*, was also increasingly used as a preservative, for example in anatomical collections. Besides these well-known preservatives, other chemical mixtures were tested in order to increase the quality of specimen preservation (Simmons 1995). Owen's liquor (see formula in the Characterization of Preservatives section) was introduced in the Anatomic Fluid Collection at the Muséum national d'Histoire naturelle de Paris (MNHN) in the years 1880–1887. It was used until the beginning of the 20th century but was abandoned and replaced with formalin thereafter (Neuville 1917). The diversity of preservative fluids in our collection reflects this testing and usage of different conservation techniques and fluids: some of the specimens are in ethyl alcohol or in Owen's liquor, others in formalin. The entry date into the collection is an important clue to indicate which preservative fluid might be contained in sealed historic jars, although specimens might not remain in their original solution but may have been transferred into other fluids. For example, some

Table 1. Information on each collection item. Invent. No. = inventory number.

Group	Invent. No.	Time in preservative solution (%)			Time of storage (yr)	pH	Species
		Alcohol	Owen's liquor	Formalin			
A	A-4802	100	—	—	168	4.1	<i>Ursus americanus eremicus</i>
B	A-5440	46	—	54	174	5.3	<i>Ursus americanus</i>
B	A-5454	50	—	50	188	4.4	<i>Panthera tigris</i>
B	1887-633	47	—	53	176	3.8	<i>Panthera leo</i>
C	1887-583	32	—	68	139	3.9	<i>Panthera leo</i>
C	1887-639	28	—	72	131	3.9	<i>Panthera leo</i>
D	A-5495	25	21	54	175	4.5	<i>Ursus arctos</i>
D	A-5441	26	21	53	178	3.8	<i>Panthera leo</i>
E	A-4851	10	25	65	146	3.6	<i>Panthera tigris</i>
F	1926-287	—	—	100	92	4.0	<i>Panthera leo</i>
F	1932-39	—	—	100	86	3.8	<i>Ursus americanus</i>
F	1945-11	—	—	100	73	3.9	<i>Ursus arctos horribilis</i>
F	1949-9	—	—	100	69	4.0	<i>Panthera leo</i>
F	1960-87	—	—	100	58	4.0	<i>Ursus arctos</i>
F	1963-247	—	—	100	55	3.8	<i>Panthera leo</i>

of the oldest specimens were transferred from alcohol to Owen's liquor and subsequently ended up in formalin, while others were moved directly from alcohol into formalin. These largely random changes of the preservative fluid, in combination with the length of time these specimens have been maintained and curated, have impacted specimen condition and quality, as well as their usefulness for future teaching and research.

In this study we used brains from our fluid collection, which harbors more than 1,000 specimens collected from the middle of the 19th to the end of 20th centuries. We selected this part of the collection because the similar composition and homogeneity of brain tissues limited potential confounding effects on data comparability due to differences in specimen or tissue types related to exposure to the preservative fluid. Preservation quality was recorded individually for each brain at different levels: (1) Macroscopic level, i.e., the visual quality of the specimen for display or teaching purposes; (2) Microscopic level, to evaluate the current state of conservation of the tissue and its potential for use in different types of research; (3) Molecular level, i.e., quality and quantity of the DNA yields (extraction and amplification) as commonly used in taxonomic studies (Hykin et al. 2015); (4) magnetic resonance imagery (MRI), i.e., a noninvasive virtual dissection of specimens widely used in anatomical research. This multifaceted approach allowed us to investigate the state of conservation of the tissue in all brain regions.

MATERIAL AND METHODS

For this study, 15 brains of large vertebrates (nine *Panthera* spp., from lions and tigers, and six *Ursus* spp., from brown and black bears) were selected (Table 1). All the brains were fixed through immersion in the preservative fluid and either stored in the same fluid or subsequently transferred into different fluids over time. Specimens were assigned to one of six specific groups representing a characteristic preservation history: (A) 100% of preservation time in ethyl alcohol; (B) 50% of preservation time in ethyl alcohol and 50% in formalin; (C) 30% of time in ethyl alcohol and 70% in formalin; (D) 25% in ethyl alcohol, 21% in

Owen's liquor, and 54% in formalin; (E) 10% in ethyl alcohol, 25% in Owen's liquor, and 65% in formalin; (F) 100% in formalin.

Group A: 100% in Ethyl Alcohol

A-4802 inventoried in 1850, 168 years in ethyl alcohol.

Group B: 50% Ethyl Alcohol and 50% Formalin

A-5454 inventoried in 1832, transferred to formalin in 1924. 1887-639 inventoried in 1887, transferred to formalin in 1924. A red undetermined substance was injected into both brains to visualize circulatory system of the brain. A-5440 inventoried in 1844, transferred to formalin in 1924.

Group C: 30% Ethyl Alcohol and 70% Formalin

1887-583 inventoried in 1887, transferred to formalin in 1924. 1887-639 inventoried in 1887, transferred to formalin in 1924.

Group D: 25% Ethyl Alcohol, 21% Owen's Liquor, 54% Formalin

A-5441 inventoried between 1830 and 1861, transferred to Owen's liquor in 1887, and to formalin in 1924. A-5495 inventoried in 1843, transferred to Owen's liquor in 1887, and to formalin in 1924.

Group E: 10% Ethyl Alcohol, 25% Owen's Liquor, 65% Formalin

A-4851 inventoried in 1872, transferred to Owen's liquor in 1887, and to formalin in 1924.

Group F: 100% in Formalin

1926-287 in formalin for 92 years. 1932-39 in formalin for 86 years. 1945-11 in formalin for 73 years. 1949-9 in formalin for 69 years. 1960-87 in formalin for 58 years. 1963-247 in formalin for 55 years.

DNA Control Sample: 100% in Formalin

In order to verify that the DNA protocol is effective on specimens stored in less acidic fluids, we took a muscle sample from two different specimens: the first stored in 10% formalin since 1960 (pH = 4.9) and the second stored in 10% buffered formalin since 2003 (pH = 5.4).

CHARACTERIZATION OF PRESERVATIVES

Alcohol Solution

The only available information from the catalog inventory is "in alcohol" without any further specification of the original concentration. By default, we assumed that it was 70% ethyl alcohol even though Pouchet (1893) during a conference referred to 90% alcohol.

Owen's Liquor

This solution was used in the fluid collection of the Laboratoire d'Anatomie comparée during the second part of the 19th century (Neuville 1917). The recipe, cited by Pouchet (1893) and Neuville (1917), was filed in one of the collection inventory catalogs and published in "Conférence d'Anatomie" (Pouchet 1893). Owen's liquor has a formula very

similar to Goadby's solution (Baird 1852) and is composed of 50 liters of water, 5 kg of marine salt (NaCl), 2.5 kg alum (potassium alum, $KAl(SO_4)_2$), and 5 g of corrosive sublimate ($HgCl_2$). Brains were directly placed in Owen's liquor (Pouchet 1893).

Formalin

The composition of this fluid was described by Neuville (1917) as a "commercial formalin" (formalin) diluted to 10% (which results in an aqueous solution containing 4% aqueous formaldehyde, water, and methanol). He did not mention whether the solution was buffered.

PROTOCOL USED TO CHARACTERIZE THE PRESERVATION STATUS OF THE PRESERVED BRAINS

Macroscopic Level

The general appearance of the brain was inspected first, noting the color, surface texture, and presence or absence of deposits. In the second step, a thick section (1.5 mm) of the spinal cord was removed, examined, and photographed under a binocular microscope at a magnification of $\times 0.63$. The surface aspect of the sections and their textures were noted. This section was subsequently used to produce histological slides for the microscopic study.

Microscopic Level and Histology

After the macroscopic examination of the thick section of the spinal cord, samples were removed from their original preservative solutions and placed in 70% ethyl alcohol for 24 hours before paraffin embedding. Five-micron thick serial sections were prepared. The tissues were deparaffinized with toluene and then hydrated using a series of decreasing concentrations of ethanol. For each specimen, hematoxylin-eosin and Giemsa staining were performed. Images were acquired using an Olympus DP72 camera coupled to an Olympus BX63 motorized microscope running cellSens Dimension (v 1.9) software. The condition and integrity of tissues and cells was recorded from each slide.

Molecular Level and Polymerase Chain Reaction

Biopsy samples of 40–80 mg were taken from the spinal cord of each brain. To remove as much formalin as possible, tissues were stepwise dehydrated and rehydrated (Hykin et al. 2015) by transferring them from the original preservation fluid to a mixture of 20% ethanol in water and back to 90% ethanol by increasing the ethanol concentration in 10% increments. Just before the DNA extraction, each sample was thoroughly dried with sterile KimwipesTM. Additionally, 20–40 ml of the original preservative fluid was sampled. We centrifuged the fluid through a series of two Amicon ultra-15 filters (YM-100 and YM-30 respectively; Merck Millipore) and rinsed them twice with EB buffer (10 mM Tris-Cl pH 8.0) and concentrated the volume to a pool of 200 μ l (100 μ l each). DNA extractions for tissue and fluid samples were performed (Nucleospin FFPE kit, Macherey-Nagel) with the following protocol modifications: omission of deparaffinization steps, twofold increased buffer volumes in all steps (lysis, decrosslinking, ethanol washing, and binding buffers respectively). The DNA extracts were eluted in 35 μ l of 10 mM Tris-Cl, 0.1 mM ethylenediaminetetraacetic acid pH8.0, 0.05% Tween-20 (TET) buffer. DNA yields were quantified by fluorescent spectroscopy (QubitTM HS kit, Life Technologies) and quantitative polymerase chain reaction (qPCR) of extracts used straight and diluted 1/10 in 10 TET buffer. The qPCR assays were specifically designed to amplify three 12S RNA fragments (spanning 67, 102, and 205 bp) from mammalian carnivores to exclude amplification of other mammals

or metazoans and to establish a degradation profile via serial qPCRs (Deagle et al. 2006). Extraction and PCR blanks were analyzed together with the samples.

Medical Imagery (MRI)

Each brain was kept in its originally sealed jar, and the MRI images were acquired using either a 3T or an 11.7T Magnet. Each dataset was acquired with a three-dimensional gradient-echo sequence. Parameters were adjusted to obtain the highest possible resolution in an overnight scan (from 25 to 450 μm isotropic; depending on sample size).

RESULTS

Macroscopic Status of the Brains

Group A.—Generally, the brain appeared to be in good condition. The different structures were macroscopically well preserved and outer tissues firm to touch. The color of the brain was uniformly creamy-white; however, occasionally small deposits could be observed on the surface of the cortex and cerebellum (Fig. 1A). The thick section sampling of the spinal cord was conducted without problems, and the texture of the sections was also firm. Different cellular structures and nuclei were readily visible under the binocular microscope without any additional staining.

Group B.—The brains in this group were less well preserved, and two main conservation issues were noted. Sample A-5454 and 1887-633 have the same creamy-white color, the tissues were very soft and appeared not to have been well fixed, while the brain A-5440 is gray-white and apparently has been more thoroughly fixed (Fig. 1B). The thick sections of spinal cord basically confirmed the expected weakness of tissues, the first two being very soft and insufficiently fixed, while the other was very brittle, not homogeneous, and difficult to cut.

Group C.—Both brains appear well preserved, the different structures are macroscopically in a good condition and very firm to the touch. The color of both brains is gray-white without any deposits on the surfaces (Fig. 1C). However, the thick section of the spinal cord of 1887-583 suffered from the same brittleness of tissues as above, while specimen 1887-639 was well preserved and easy to cut.

Group D.—At first glance, both brains had a fairly good overall appearance, but the tissues seemed baked. A gray rather than gray-white color was typical for this group (Fig. 1D). The section of the spinal cord was not easy to cut, and the tissue was very brittle, not homogeneous, and without strong cohesion.

Group E.—As in group D, the brains appeared in good shape, their color was more creamy-white compared to the former group (Fig. 1E). The sectioning of the spinal cord was difficult, and the tissue was brittle and not homogeneous, but its cohesion appeared to be better.

Group F.—All brains in this group were in good condition, the tissues well fixed and creamy-white in color (Fig. 1F). The sectioning of the spinal cord was easy to perform, and the different structures of the medulla were readily visible under the binocular microscope without any staining.

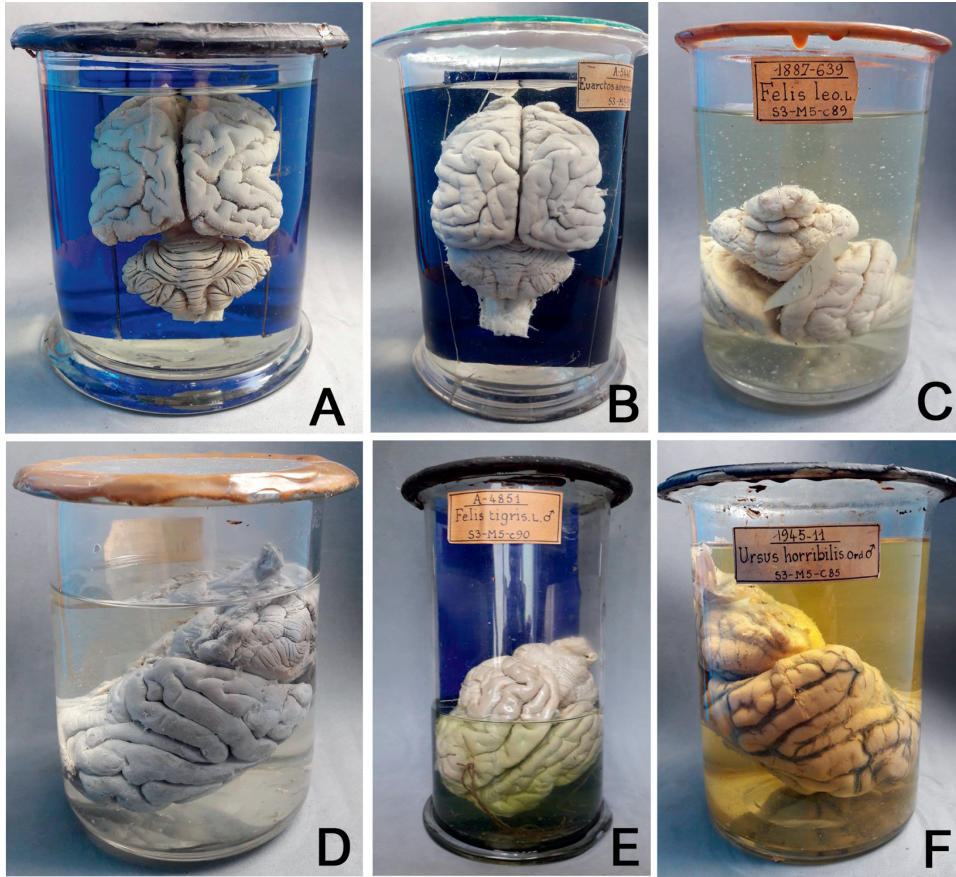


Figure 1. Representative specimens from each group. (A) group A, 100% in ethyl alcohol; (B) group B, 50% in ethyl alcohol and 50% in formalin; (C) group C, 30% in ethyl alcohol and 70% in formalin; (D): group D, 25% in ethyl alcohol, 21% in Owen's liquor, and 54% in formalin; (E) group E, 10% in ethyl alcohol, 25% in Owen's liquor, and 65% in formalin; (F) group F, 100% in formalin.

Microscopic Level and Histology

Group A.— Because of the tissue hardness and brittleness in this specimen, tissue sections were torn. At low magnification, fairly good staining exactly disclosed the limits of different cellular structures of the medulla, and the white and gray matter can be observed clearly (Fig. 2A). At higher magnification, the tissue seems perforated with little holes. Neurons, motor neurons, and fibers are mostly visible but not quantifiable (Fig. 3A).

Group B.— This nonhomogeneous material also produced torn sections. At the macroscopic level, two specific states of preservation could be observed in the medullar tissue. At low magnification, the softer tissues of A-5454 and 1887-633 are well stained and covered with many holes, and even though the medullar tissue has not been very well fixed, it is preserved better at the center than at the periphery of the spinal cord. The tissue of the third medulla (A-5440) is better fixed but also appears perforated by many holes strewn over the entire section (Fig. 2B). Observation at higher magnification confirms this finding,

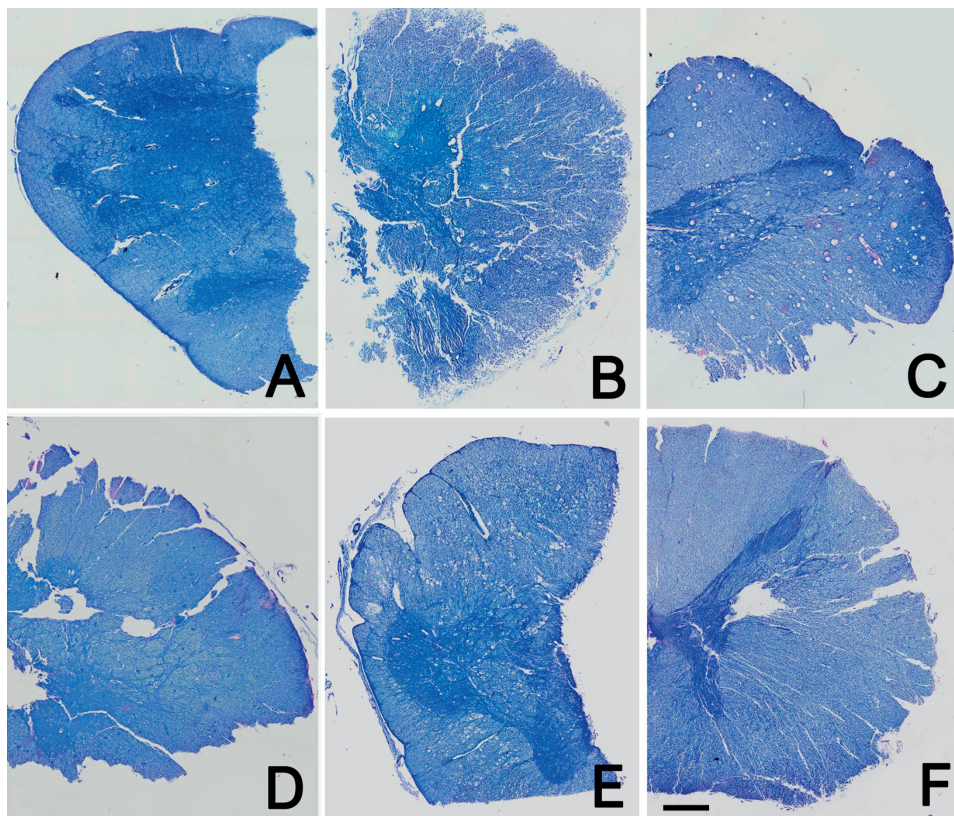


Figure 2. Microscopic observations of spinal cord section in Giemsa stain at low magnification. (A) group A; (B) group B; (C) group C; (D) group D; (E) group E; (F) group F. In each group it is easy to discriminate the gray and white matter. Scale bar = 1 mm.

while neurons, motor neurons, and some bundles of fibers (only in A-5440) are observable (Fig. 3B).

Group C.—The hard and brittle material resulted in torn sections. At low magnification, the different structures of the medulla are fairly well stained and the white and gray matter can be clearly observed. However, the tissue seems extensively perforated by small holes (Fig. 2C). At higher magnification the neurons and motor neurons appear retracted and are not always clearly visible, but still quantifiable. Many fiber bundles are easily identifiable (Fig. 3C).

Group D and E.—As in the previous group, all the sections are torn. At low magnification, as above, the white and gray matter is easily recognizable, but the tissue is extensively perforated (Fig. 2D, E). At higher magnification, in addition to neurons, motor neurons, and fiber bundles, some glial cells are discernible, and in a few neurons, stained nuclei can be observed (Fig. 3D, E).

Group F.—This well-fixed material allowed preparation of undamaged brain sections of the highest quality. At low magnification, the different structures of the medulla stain

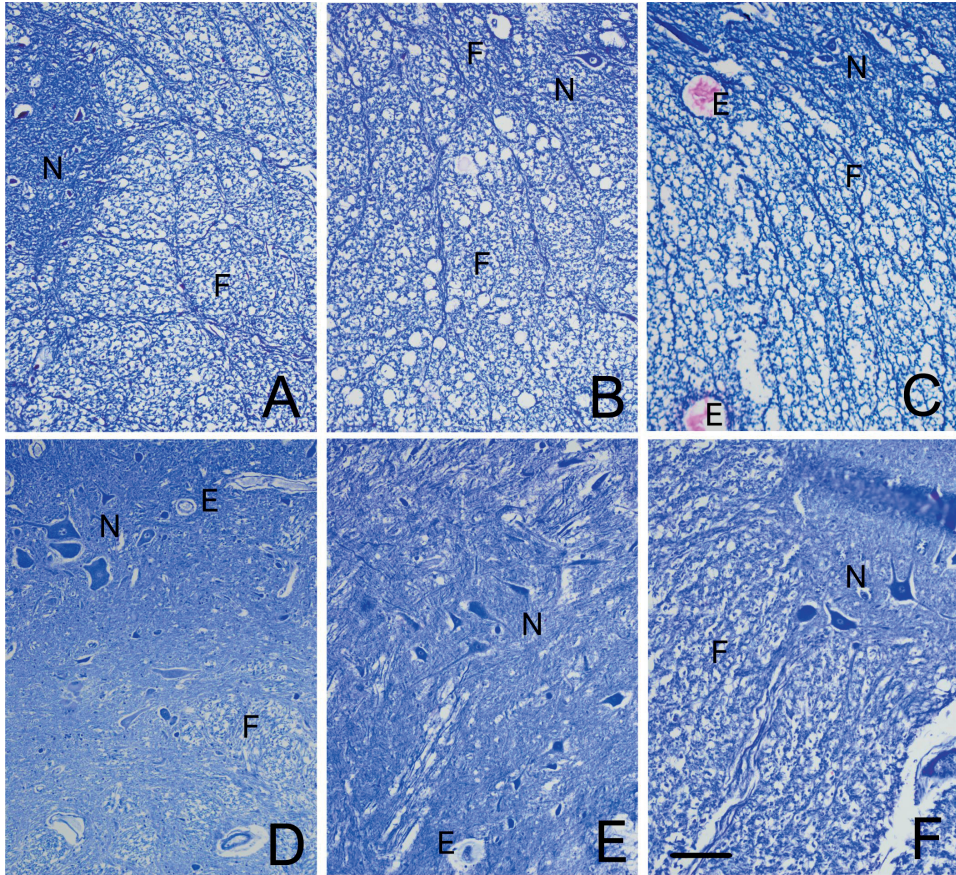


Figure 3. Microscopic observations of spinal cord section in Giemsa stain at high magnification. (A) group A; (B) group B; (C) group C; (D) group D; (E) group E; (F) group F. E, erythrocyte in vessel; F, fiber; N, motor-neuron or neurons. Scale bar = 0.1 mm.

well and are clearly recognizable (Fig. 2F). At higher magnification the homogeneity of tissue is much better than in all other groups (few tissue perforations), and all nerve cells are identifiable and quantifiable. Nuclei and nucleoli are also noticeable (Fig. 3F).

Molecular Level and PCR

Groups A, B, C, D, E, and F.—Despite repeated qPCR amplification attempts (three replicates per fragment length and extract), none of the tested brains yielded any amplifiable DNA in real-time PCR, whereas amplifiable DNA was obtained by the same methods from two control samples of muscle preserved in less acidic formalin (Fig. 4). To rule out the possibility of inhibition of the Taq polymerase as the source for this outcome, we performed an inhibition test for each DNA extract by spiking an internal positive control with the same amount of DNA used in PCR and assessed the amplification curve derived from spiked and nonspiked controls (see King et al. 2009 for details). Although a slight level of inhibition was observed in a few cases (four extracts), all the others yielded no obvious artificial effects that would have impacted the success of real-time PCR amplification. In addition, all QubitTM readings returned nonmeasurable DNA yields in all extracts. Except

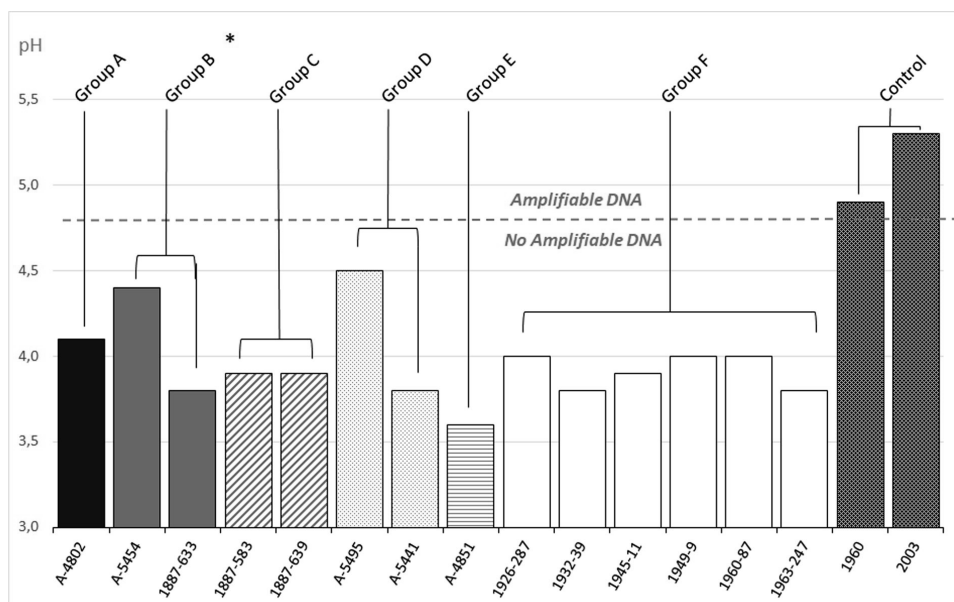


Figure 4. Impact of the pH of the preservative solution on DNA extraction. The dotted line indicates the threshold for DNA extraction/amplification; Beneath this line none of the samples yielded any amplifiable DNA, while above the line DNA was successfully amplified. The bars show the pH of each groups of specimen. Briefly, the figure show that none of the brain samples allowed DNA extraction except for the control samples. *The fluid of one of the specimens of Group B (A-5440) had a pH of 5.4, but the original preservative fluid was replaced by buffered formalin in 2013.

for the two control samples where the pH was higher (4.9 and 5.4), the pH of the preservation fluid of each brain sample was below pH 4.5. Because the original formalin of A-5440 was replaced with buffered formalin solution at pH 6.4 several years previously, the pH of this sample was not considered.

Medical Imagery (MRI)

Group A.—The brain immersed in alcohol had strong tissue signal variations (Fig. 5A). The signal-to-noise ratio (SNR) was lower compared to fluids in other groups, gave less contrast, and resulted in a lower read of slides. The brain also showed many dark-star artifacts in large parts of the brain sections.

Groups B and C.—The brains originally immersed in alcohol and subsequently moved to formalin had different tissue signal variations compared to group A (Fig. 5B, C). The overall SNR of both groups was higher than alcohol, and the white/gray matter contrast was lower in the 50/50 than in the 30/70 group (alcohol/formalin exposure time). Several dark spots were visible in both groups, while apparently shorter alcohol exposure seemingly resulted in fewer dark spots.

Groups D and E.—The brains exposed to various preservation fluids, i.e., alcohol, Owen's liquor, and formalin as the final stage, showed similar results to groups B and C (Fig. 5D, E). However, in images of brains that were kept longer in formalin, white/gray

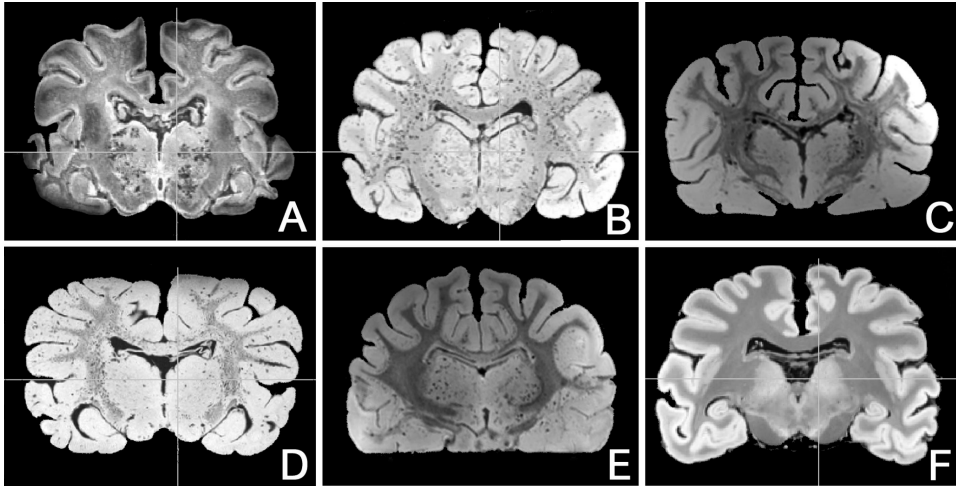


Figure 5. MRI of a brain from each group. (A) group A; (B) group B; (C) group C; (D) group D; (E) group E; (F) group F. Grey and white matter are well contrasted, and it is possible to identify the architecture of several nervous structures. Note the low density of dark spots in group F.

matter had better contrast. Dark spots were perceivable in both groups but without much variation among both groups.

Group F.—The brains kept in formalin from the beginning had a high SNR and grey/white matter contrast and consequently delivered images in the best quality (Fig. 5F). However, they also had some dark spots, but fewer compared to all other groups.

DISCUSSION

The diverse analytical methods applied here allowed a thorough evaluation of the preservation quality of investigated specimens in our collection. In isolation, any single approach would have failed to give a comprehensive picture of the quality of these historic specimens and would have been insufficient to evaluate the state of preservation and determine the range of potential uses of the specimens.

While the macroscopic comparison of brains in each group showed that almost all brains appear in good shape despite their age (Fig. 1), a closer examination revealed that the structural integrity had been compromised in some, correlating with their preservation history. Long preservation in alcohol affects the appearance of the brain and seems to “cook” and harden the tissues. In the same way, the subsequent transfer to Owen’s liquor seems to deposit a gray shading on the brain, probably from exposure to the mercuric chloride. This is contrasted by the well-preserved color of brains and tissues fixed and kept in formalin for a long time, which applies to all the brains selected for this study

The poor condition of the injected brains in group B (A-5454, 1887-633) surely can be attributed to the composition of the solution injected in brains stored in pseudo-fixative alcohol (Milne-Edwards 1858). Macroscopic evaluation suggests that changing of the preservative solution over different periods of time did not affect the visual outer appearance of the brains, and that these have maintained their usefulness as exhibition objects for public display.

On closer examination at the microscopic level, the preservation quality and integrity of tissues was less optimal in some samples, rendering some specimens unsuitable as anatomical specimens for future research (Fig. 2). This is in contrast with the uniformly good results in all groups apparent at the macroscopic level. While low magnification still revealed the identifiable structures in the spinal cord regardless of the preservation history of the individual samples, tissues that have been in formalin longer seem to have less structural damage in white and gray matter. It was not possible to establish a definite correlation between the degree of perforation of thin sections and the preservation history of the samples, but the size and magnitude of cellular tissue damage probably corresponds to the lag between the time of animal death and first contact with the preservative fluid. Because the elapsed time before fixation varies for each brain, the amount of perforation might not be a good indicator for induced damage during subsequent preservation. However, irrespective of their preservation, most brains permitted observation, identification, and repartition of neurons, motor neurons, axons, and bundles of fibers. Considering the age of these brain specimens, the degree of preservation of the nerve cells and the ability to identify the nuclei and even the nucleoli in many of them was an unexpected finding (Fig. 3). However, the preservation of cells and their cyto-architecture in analyzed brains increased the longer the brain was exposed to formaldehyde. The most degraded tissue on the macroscopic level was recorded in group A, which had exclusively been maintained in alcohol, and the condition on the microscopic level was worse in the injected brain A-5454. In contrast, the best results were obtained with group F (100% formalin), with even small glial cells visible in the preserved brain tissue. This excellent preservation of fine and even cellular details in the tissue underlines the potential of group F brains for histochemical studies—as long as they are fixed rapidly after the death of the organism. In summary, the microscopic analysis shows that parts of each group could still be used for comparative micro-anatomical studies, but the usefulness of the samples strongly correlates with the length of time they have been preserved in formalin (the longer, the better).

The third assessment on the molecular level to quantify the DNA degradation caused by exposure to the different preservation fluids clearly showed that, irrespective of individual groups, none of the brains contained amplifiable DNA (Fig. 4). This confirms previous work on historical collection objects stored in unbuffered preservative fluids such as formalin (Schweiger et al. 2009, Hykin et al. 2015). Even though DNA has been successfully amplified from formaldehyde-preserved tissues repeatedly (Totou et al. 2020), the long exposure to formaldehyde (94–55 yr) and other degrading agents such as mercury (corrosive sublimate in Owen's liquor) most likely caused severe degradation of DNA molecules in the MNHN brain samples. Acidification of the preservative fluid induced by natural chemical degradation of formaldehyde into formic acid and degradation of fats into fatty acids lowered the pH in the samples analyzed in this study to pH 4.2 or below. So far, we have been successful in recovering DNA from formalin-preserved specimens at the MNHN to a pH as low as pH 4.5, but not lower. A decrease of the pH of the solution accelerates DNA hydrolysis and triggers loss of nucleobases from the DNA molecule. This adds to the normal difficulties of recovering DNA from formaldehyde-fixed tissues caused by the covalent bonding and crosslinking of the formaldehyde in the DNA helix and proteins.

The last assessment to evaluate specimen quality in this study is complementary to the microscopic observations. The noninvasive MRI and advanced analytical methods allow new insights into the architecture and connectivity in the brain, compared to classical histological techniques (Sébille et al. 2019). To some extent the MRI results overlap with the microscopy findings for the various groups, in which the noticeable degree of perforation in

each group was not correlated with preservation history of the specimens but rather seemed to be associated with the fixation condition of the brains. With MRI, a similar effect was observed, where dark spots more or less corresponded to the presence of erythrocytes in the cerebral blood vessels. Apparently, and this is another interesting finding, the presence of blood cells seems to decrease with increased exposure to formaldehyde over time. Finally, also congruent with the microscopic results, the contrast was reduced and the lowest read qualities were obtained for specimens stored in alcohol. Conversely, the best contrast was observed in group F or after prolonged subsequent exposure to formaldehyde in groups D and E (Fig. 5). Independent computed tomography scans of other primate brains from the 1960s showed the same type of results, i.e., higher contrast and resolution in brains preserved in formalin compared to those stored in alcohol (personal unpublished results).

CONCLUSION

This comparative study underscores that historic fluid collection specimens—irrespective of their preservation history—are still valuable in natural history collections for research and other uses such as teaching or display. Advanced medical imaging techniques such as MRI are useful for measuring and modeling the complex structures of such specimens and ideally allow for virtual dissection (Herbin et al. 2010). However, as the old adage says, “The clothes do not make the man,” and a nice appearance does not necessarily imply that the tissues are well fixed and that the specimen is suited for histologic research. However, our results show that prolonged formaldehyde exposure seems beneficial in this context and enhances their potential use for different research disciplines. To increase the usefulness of specimens for other areas such as genomic research, the formaldehyde should be buffered to reduce DNA degradation. Other preservative fluids such as glycerol or Kaiserling III (without formalin) have been advocated, but so far, no studies have analyzed the potential effects of these solutions on the quality and integrity of specimens, tissues, DNA, or RNA in the long run. Because of the scarcity of analytical studies investigating potential impacts of the transfer of specimens from alcohol or formaldehyde to glycerol, we intend to assess specimen tissue quality for such cases in the future using similar methodology.

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RÉSUMÉ

Au cours des époques, les solutions de conservation utilisées dans les collections en fluides ont changé avec les progrès de la chimie. Le choix initial de la solution de conservation est souvent étroitement lié à, l'époque, et à l'utilisation originale des échantillons. Par conséquent, dans une collection donnée, plusieurs changements de solution de conservation ont pu avoir lieu au cours du temps. L'analyse macroscopique, microscopique et moléculaire de l'état de conservation des cerveaux, nous a permis d'évaluer les effets des différents traitements appliqués au cours du temps à ces collections en fluide. Nos résultats confirment que la durée d'exposition au formaldéhyde a clairement un effet sur les tissus préservés. Malgré les controverses associées à la qualité ou l'utilisation de certains fixateurs historiques, les méthodes analytiques modernes telles que l'imagerie médicale prouvent la qualité de conservation des spécimens historiques et leur potentiel pour leur utilisation future en recherche. Cependant, le choix du fluide de fixation et de conservation est d'une importance cruciale car les choix d'aujourd'hui influenceront l'utilisation des spécimens à l'aide des nouvelles méthodes d'analyse de demain.

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