Leaching of Trace Elements from Biological Tissue by Formalin Fixation

By: Kristin Gellein, Trond Peder Flaten, Keith M. Erikson, Michael Aschner, and Tore Syversen

Gellein, K., Flaten, T.P., Erikson, K.M., Aschner, M., Syversen, T. (2008) Leaching of trace elements from biological tissue by formalin fixation. Biological Trace Element Research. 121:221-225.

Abstract:
In studies of trace elements in biological tissue, it is imperative that sample handling does not substantially change element concentrations. In many cases, fresh tissue is not available for study, but formalin-fixed tissue is. Formalin fixation has the potential to leach elements from the tissue, but few studies have been published in this area. The concentrations of 19 elements were determined by high-resolution inductively coupled plasma mass spectrometry in formalin in which human and rat brain samples had been stored for different time durations ranging from weeks up to several years. Additional analysis was carried out in fixed brain samples. There was substantial leaching of elements from the tissue into the formalin, and the leaching varied considerably between different elements. For example, formalin concentrations of As, Cd, Mg, Rb, and Sb increased more than 100-fold upon long-term (years) storage, while for Ni and Cr, the leaching was negligible. The degree of leaching was strongly time-dependent. In conclusion, formalin fixation and storage of biological tissue has the potential to leach substantial fractions of several trace elements from the tissue. The potential of leaching must be critically considered when using formalin-fixed biological tissue in trace metal analysis.
Keywords: Formalin fixation, Biological tissue preservation, Trace elements, Leaching, Brain tissue

Article:
Introduction
A major concern in studies of trace elements in biological materials is the potential for sample contamination with the elements to be analyzed, during sample collection, handling, and analysis [1]. However, the converse may also be true under certain conditions, that is, that some of the elemental content is lost in the process. In the present paper, our aim is to draw attention to the fact that standard formalin fixation may leach considerable amounts of elements from the biological tissue. Knowledge of the effects of formalin fixation on tissue elemental concentrations is important because in many cases, fresh (frozen) tissue may not be available for study, but formalin-fixed tissue is (e.g., archival samples utilizing routine formalin fixation required for histopathological diagnosis).

To date, there have been few systematic studies of the effects of formalin fixation on trace element concentrations in human tissue [2-5], and most of these have determined only one or two elements [3-5]. In general, few dramatic differences between fresh and formalin-fixed tissue concentrations were reported. However, Bush et al. [2] reported that formalin storage resulted in decreased Mn and Mg and increased Al tissue levels, and Meldrum [4] reported decreases in Co and Al concentrations. Others have reported decreased Cu levels in liver tissue from an antelope species [6] and in woodlice [7] as an effect of storage in formalin. Herein, we present data from two different studies in which brain tissues were stored on formalin, one of human brain samples [8] and one of rat brain samples [9]. In these studies, preliminary tests indicated substantial concentrations of several elements in the formalin, so we decided to investigate this at greater detail. Our results extend the observations from earlier studies and emphasize the importance of elements leaching from tissue into formalin; specifically, leaching is time-dependent and varies considerably between different elements.

Materials and Methods

Brief Description of the Two Studies

In the first study [8], human brain samples were collected during the period 1979-1983. The brains were stored as whole organs in formalin until 2000, when tissue pieces of 0.5— 1.0 g were dissected out. Three different types of formalin were analyzed: (1) fresh formalin of the same brand (Baker) that was originally used to fix the brains; (2) the formalin used for shipping the dissected brain pieces from the USA to Norway, sampled after about 18 months; (3) aliquots of the formalin in which the whole brains had been stored for several years (this was not the formalin originally used at the autopsies in 1979-1983; this formalin had been changed during storage). For analysis, 3 ml of the formalin was diluted to 10 ml with ion-exchanged water (Milli-Q, Millipore). The second study was performed on formalin-fixed rat brains [9]. These brains were stored in formalin for 12 weeks. For analysis, 0.2 ml of the formalin was diluted to 12.2 ml with ion-exchanged water.

Inductively Coupled Plasma Mass Spectrometry Analysis

Multielement analysis was performed using a Thermo Element high-resolution inductively coupled plasma mass spectrometry (ICP-MS) instrument (Bremen, Germany) with seven scans per sample. The radiofrequency power was 1, 150 W. Samples were introduced with a CETAC ASX 500 autosampler (Omaha, NE, USA) with a peristaltic pump (pump speed, 1 ml/min). The instrument was equipped with a concentric Meinhart nebulizer connected to a Scott spray chamber, and a quartz burner with a guard electrode. The argon nebulizer gas flow was adjusted to give a stable signal with maximum intensity for the nuclide ^{115}In. For details of the analytical methods, see [8, 9].

Results and Discussion

The results of the ICP-MS analysis of the formalin solutions from the two studies are given in Tables 1 and 2. Table 1 clearly shows that the degree of leaching varied considerably between the different elements. The concentrations of As, Cd, Mg, Rb, and Sb in the formalin in which the human brains had been stored for several years were more than 100 times their concentrations in fresh formalin. For Cr and Ni, the leaching was negligible. For the essential elements Fe, Zn, and Cu, the results were intermediate, the concentration ratio between the stored formalin and the fresh formalin being about 20. It is evident that the leaching was not a simple function of the elemental concentration in the tissue: Some elements present at high tissue levels showed little apparent leaching, while others present in relatively low abundance readily leached out. It seems reasonable to assume that such differences would largely depend on the strength and mode of binding of the different elements in the tissue. For example, the metals Ag, Hg, Ni, and Pb, which are known to be strongly bound to sulfhydryl groups (largely present in proteins), were leached from tissue to formalin to a lesser degree than most other elements (Table 1).

Seemann et al. [5] studied Cr and Ni in lung tissue fixed and stored in formalin and reported negligible leaching of the two metals to the formalin in which they were conserved. Our results are consistent with this (Table 1), indicating that Cr and Ni are probably generally strongly bound in tissue.

Bush et al. [2] compared the concentrations of Cu, Fe, Ca, Mg, Zn, Cd, Hg, Al, and Mn in fresh tissue with concentrations in tissue fixed in formalin for 1 week, 6 months, and 12 months. The concentrations of Mg decreased by 18-41% in seven different types of tissue after 1 week's storage, but remained constant in bone. Long-term storage lead to gradually increasing concentrations of Al, from 0.9 µg/g at time zero to 2.3 µg/g after 12 months, while there was a significant decrease in Mn concentration from 6 to 3.8 µg/g after 1 year of storage. For the other elements, there were no detectable changes. In our study, substantial amounts of Mg and Mn were leached to the formalin (Table 1), in accordance with the results of Bush et al. [2].

While Bush et al. [2] reported no detectable decreases in Cu concentrations in tissues upon formalin storage, Quan et al. [6] found that 4 1/2 months of formalin storage significantly reduced the Cu concentration in liver samples from blesbok, a South African antelope species. In addition, Hendrickx et al. [7] reported that 14 days of formaldehyde storage substantially reduced (up to 40%) the Cu concentrations in three species of woodlice.

Table 1 Average Concentrations of Elements in Formalin Solutions and in Fixed Human Brain Tissue Samples [8]

	µg/l			µg/kg Dry weight
	Fresh[a]	18 months[b]	Long term[c]	Brain content[d]
Ag	0.016	0.016	0.050	39
As	0.13	0.53	31	64
B	0.6	7.0	16.8	1,030
Cd	0.01	0.22	2.61	92
Co	0.06	0.17	0.58	37
Cr	1.9	0.4	3.5	469
Cu	5.2	16	98	23,700
Fe	31	30	565	313,000
Hg	0.07	0.10	0.33	204
Mg	11	474	1428	31,200
Mn	0.8	11	31	790
Ni	3.0	2.4	2.6	350
Pb	0.11	0.21	1.23	1,310
Rb	0.03	1.5	10.9	150
Sb	0.04	0.27	7.54	350
Sn	0.0	0.0	0.1	93
Sr	0.3	10.4	17.7	389
V	0.01	0.14	0.37	211
Zn	19	373	355	50,400

[a] Formalin not used for tissue fixation (Baker, reagent grade; n=4).

[b] Formalin used for shipping the dissected tissue pieces to Norway (n=2).

[c] Formalin in which the whole brains were stored (n=10).

[d] Average values for 30 brain samples

It is conceivable that, in addition to changing the total amounts of elements present in tissue, formalin fixation can also change the physicochemical form (speciation) of some elements. Chuaanusorn et al. [3] studied Fe-loaded spleen tissue immersed over a period of 200 days. Over the first 60 days, there was a steady leakage from the tissue until 3% of the Fe had been lost, but thereafter, no further leaching was detected. Mössbauer spectroscopy indicated no evidence of chemical transformation of the iron remaining in the tissue. In contrast, another Mössbauer spectroscopy study utilizing brain tissues from Parkinsonian patients indicated chemical transformation of some of the ferritin-bound Fe to some other unidentified form of Fe during the storage of the tissues in formalin from 1 to 10 years [10]. This last study seems to indicate that very long-term storage of tissue in formalin may change the tissue speciation of certain elements.

When comparing tissue from diseased individuals with tissue from control persons, identically preserved and processed, there would seem to be little reason to suspect that the degree of leaching should depend on the disease status of the individuals [11], but this possibility can of course not be ruled out. In our second study, using several different treatment paradigms in experimental rats, there were substantial differences in element concentrations between the treatment groups and the normal rats [9]. However, the rate of release from the tissue to the formalin was the same regardless of the treatment paradigms, indicating that the differences in elemental concentrations were not related to differential leaching, and that the use of formalin-fixed tissue was valid in this case.

Table 2 Average Concentrations (Ranges in Brackets) of Metals in Formalin Solutions and in Fixed Brain Tissue Samples from Rats [9]

Metal	Formalin solutions (µg/l)		Brain content (µg/kg; wet weight; n=100)
	Fresh[a] (n=10)	Stored[b] (n=100)	
Cu	0.06 (0.03–0.10)	0.46 (0.14–3.18)	2,010
Fe	7.6 (4.3–9.8)	8.2 (4.7–32.4)	13,500
Mn	0.10 (0.08–0.12)	0.52 (0.30–1.29)	465
Zn	1.5 (1.0–1.9)	8.6 (3.2–24.6)	15,700

[a] Formalin not used for tissue fixation

[b] Formalin in which the brain tissue had been stored for 12 weeks

In conclusion, it is evident that formalin fixation and storage of biological tissue has the potential to leach substantial fractions of certain trace elements from the tissue. The degree of leaching is very different for different elements and is probably mainly determined by the strength and mode of binding of the particular element in the tissue. The degree of leaching is time-dependent; long-term storage (years) has a considerably larger influence on trace metal concentrations than short-term storage (weeks). The potential of leaching should always be critically considered when using formalin-fixed biological tissue in trace metal analysis. If available, it is generally strongly advisable to use fresh/frozen tissue.

References

1. Versieck J, Cornelis R (1989) Trace elements in human plasma or serum. CRC Press, Boca Raton
2. Bush VJ, Moyer TP, Batts KP, Parisi JE (1995) Essential and toxic element concentrations in fresh and formalin-fixed human autopsy tissues. Clin Chem 41:284–294
3. Chua-anusorn W, Webb J, Macey DJ, Pootrakul P, St. Pierre TG (1997) The effect of histological processing on the form of iron in iron-loaded human tissues. Biochim Biophys Acta Mol Basis Dis 1360:255–261
4. Meldrum RD (2001) Erroneous aluminum and cobalt tissue concentrations from using formalin. J Biomed Mater Res 57:59–62
5. Seemann J, Wittig P, Kollmeier H, Müller K-M, Schejbal V (1990) Trace metal analysis of chromium and nickel in lung tissue fixed and stored in formalin. Pathol Res Pract 186:197–201
6. Quan M, Mülders MS, Meltzer DGA (2002) The effect of storage conditions on samples for the evaluation of copper status in blesbok (Damaliscus pygargus phillipsi). J S Afr Vet Assoc 73:111–114
7. Hendrickx F, Maelfait J-P, De Mayer A, Tack FMG, Verloo MG (2003) Storage mediums affect metal concentration in woodlice (Isopoda). Environ Pollut 121:87–93
8. Gellein K, Garruto RM, Syversen T, Sjøbakk TE, Flaten TP (2003) Concentrations of Cd, Co, Cu, Fe, Mn, Rb, V, and Zn in formalin-fixed brain tissue in amyotrophic lateral sclerosis and parkinsonism– dementia complex of Guam determined by high-resolution ICP-MS. Biol Trace Elem Res 96:39–60
9. Erikson KM, Syversen T, Steinnes E, Aschner M (2004) Globus pallidus: a target brain region for divalent metal accumulation associated with dietary iron deficiency. J Nutr Biochem 15:335–341
10. Galazka-Friedman J, Bauminger ER, Friedman A, Barcikowska M, Hechel D, Nowik I (1996) Iron in parkinsonian and control substantia nigra: A Mössbauer spectroscopy study. Mov Disord 11:8–16
11. Uitti RJ, Rajput AH, Rozdilsky B, Bickis M, Wollin T, Yuen WK (1989) Regional metal concentrations in Parkinson's disease, other chronic neurological diseases, and control brains. Can J Neurol Sci 16:3 10– 314