DNA/RNA Degradation Rate in Long Term Fixed Museum Specimens

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ABSTRACT

In today's research driven society, it has become commonplace for institutions to rely upon DNA and RNA extraction techniques to help obtain genomic data from old specimens. Generally, specimens were commonly preserved for future gross examination and/or teaching. Using histological examination of specimens from museum jars from the Pathology Department at the Indiana University School of Medicine, the sequential and chronological degradation of DNA and RNA has been studied. We examined gross specimens from nine decades from 1920 until 2000. We evaluated histologic preservation of kidney, liver, heart, lung, spleen, uterus, and brain for nuclear structure in these samples. Nuclear preservation was based on amount of nuclei per 20x microscopic field and the crispness of the nuclear membrane and internal features. The nuclei in high lipid tissues such as the brain were found to degrade at a quicker rate than dense tissues such as the heart and uterus. Our study has shown specimens preserved beyond fifty years were likely to have little to no nuclei left, thus indicating that there was little to no DNA and RNA remaining. This technique of histologic evaluation first is an important finding and a general guideline which may save research institutions from the expensive process of DNA and RNA extraction.

INTRODUCTION

Historically, a variety of fixatives – such as various concentrations of alcohols and formalin – have been used; presently, the accepted fixative is 10% neutral buffered formalin. Formalin has a primary mechanism of fixation by way of cross linking proteins thereby causing the fixed substance to become firmer than when in situ. Within reasonable time frames formalin may also still provide a suitable medium for genomic study such as DNA and RNA extraction.
First prepared in 1859, formalin did not undergo commercial production in the United States until 1901. Formalin was first used for preservation of tissue samples for purposes such as histology, gross anatomy, cytology, and taxonomy. Only within the past two decades has formalin begun to be used for archival collections for DNA-related purposes. Within 6-8 months, formalin begins the chemical breakdown process into formic acid; further reactions due to long exposure to formalin have not been well characterized. Due to the nature of the breakdown process of formaldehyde, it is not a suitable medium for preserving DNA. Instead, tissue for molecular studies is commonly either frozen or fixed in alcohol. Even short term specimen storage in formalin have been shown to significantly reduce DNA and RNA solubility. There is considerable evidence to show formaldehyde induces DNA and RNA degradation.
Upon removal from the body, tissues undergo an autolytic process initiated soon after cell death causing breakdown of proteins and eventual liquefaction of the cell. Fixation is a preservation method commonly used to preserve cells and tissues in as close to a life-like state as possible while still allowing them to undergo further preparative procedures and examinations without change. As fixation stabilizes cells and tissues through cross linking proteins, autolysis and bacterial decomposition is arrested. Fixation rate in dense tissues heart and uterus can be very slow; direct injection of fixative can increase the rate of fixation.
Our study sought to discover what a "reasonable time frame" is for specimens stored in formalin that may still allow DNA and RNA extraction. Although there is variance between lipid and protein based structures, our study found that specimens preserved roughly beyond fifty years are likely to have little to no nuclei and consequentially a low likelihood of success for DNA and RNA extraction. Other factors besides length of storage time and biological make up of the fixed specimen influence whether or not DNA and RNA extraction is successful; these factors include size of specimen, duration of tissue hypoxia, and chemical composition of fixative (refer to graph 1).

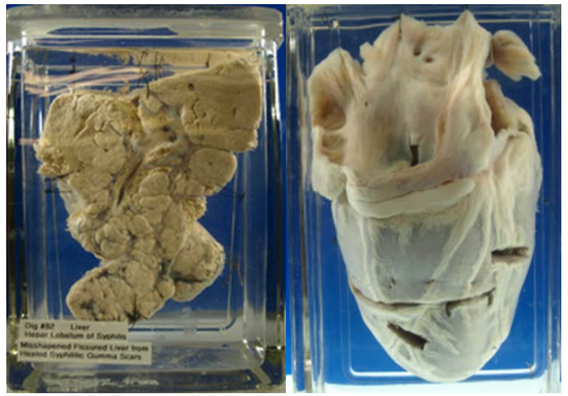


Figure 1. Museum jars from Indiana University School of Medicine. Preserved organs such as the liver and heart had higher rates of cellular structural preservation compared to those of organs such as the brain.

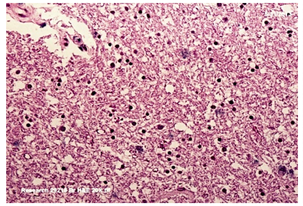


Figure 2. Brain tissue with minimal nuclei degradation (15 year storage, 1980's) 20X

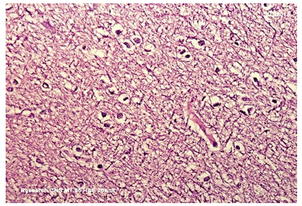


Figure 3. Brain tissue with moderate nuclei degradation (25 year storage, 1970's) 20X

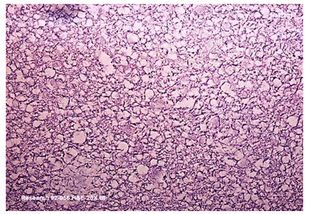


Figure 4. Brain tissue with complete nuclei degradation (80 year storage, 1920's) 20X

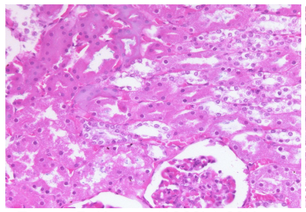


Figure 5. Kidney (1960's) 20X

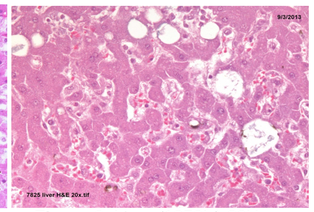


Figure 6. Liver (1950's) 20X

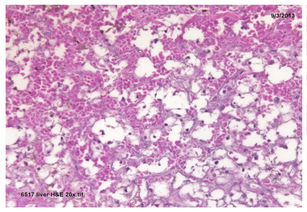


Figure 7. Liver (1940's) 20X

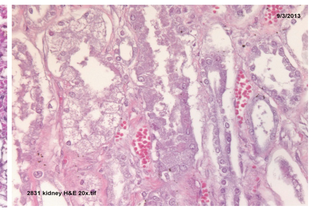
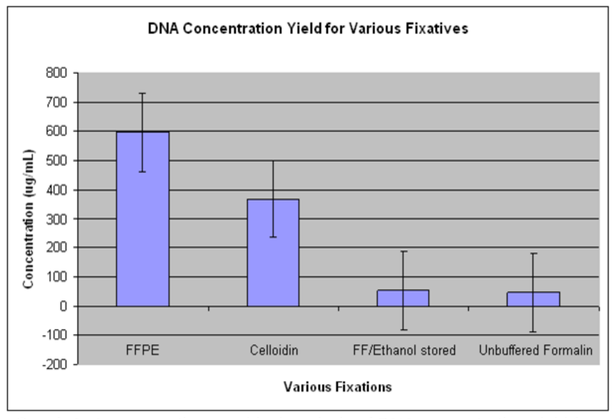


Figure 8. Kidney (1930's) 20X



Graph 1. A comparative graphical analysis of DNA concentration yield for four different tissue fixation methods.

MATERIALS & METHODS

All of the data was collected after a detailed IRB approved protocol. Upon breaking the seal on the museum jars, sections of tissue were taken for processing and analysis. 10 specimens from each decade consisting of various organs such as brain, kidney, liver, heart, lung, spleen, and uterus were included in the study. Grossly all organs looked intact and lesions (hemorrhages, necrosis, inflammation and tumors) could still be seen in the tissues.
Tissue Processing: The tissues are fixed overnight in neutral buffered formalin and then transferred to 70% ethanol prior to processing to a paraffin block. The 3.5 x 2.5 slides were then baked overnight @ 59 degrees C in an oven before staining with H&E (Hematoxylin and Eosin).
Slide Evaluation: Three investigators QCd the various slides by light microscopy to evaluate the following information. Our histology specifications for histologic scoring were the following: The nuclear preservation and/or the loss of cellular nuclei on a slides were scored as 0= negative or no loss, 1= minimal (less than 25% nuclei affected) , 2= moderate (25 to 50% nuclei affected), 3= severe (50 to 75% nuclei affected), and 4 = marked(all nuclei affected).
Molecular Analysis: *DNA Extraction on FFPE Tissues:* The block was microtomed to cut 10-40 mg of tissue. The tissue was placed in 2 ml cryovials and rinsed with xylene. The vials were vortexed and centrifuged at 13 rpm. The supernatant was removed, and ethanol rinse was repeated. The cryovials were left uncovered for 15 minutes before 1 ml of isopropanol was added. The vials were soaked for 14 hours. The samples were centrifuged and the supernatant was decanted, leaving only tissue. The tissue was homogenized for 30 seconds. 240 µL of M1 reagent and 250 µL of M2 solution from the Autogen kit AFKT-FXTD were added to all cryovials and PK solution was made with a concentration of M1 solution to 50 mg of PK. Ten µL of the PK solution was added to all tissue samples, and the vials were left to rotate in an incubator overnight at 55 C. One ml was removed from all samples and added to the Autogen Flexstar machine for DNA extraction. The procedure was completed according to the Autogen manufacturer's recommendations. Similar procedure was done for liquid storage of tissues. DNA integrity and yield were assessed by determining sample absorbance at 260 and 280nm on the Nanodrop 1000.

RESULTS

The tissues (kidney, liver, heart, lung, spleen, uterus, and brain) were in a state of good histologic preservation with retention of nuclei, cytoplasm and cell membrane in most tissues after 1960. In general, tissues collected before 1960 and earlier than1950 showed cell structure degradation with dissolution of some nuclei, washed out cell cytoplasm, and some cell membrane dissolution. These degenerative features were observed to be more severe in the kidney, liver, lung, spleen, and brain than the heart and uterus. The heart and uterus had moderate nuclear loss at the 1920 time frame (see figures 1-8).
The fixation preservation in which the tissues were preserved was also considered when analyzing the molecular structural integrity of the nucleic acids. The highest percentage of DNA yield and concentration was found in specimens which were formalin fixed/ paraffin embedded. These samples were followed by specimens fixed in celloidin. Specimens stored in both formalin fixed/ethanol stored and unbuffered formalin both have been found to have a significantly lower DNA concentration than the preceding two preservation methods (see to graph 1).

CONCLUSIONS

Through chronological analysis our study has shown H&E can reliably predict DNA and RNA status – if nuclei are gone, both DNA and RNA are degraded. Extrapolating from this finding, our study may consequentially be used to help predict whether or not it is worth a research institution's money to attempt extraction. Lastly, DNA and RNA were found to degrade more rapidly in lipid based tissues such as the brain versus dense tissues such as the heart and uterus.

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