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Determination of Trace and Minor Metals in Benign and Malign Human Thyroid Tissues

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There is a need for the analysis of essential and toxic metals in cancerous and non-cancerous human thyroid tissues because the relationship between these elements and the mechanism of cancer development and inhibition were reported in the literature. Selected metal concentrations including Ni, Fe, Mg and Ca in both cancerous (malign) and non-cancerous (benign) thyroid tissues were determined by atomic absorption spectrometry. For this purpose, wet tissues were digested by using microwave energy. The calcium concentrations in the malign thyroid tissues were found to be lower significantly than in the benign thyroids. It was observed that there are slightly the reverse-proportional relationship between the changes in iron concentrations and the changes in nickel concentrations regarding cancerous and non-cancerous thyroid samples. Therefore, it is understood that the decreases in the calcium levels in the cancerous thyroid samples in compared to non-cancerous tissues and the interaction between nickel and magnesium are very important for the investigation of cancer mechanism and may be important for cancer diagnosis.

Key Words: Cancer, Metals, Human thyroid, Microwave energy, Atomic absorption.

INTRODUCTION

The importance of the essential trace metals in health and disease is indisputable because of their both essential roles in specific concentration ranges and toxic roles in relatively high levels. It may be expected that the deficiency of essential trace metals as cofactors of enzymes could impair the host's resistance against carcinogenic stress[1]. Furthermore, the roles of metals in the development and inhibition of cancer have a complex character and have risen many questions because of their essential and toxic effects on human health. In the last three decade, cadmium, nickel, arsenic, beryllium and chromium(VI) have been recognized as human or animal carcinogens by International Agency for Research on Cancer (IARC)[2-6]. It was reviewed that the metal carcinogenesis is mediated either by the increased generation of reactive oxygen species (ROS) or by interference with DNA repair processes[7] since the numbers of metals are able to generate free radicals such as ROS.

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Nucleotide excision repair (NER), the major repair system, is inhibited at low levels such as non-cytotoxic concentrations of Ni(II), Cd(II), Co(II) and As(III). The repair inhibition is attributed to the displacement of Zn(II) and Mg(II) by Ni(II) and Cd(II) ions due to the fact that Mg and Zn being cofactors for DNA polymerase are effective protectors against carcinogenesis *in vivo*[4,8]. Furthermore, magnesium stabilizes DNA structure on the natural conformation and prevents also DNA from alkylation. In magnesium-deficient animals, there is an increasing in the production of free radicals[5]. Ni may be an essential trace metal, but its role in the body is completely unknown. Although, small amounts of Ni are probable needed by the human body to produce red blood cells, long-term exposure can cause decreased body weight, heart and liver damage and skin irritation. High levels of Ni in the diet may be associated with an increased risk of thyroid problems, cancer and heart disease. The International Agency for Research on Cancer (IARC) evaluated the carcinogenicity of Ni in 1990 and suggested that all nickel compounds except metallic Ni were carcinogens to humans[6].

Although iron participates in a number of critical physiological processes, such as oxygen transportation, xenobiotic metabolism and oxidative phosphorylation. It is also a prosthetic group in many enzymes and its contents in excess of the organism's binding capacity, may be highly toxic as indicated by iron overload-related diseases including cancer development. In addition, excess iron, like iron deficiency, also leads to oxidative DNA damage. Reducer active iron ions, besides copper ions, play a role in the increase of the ROS production by transforming molecular oxygen (Fenton and Haber-Weiss reactions) in biological systems[5,9]. Recent studies[7] have shown that the differences in the toxic effects of trace metals such as Fe, Cu, Cr, Ni, Cd and V depend on their contribution to the rate of reactive oxygen species (ROS) generation, solubility and the formation of complexes in cell. Calcium contributes to the formation of intracellular cement and the cell membranes, besides its many numbers of functions.

The thyroid tissue has also been well studied tissue for trace metal determinations because this tissue could accumulate both highly toxic and essential metals[10]. Reddy *et al.*[11] have reported higher levels of Ti, V, Cr, Mn, Fe, Co and Sr in the cancerous thyroid tissues than in the normal thyroids, whereas the lower concentrations of Ca, Cu, Zn, As and Hg in carcinoma thyroid than in the normal thyroid tissues were found. On the contrary these results mentioned above, Maeda *et al.*[12] observed the lower iron concentrations in carcinoma thyroid tissues in compared to the normal thyroid. According to a study made by Yaman and Akdeniz[13], while Cu concentrations in cancerous thyroid tissues were higher than those in the non-cancerous (benign) tissues ($p < 0.05$), slightly lower Zn contents in corresponding samples were found. The observed lower concentrations in their study in compared to the other studies were attributed to the differences at the treatment of tissues such as formalin-fixed and freeze-drying procedure. The probable reasons of the increases and decreases in trace metal concentrations of cancerous and non-cancerous thyroid were detailed

in the literature[7]. Trace metal levels reported in the literature for cancerous and non-cancerous thyroid tissues were summarized in the Table-2. The reported metal concentrations[16-19] are seen to be in the ranges as cancerous/non-cancerous (mg kg^{-1}): 244-1397/182-570 for Fe and 72-101/87-394 for Zn according to dry weight basis. As it can be seen from these results, the reported metal concentrations are different from each others. Thus, there is a requirement to determine the decisive metal concentrations in thyroid samples considering the cancerous and non-cancerous state. In our laboratory, trace metals in human tissue samples were successfully determined by using atomic absorption spectrometry[13-17].

In this study, the concentrations of selected metals, including Ni, Fe, Mg and Ca in malign and benign thyroid tissues, were determined by atomic absorption spectrophotometry. For digestion of the tissues, a microwave oven was used.

EXPERIMENTAL

An ATI UNICAM 929 model flame atomic absorption spectrophotometer (FAAS) equipped with ATI UNICAM and COTTO hollow cathode lamps was used for the metal determinations. The optimum conditions for FAAS are given in Table-1. A Domestic microwave oven (Kenwood) was used for the digestion of the tissues.

TABLE-1
OPERATING PARAMETERS FOR FAAS

Parameter	Ni	Fe	Mg	Ca
Wavelength (nm)	232.0	248.3	285.2	422.7
HCl current (mA)	7.5	15	15	6
Acetylene flow rate (L/min)	0.5	0.5	0.5	4.2
N_2O flow rate (L/min)	-	-	-	4.7
Air flow rate (L/min)	4.0	4.0	4.0	-
Slit (nm)	0.2	0.2	0.5	0.5

Unless stated otherwise, all chemicals used were of analytical-reagent grade. Throughout the analytical work, doubly distilled water was used. All glass apparatus (Pyrex) were kept permanently full of 1 mol L^{-1} nitric acid when not in use. In the digestion procedures, concentrated nitric acid (65 %, Merck) and hydrogen peroxide (35 %, Merck) were used. Stock solutions of metals (1000 mg L^{-1}) were prepared by dissolving their salts in 1.0 mol L^{-1} nitric acid.

Preparation of samples: Due to the fact that unprocessed specimens are preferable, the fresh tissues were chosen. It was proved that fresh and formalin-fixed tissues yielded virtually the same results for essential and toxic metals including Ca, Mg, Fe, Cu, Zn, As, Cd, Hg and Pb[22]. In this study, the samples were obtained in the formaldehyde solution from private pathology laboratories and the pathology laboratory at Firat University in Elazig, Turkey, after surgery and histopathologic examination. Among total 19 patients, 5 of thyroids were taken from cancerous (malign) tissues of patients. In addition, 13 of thyroids were taken from noncancerous (benign)

tissues of patients. These samples were described as the independent samples. Only one of thyroid sample was examined for both cancerous (malign) part and non-cancerous (normal) part of tissue taken from areas close to tumours, described as the paired sample. Among total 19 patients, 16 samples were taken from females and 3 samples from male. The ages of subjects were observed to be between 18 to 51 (Table-2). The studied samples have not occupational exposition. The wet tissue samples were cut into small pieces with a stainless-steel knife and were transferred into beakers.

TABLE-2
TRACE METAL LEVELS REPORTED IN THE LITERATURE FOR CANCEROUS AND NON-CANCEROUS THYROID TISSUES (mg kg^{-1})

Tissue type	Sample type	Sample amount	Analyses method	Fe	Zn	Cu	Se	Cd	Pb	Ca	Cr	Ref.
Non-cancer	Dry	10-30 mg	ICP-MS/ NAA	182/ 191	341/ 394	–	1.76/ 1.33	–	–	3600/ 3800	2.1/ 2.0	18
Cancerous	Freeze-dried	-	PIXE	1397	72	12.9	–	–	18	393	29.8	11
Normal				570	150	54.9	–	–	17	688	6.2	
Malign	Dry	50 mg	NAA	244	101	–	3.02	–	–	–	0.6	10
Benign				205	93	–	2.00	–	–	–	0.5	
Healthy				237	87	–	2.60	–	–	–	0.8	
Normal-autopsy	Dry	0.25 g	NAA	183	129	-	3.7	16.1	-	-	-	19
Cancer	Wet	0.30 g 0.75 g for Cd, Pb	AAS	–	12	1.2	–	0.11	0.19	–	–	13, 15
Benign				–	15	0.7	–	0.22	0.12	–	–	
Normal-Autopsy	Wet	–	NAA GF-AAS	–	–	–	0.42	1.66	0.05	–	–	20
Normal-Autopsy	Wet	1-2 g	AAS					11.9				21

Digestion by using microwave oven: It was reported[11-16] that the tissue digestion by using closed-vessel digestion with microwave oven was found to be simple, rapid and practical as well as having very low blank values and reduce the risk of metal loss or contamination. Thus, microwave oven was chosen to digesting the tissue samples. For this purpose, the followed steps were applied as described in elsewhere[13,15] by using 1.0 g of wet thyroid tissue. The blank digests were carried out in the same ways.

Calibration graphs: Calibration curves are obtained by using the solutions of the studied elements at different concentrations. The obtained graphs are linear in the concentration ranges described below and the equations of the curves are as follows:

$Y= 85 X + 0.5$	$R^2 = 0.99$	for Ni (0.1-2.0 mg/L)
$Y= 64 X + 0.43$	$R^2 = 0.99$	for Fe (0.20-3.0 mg/L)
$Y= 515 X + 7.0$	$R^2 = 0.99$	for Mg (0.25-2.0 mg/L)
$Y= 305 X + 39$	$R^2 = 0.99$	for Ca (0.25-2.0 mg/L)

RESULTS AND DISCUSSION

Analytical performance: The accuracy of the method was studied by examining the recovery of the metals from thyroid samples fortified with various amounts of the studied metals. The following metal amounts were added: 100 ng/g of Ni, 10 mg/kg of Fe, 100 mg/kg of Mg and 200 mg/kg of Ca. After digestion by microwave oven, the recoveries were found to be, at least, 90 % for Ni and 95 % for Fe, Mg and Ca. In addition, the standard additions method was used to investigate possible interferences caused by the matrix. The slopes of the calibration curves for all studied elements were compared to the slopes obtained by the standard additions method. The slopes of the calibration curves were found to be the same as those obtained with the standard additions method. In other words, all of standard additions curves were parallel to the calibration curves. These results indicate absence of chemical interferences.

Levels of the metals including Ni, Fe, Mg and Ca in the reagent blanks in total analytical steps were found to be 25; 50; 110 and 200 ng mL^{-1} with standard deviations of 4.0; 8.0; 20 and 30, respectively. Therefore, the detection limits for these elements, defined as three times the s values of blanks were calculated as 12; 24; 60 and 90 ng mL^{-1}. Related with precision, the standard deviations for 10 samples of the same tissue were found less than 10 % for all studied elements.

The effect of contamination was eliminated by subtracting the values obtained for blanks.

Comparison of metal levels in the malignant and benign tissues: In the literature, it is described that trace element concentrations of healthy adult thyroid glands were not depend on sex and age[10]. Contradictory results were reported as related to the changing of Ni concentrations between cancerous and non-cancerous human tissues[15,16]. From the Table-3, although Ni concentrations in the cancerous tissues were found to be lower than in the non-cancerous tissues, these differences are statistically not important because of $p > 0.1$. On the other hand, it was observed that there is slightly the reverse proportional relationship between Mg and Ni concentrations in non-cancerous thyroid samples (Fig. 1). In our another study[15], excessive Cd concentrations (450 and 700 ng g^{-1}) were found in these two same thyroid samples, in comparison with the other samples (between 95 and 302 ng g^{-1}). In brief, Mg concentrations in non-cancerous thyroid (benign) tissues change inversely with Ni and Cd concentrations in these samples (Table-3). This is attributed to the displacement of Mg(II) with Ni and Cd as described in the literature[4]. These results agreed to the information that there is the competition between Ni and Mg in tissues. Furthermore, the protective role of Mg in Ni-induced cytotoxicity and genotoxicity can be attributed to its ability to reduce either the intracellular Ni concentration or ROS formation[23]. On the other hand, Mg concentrations of cancerous (malign) thyroid tissues do not significantly change in compared to the non-cancerous tissues (Fig. 2).

TABLE-3
TRACE METAL CONCENTRATIONS IN THE CANCEROUS AND NON-CANCEROUS TISSUES OF THYROID. THE RESULT ARE FRESH WEIGHT BASIS, n = 3, CANCEROUS AND NON-CANCEROUS TISSUES BELONG TO DIFFERENT PERSONS (WITH ONE EXCEPTION)

Tissue number (age of can-non-can)	Ni (ng/g)		Fe (mg/kg)		Mg (mg/kg)		Ca (mg/kg)	
	C	NC	C	NC	C	NC	C	NC
1 (18-26)	700±100	1160±120	21±3	26±6	72±6	65±5	212±20	500±65
2 (35-41)	450±60	1300±120	32±5	22±2	90±10	80±8	200±21	400±35
3 (45-51)	170±20	770±90	42±6	24±6	108±11	87±7	300±40	450±50
4 (40-23)	355±50	315±34	45±5	32±10	172±21	53±8	151±20	340±42
5 (23)		495±52		45±4		54±7		228±25
6 (32)		110±17		16±3		60±8		300±38
7 (37)		100±13		13±1		50±7		295±33
8 (29)		100±14		46±7		250±33		400±42
9 (39)		<100		48±6		302±36		600±51
10 (32)		<100		56±7		190±20		450±51
11 (40)		510±70		35±5		265±32		750±71
12 (28)		430±53		20±3		216±22		235±30
13* (36)	350±50	281±35	30±2	36±4	238±20	243±25	240±30	275±20
Mean	**405**±194	**506**±413	**34**±10	**32**±13	**136±68**	**147±97**	**221±55**	**402±151**
	p = 0.2584				**p = 0.4435**		**p = 0.0009**	

C = Cancerous; NC = Non-cancerous.
*Both benign and malign samples in this line belong to the same person.

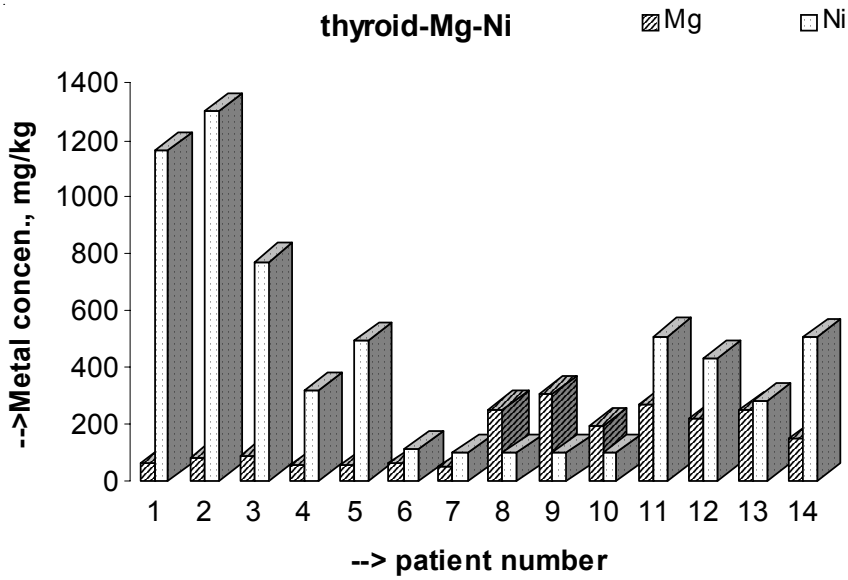

Fig. 1. Comparison of Ni and Mg concentrations in non-cancerous (benign) thyroid tissues. The concentration of number 14 is average value

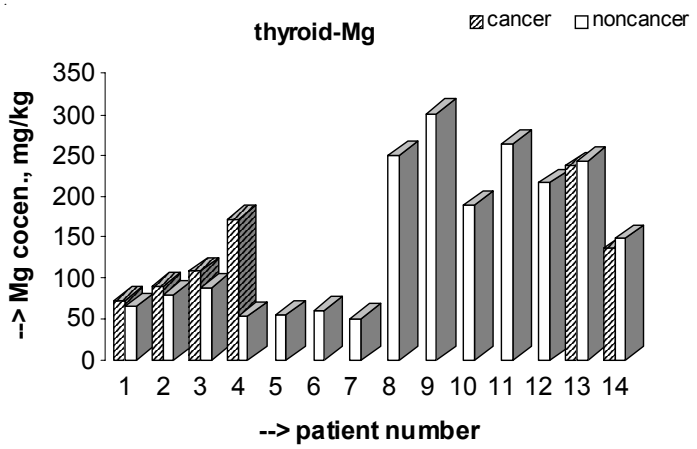

Fig. 2. Comparison of Mg concentrations in cancerous and non-cancerous thyroid tissues. The concentration of number 14 is average value

Although Fe is an essential nutritional element for all life forms; it is known that excess iron, such as iron deficiency, also leads to oxidative DNA damage[24]. In the current study, iron concentrations in the cancerous tissues were slightly higher than in the non-cancerous (benign) tissues (Table-3). Uda and coworkers[25] observed that the deficiency or excess of trace elemental concentrations in cancerous tissues of different organs change from one organ to another organ. Reddy *et al.*[11] have reported higher levels of Fe (1397.1 mg/kg on basis freeze dried) in the tissue of cancerous thyroid in comparison to normal thyroid (569.6 mg/kg) and adenoma thyroid (525.1 mg/kg). Boulyga and coworkers[18] reported the iron concentration of 182 mg kg^{-1} on dried basis, the very high values in comparison with our concentrations (*ca.* 30 mg/kg) can be attributed to the dried basis of their samples. On the contrary Reddy *et.al.*[11] and Maeda *et al.*[12] found the lower iron concentrations in carcinoma thyroid tissues in compared to the normal thyroid.

It has also been described that Ca concentrations of the cancer thyroid tissues were lower than the adenoma and normal thyroid tissues[11]. Boulyga and coworkers[18] reported the calcium concentration of 3600 mg kg^{-1} on dried basis. Similarly, it is seen from the Table-3, the Ca concentrations in cancerous (malign) tissues were found to be significantly lower ($p < 0.05$) than in the non-cancerous (adenoma) tissues (Fig. 3). The possible reasons of the low level of Ca in carcinoma can be attributed to the following reasons[11]. The normal thyroid gland is made up of 20-40 follicles lined by columnar epithelium. The thyroid follicles are separated from each other by connective tissue called "intra-follicular stroma" which contains *para*-follicular cells also called "c" cells. These cells secrete the hormone calcitonin which promotes the absorption of calcium by the skeletal system of the human body. In cancerous thyroid, normal thyroid tissue is destroyed and the calcium level falls.

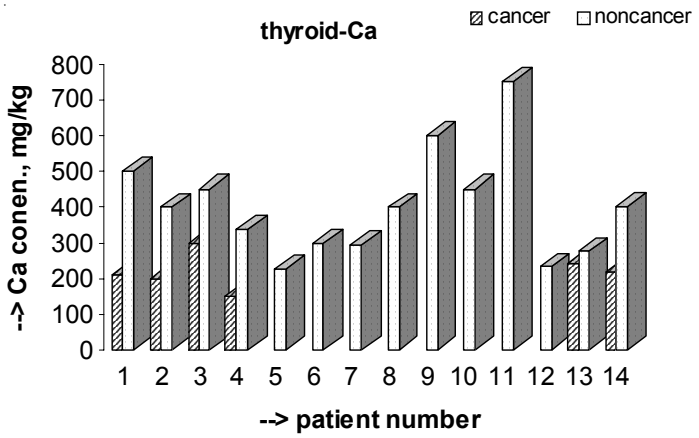

Fig. 3. Comparison of Ca concentrations in cancerous and non-cancerous thyroid tissues. The concentration of number 14 is average value

Conclusion

The calcium concentrations in malign thyroid tissues were found significantly lower than in the benign thyroid tissues. The decrease in Ca levels is very important for the investigation of cancer mechanism and probably for cancer diagnosis. Although a decrease tendency in Ni and Mg concentrations of cancerous thyroids are seen, it is estimated that this decrease is not important, compared to non-cancerous samples. As similar to the recently reported literature results[16,17], the increase and/or decrease observed in metal levels in cancerous tissues in comparison with non-cancerous samples are very important to investigate the cancer mechanism and may provide a new tool to evaluate the biochemical status of cancer tissues.

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REFERENCES

1. G.N. Schrauzer, *Trace Elem. Anal. Chem. Med. Biol.*, 183 (1980).
2. L. Tomatis, *Mutation Res.*, **462**, 407 (2000).
3. IARC, Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Human, Lyon: IARC, Vol. 23 (1980).
4. A. Hartwig, *Pure Appl. Chem.*, **72**, 1007 (2000).
5. B. Desoize, *Crit. Rev. Oncol./Hematol.*, **42**, 1 (2002).
6. IARC Working Group, Nickel and Nickel Compounds, In: IARC Monographs on the Evaluation of Carcinogenic Risks of Chemicals to Humans, Lyon, Vol. 49, pp. 257-446 (1990).
7. M. Yaman, *Curr. Med. Chem.*, **13**, 2513 (2006).
8. K.S. Kasprazak, W. Bal and J. Lukszo, *Mut. Res.*, **533**, 67 (2003).
9. K.H. Cheeseman and T.E. Slater, *Br. Med. Bull.*, **49**, 491 (1993).
10. V.Y. Zaichick, A.F. Tsyb and B.M. Vtyurin, *Analyst*, **120**, 817 (1995).

11. S.B. Reddy, M.J. Charles, M.R. Kumar, B.S. Reddy, C. Anjaneyulu, G.J.N. Raju, B. Sundareswar and V. Vijayan, *Nucl. Instr. Meth. Phys. Res. B*, **196**, 333 (2002).
12. K. Maeda, Y. Yokode, Y. Sasa, H. Kusuyama and M. Uda, *Nucl. Instr. Meth. Phys. Res. B*, **22**, 188 (1987).
13. M.Yaman and I. Akdeniz, *Anal. Sci.*, **20**, 1363 (2004).
14. M. Yaman, D. Atici, S. Bakirdere and I. Akdeniz, *J. Med. Chem.*, **48**, 630 (2005).
15. M. Yaman, *Anal. Biochem.*, **339**, 1 (2005).
16. M. Yaman, G. Kaya and H. Yekeler, *World J. Gastroentor.*, **13**, 612 (2007).
17. M. Yaman, G. Kaya and M. Simsek, *Int. J. Gynec. Cancer*, **17**, 220 (2007).
18. S.F. Boulyga, J.B. Becker, A.F. Malenchenko and H.J. Dietze, *Mikrochim. Acta*, **134**, 215 (2000).
19. Y. Katoh, T. Sato and Y. Yamamoto, *Biol. Trace Elem. Res.*, **90**, 57 (2002).
20. I. Falnoga, M. Tusek-Zinidaric, M. Horvat and P. Stegnar, *Environ. Res.*, **84A**, 211 (2000).
21. M. Uetani, E. Kobayashi, Y. Suwazono, R. Honda, M. Nishijo, H. Nakagawa, T. Kido and K. Nogawa, *Biometals*, **19**, 521 (2006).
22. V.J. Bush, T.P. Moyer, K.P. Batts and J.E. Parisi, *Clin. Chem.*, **41**, 284 (1995).
23. E. Rojas, L.A. Herrera, L.A. Poirier and P.O. Wegman, *Mutation Res.*, **443**, 157 (1999).
24. B.N. Ames, **475**, 7 (2001).
25. M. Uda, K. Maeda, Y. Sasa, H. Kusuyama and Y. Yokode, *Nucl. Instr. Meth. Phys. Res. B*, **22**, 172 (1987).

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