

10-14-1996

## Relative Intranuclear Magnesium and Phosphorus Contents in Normal and Tumor Cells of the Human Thyroid Gland as Revealed by Energy-Dispersive X-Ray Microanalysis

Geza L. Lukacs  
*University Medical School, Hungary*

Imre Zs.-Nagy  
*University Medical School, Hungary*

Janos Steiber  
*University Medical School, Hungary*

Ferenc Gyori  
*University Medical School, Hungary*

Gyorgy Balazs  
*University Medical School, Hungary*  
Follow this and additional works at: <https://digitalcommons.usu.edu/microscopy>



Part of the [Biology Commons](#)

---

### Recommended Citation

Lukacs, Geza L.; Zs.-Nagy, Imre; Steiber, Janos; Gyori, Ferenc; and Balazs, Gyorgy (1996) "Relative Intranuclear Magnesium and Phosphorus Contents in Normal and Tumor Cells of the Human Thyroid Gland as Revealed by Energy-Dispersive X-Ray Microanalysis," *Scanning Microscopy*. Vol. 10 : No. 4 , Article 24.

Available at: <https://digitalcommons.usu.edu/microscopy/vol10/iss4/24>

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Scanning Microscopy by an authorized administrator of DigitalCommons@USU. For more information, please contact [digitalcommons@usu.edu](mailto:digitalcommons@usu.edu).



## RELATIVE INTRANUCLEAR MAGNESIUM AND PHOSPHORUS CONTENTS IN NORMAL AND TUMOR CELLS OF THE HUMAN THYROID GLAND AS REVEALED BY ENERGY-DISPERSIVE X-RAY MICROANALYSIS

Géza L. Lukács, Imre Zs.-Nagy<sup>1,\*</sup>, János Steiber<sup>1</sup>, Ferenc Győri and György Balázs

First Department of Surgery, <sup>1</sup>Verzár International Laboratory for Experimental Gerontology (VILEG),  
Hungarian Section, University Medical School, H-4012 Debrecen, Hungary

(Received for publication June 26, 1996, and in revised form October 14, 1996)

### Abstract

Energy dispersive X-ray microanalysis was performed on altogether 42 surgically removed tissue specimens of 32 patients, which were taken either from intact thyroid parts or various histopathologically verified tumors of the thyroid gland. The tissue specimens were processed with the freeze-fracture-freeze-drying technique and then analyzed in the so-called bulk specimen form. The studies were carried out during the years 1980-81, when intranuclear monovalent ionic composition was studied in detail. From the retained total elemental peak list, it was possible to calculate retrospectively the relative intranuclear Mg and P contents. The data processed by nested (hierarchical) analysis of variance show that the intranuclear Mg content of the 5 diagnostic groups (normal thyroid tissue, thyroiditis, benign adenomas, differentiated carcinomas and undifferentiated thyroid tumors) increases significantly, in parallel with the increasing malignancy, but the P content remains unchanged. One can conclude that the elevated intranuclear Mg content in the tumors of high malignancy may be of diagnostic importance, and a warning signal for the therapeutic approaches based on Mg-supplementations.

**Key Words:** Intranuclear Mg content, intranuclear P content, thyroid gland, thyroid tumors, X-ray microanalysis of bulk specimens.

### Introduction

Magnesium (Mg) is a divalent cation of vital importance. Its ability to influence *in vitro* protein synthesis has been recognized already during the early experiments with ribosomes (Zamecnik, 1960). It has also been revealed that Mg is involved in the initiation of DNA replication in dividing cells, i.e., its regulatory role of growth in normal and cancer cells is obvious (Heeley *et al.*, 1974; Rubin *et al.*, 1979; Terasaki and Rubin, 1985; Walker, 1986; Cameron and Smith, 1989a,b).

For obvious methodological reasons, the majority of human investigations refer to Mg concentrations in blood serum. Attention has recently been paid to the fact that no information is available even on the serum Mg levels in a great percentage of the patients, since Mg level-measurements are not part of routine laboratory examinations (Whang and Ryder, 1990). It is also a generally accepted view that the connection between magnesium and tumorous diseases is very complex: administration of Mg is capable of producing anticarcinogenic effects in some cases, whereas in other ones, just the opposite effect was observed both *in vivo* and *in vitro* (Durlach *et al.*, 1986).

Although it would be important to monitor the intracellular Mg contents in various phases of normal and tumorous growth, data on this parameter are rather scarce. This is due to multiple methodological limitations as explained below.

(1) The atomic absorption spectroscopic techniques applicable to blood samples and extracts of tissue homogenates (see e.g., Szmaja and Konczewska, 1986) can reveal only serum, total tissue or tumor Mg-contents. The situation is further complicated by the fact that the serum contains usually only about 1-2% of the total Mg content of the organism (Holtmeier, 1988). Therefore, we can hardly draw well-founded conclusions regarding the role and metabolism of Mg without knowing the intracellular Mg content. The necessity of such knowledge was emphasized by others, too (Elin and Hosseini, 1985; Dolev *et al.*, 1988).

\*Address for correspondence and present address:

Imre Zs.-Nagy

DOTE, VILEG Hungarian Section

University Medical School

H-4012 Debrecen, Hungary

Telephone/ FAX number: +(36-52) 418-470

(2) Although the energy dispersive X-ray microanalytic technique available for biological purposes since the early seventies, is suitable to reveal the intracellular elemental composition, including Mg concentrations (see for references: Zs.-Nagy, 1983, 1989; Cameron and Smith, 1989b; Zs.-Nagy and Casoli, 1990), it could not gain a sufficiently wide application because of the need of complicated preparative procedures and expensive equipment, as well as well trained persons. Due to this situation, the available papers regarding intracellular Mg contents in various intermitotic or mitotic cell types concern only animal tissues, as listed below: (i) normal and cancerous liver cells of rat (Smith *et al.*, 1978; Cameron *et al.*, 1979a); (ii) enterocytes of female C3H mice (Cameron *et al.*, 1979b); (iii) skeletal muscle cells of normal and cardiomyopathic hamsters (Cameron *et al.*, 1990); (iv) normal and tumorous mammary epithelium of mouse and rat (Cameron *et al.*, 1980); and (v) normal rat and pig thyroid follicular cells and the colloid taken from *in vivo* glands or kept in culture (Wroblewski *et al.*, 1991; Wroblewski, 1994; Wroblewski and Wroblewski, 1994).

The significance of the Mg content of cells was reviewed by Cameron and Smith (1989a): they reached the conclusion that while the average intracellular Mg content amounts to  $50 \pm 3.1$  mmole/kg dry weight in slowly dividing cell types (hepatocytes, lactating mammary epithelium, smooth muscle cells, cardiac myocytes from 32- or 64-day-old mice, pancreatic acinar cells, bladder epithelium and medullary thymocytes, all showing a cell turnover time longer than 7 days), this parameter is slightly but significantly higher ( $63 \pm 3$  mmole/kg dry weight) in rapidly dividing cells (duodenal crypt cells, cardiac myocytes from 2- or 4-day-old mice, colon crypt cells and cortical thymocytes, all showing a cell turnover time shorter than 7 days).

To the best of our knowledge, no data are available comparing the intracellular or intranuclear Mg and P contents of the human thyroid tissue and its tumors. This fact prompted us to perform a retrospective analysis in our earlier human biopsy material, as described in this paper.

During the early eighties, we investigated the changes in intracellular monovalent ionic composition in thyroid tumors of various levels of malignancy by means of the bulk specimen X-ray microanalysis (Lukács *et al.*, 1983; Zs.-Nagy *et al.*, 1983). That time, however, we did not evaluate the X-ray spectra for Mg and P. Since the computerized energy-dispersive X-ray microanalysis gave us a full list of the elements in the specimen, it was possible now to review our previously recorded peak lists of the X-ray spectra also for Mg and P. We could calculate the relative changes in Mg and P content for normal and tumor cells showing different stages of

malignancy. On the basis of the results reported in the present communication, one can assume that the investigation of intracellular Mg content may be one of the subjects of tumor research in the near future. This claim is underlined by the fact that in spite of the contradictory judgements and unclear status of the role of Mg in tumor cell growth (Durlach *et al.*, 1986), cancer therapy in humans already applies adjuvant treatments based on Mg supplementation.

## Materials and Methods

Our criteria for surgical sampling are dealt with in detail in earlier communications (Lukács *et al.*, 1983; Zs.-Nagy *et al.*, 1983). The samples excised in the course of surgical intervention can be divided into the following five groups. It should be stressed that parallel histopathology has confirmed the diagnosis for each sample removed.

(1) Normal thyroid tissue (Group C): these samples were taken when, because of the nature of surgical intervention, solitary, so-called cold nodules were removed by deep incision into the intact tissue, or the tumor on one side had to be removed together with the virtually intact contralateral lobe. The intact structure of the control tissue specimens was checked in every case by histopathological methods and also using the morphological picture obtained by scanning electron microscopy. We are completely aware of the possibility that the tissue samples used as controls may perhaps be affected in some way by the presence of tumors in the given individual, even if the microscopical structure proved to be intact. Nevertheless, this compromise must be accepted, since, for ethical reasons, one cannot obtain entirely intact human thyroid tissue for experimental purposes from healthy persons.

(2) Cases of thyroiditis (Group T) which were operated because of suspicion of malignancy, however, histopathology could not prove the presence of it later. We had only three such cases.

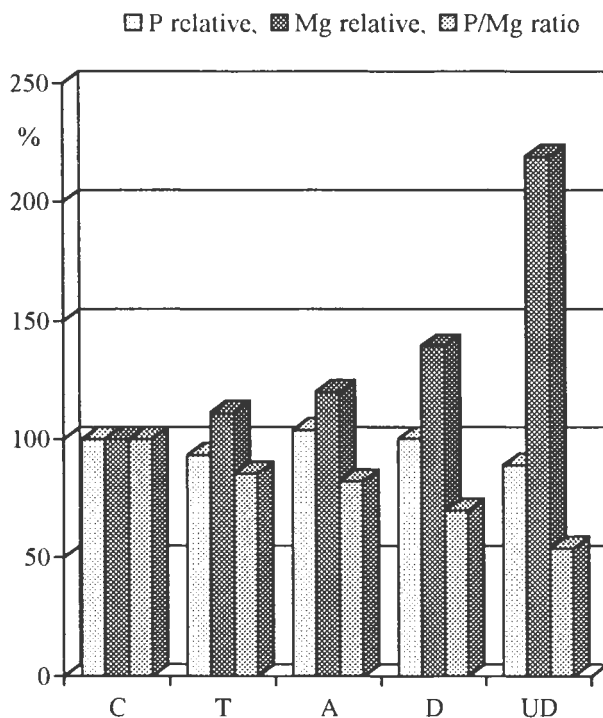
(3) Benign adenomas (Group A).

(4) Highly differentiated carcinomas (Group D). In this group, follicular and papillary carcinomas, their mixtures and one case of medullary carcinoma were classified.

(5) Undifferentiated tumors (Group UD). Anaplastic and planocellular carcinomas have been classified in this group. In addition, one immunoblastic lymphoma was also included. The pronounced proliferativity and clinical malignancy of these cases have been proven also by the postoperative follow-up.

### X-ray microanalysis

The detailed description of this method is contained in previous communications (Zs.-Nagy *et al.*, 1977,



**Figure 1.** The main trends of changes in the relative P and Mg concentrations as well as the P/Mg ratios. P and Mg values  $\pm$  standard error of the mean (S.E.M.) are given numerically in Table 4. P/Mg values were calculated from the individual averages (Tables 1 and 2) and group averages are shown. It is obvious that the increase in Mg content with increasing malignancy is the main cause of the decrease in the P/Mg ratio.

1983; Zs.-Nagy, 1983, 1989), therefore, we summarize here only the major steps: (a) The excised piece of the specimen, cut to the shape of a piece of match is immediately frozen by immersion into isopentane cooled to its freezing point ( $-165^{\circ}\text{C}$ ) with liquid nitrogen. (b) The elongated specimens, while still in the cooled isopentane, are broken with scissors in the transversal direction. In this way, inner broken surfaces of the specimen are revealed which did not suffer any damage during the excision. (c) The broken specimens are frozen-dried until complete dryness. (d) The freeze-fractured frozen-dried (FFFD) (Zs.-Nagy *et al.*, 1977) tissue specimens are fixed to appropriate sample holders then, without any further procedure, put into the scanning electron microscope, where X-ray microanalysis is performed at a 10 kV accelerating voltage in the morphologically well-recognizable cell nuclei at  $\sim 450$  cps for 40 seconds. (e) The X-ray spectrum obtained is processed by a computer program calculating the background intensity (B) (taken between 4 and 6 keV of energy, where no characteristic

elemental peaks are present) and the specific elemental peak intensity ("P"). The quotient of these parameters, the "P/B ratio", is proportional with the relative differences in concentration according to the mass fraction method (Hall *et al.*, 1973; Zs.-Nagy and Casoli, 1990). If standards are available, absolute concentrations can be calculated. It should be noted, however, that at the time of our investigations, we did not use Mg- or P-standards. Because during the more than 15 years since those investigations, spectrometer parameters changed (e.g., the so-called "dead silicone layer" of the semiconductor detector increased, the Be window had to be renewed), Mg and P standard measurements performed now would not be quantitatively accurate for the old data. Therefore, we decided to present our results on Mg and P contents only in relative terms. This method is perfectly suitable to the actual purpose of our studies.

The data obtained have been presented first individually for each diagnostic class (Tables 1 and 2). However, since there was substantial variation between individual means even within the same diagnostic groups, the method of nested (hierarchical) analysis of variance (Sokal and Rohlf, 1981; Zar, 1984) seemed to be the correct method of statistical evaluation, as explained in Results. On the basis of the results of the nested analysis of variance (ANOVA), we have also performed the two-tailed Student's t-test for the total pool of measured nuclei in each tumor class against the controls.

## Results

Tables 1 and 2 show the "P/B ratios" of Mg and P in the cell nuclei found in the FFFD samples of normal thyroid cells of 10 different patients (Group C), compared to those obtained in tissue samples classified as thyroiditis (Group T) by the histopathological analysis. These data represent quite a homogeneous pool, although some differences can be encountered even within the same diagnostic group.

Tables 1 and 2 present the same parameters found in the cell nuclei of thyroid tumors of various levels of malignancy, too. The "P/B ratios" are proportional to the absolute element contents, and they are directly comparable to each other within our experiments, since the analytical conditions used were identical for each of them. Since, however, other authors used different analytical conditions, direct comparison of our "P/B" data is not possible with the data found by others in animal tissues, and cited in the Introduction.

Since the surface of the FFFD specimen is usually not a smooth one, in order to minimize the eventual analytical errors caused by this roughness, we have calculated the average P/Mg ratios. They are presented graphically in Figure 1 for all groups of diagnoses.

**Table 1.** The average peak intensity to background intensity ratio (P/B) of magnesium in the nuclei of intact thyroid, thyroiditis, and thyroid tumor cells of various levels of malignancy (n = number of measured cells).

Ident.	Sex	P/B	± S.D.	± S.E.M.	n	PS
<b>Control thyroid tissue (C)</b>						
1.	F	0.0120	0.0173	0.0015	125	
2.	F	0.0099	0.0133	0.0017	60	
3.	F	0.0076	0.0146	0.0024	38	
4.	F	0.0105	0.0121	0.0022	30	
5.	F	0.0056	0.0085	0.0011	60	
6.	F	0.0118	0.0123	0.0023	81	
7.	F	0.0156	0.0153	0.0024	40	
8.	F	0.0137	0.0169	0.0029	33	
9.	M	0.0173	0.0176	0.0023	58	
10.	M	0.0114	0.0123	0.0028	20	
Group mean		0.0116	0.0149	0.0006	545	
<b>Thyroiditis (T)</b>						
1.	F	0.0075	0.0102	0.0013	65	
2.	F	0.0148	0.0162	0.0022	52	
3.	F	0.0179	0.0174	0.0025	48	
Group mean		0.0128	0.0151	0.0012	165	
<b>Benign adenomas (A)</b>						
1.	F	0.0154	0.0191	0.0028	47	
2.	F	0.0105	0.0127	0.0014	80	
3.	F	0.0141	0.0123	0.0013	97	
4.	F	0.0110	0.0136	0.0017	65	
5.	F	0.0155	0.0171	0.0014	149	
6.	M	0.0151	0.0152	0.0017	82	
Group mean		0.0138	0.0152	0.0007	520	
<b>Differentiated carcinomas (D)</b>						
1.	F	0.0099	0.0136	0.0019	49	24
2.	F	0.0112	0.0118	0.0012	98	
3.	F	0.0106	0.0145	0.0015	98	
4.	F	0.0124	0.0155	0.0013	149	
5.	F	0.0175	0.0171	0.0017	99	132
6.	F	0.0232	0.0178	0.0027	43	
7.	M	0.0311	0.0187	0.0023	67	
8.	M	0.0245	0.0139	0.0020	50	
Group mean		0.0161	0.0167	0.0007	653	
<b>Undifferentiated tumors (UD)</b>						
1.	F	0.0099	0.0106	0.0015	51	2
2.	F	0.0275	0.0185	0.0018	101	12
3.	F	0.0361	0.0426	0.0029	223	0
4.	M	0.0212	0.0187	0.0011	285	1
5.	M	0.0157	0.0166	0.0020	69	48
Group mean		0.0253	0.0290	0.0011	729	

Notes: F = female, M = male; S.D. = standard deviation; S.E.M. = standard error of the mean; PS = postoperative survival in months (if no figure is given, the patient is alive at the moment of the closure of this manuscript). Group means are calculated from the total cell pool of the group, i.e., not as the average of the individual means.

# Internuclear Mg and P in human thyroid tumors

**Table 2.** Average P/B values of phosphorus in the nuclei of intact thyroid, thyroiditis, and thyroid tumor cells of various levels of malignancy (n = number of measured cells).

Ident.	Sex	P/B	± S.D.	± S.E.M.	n	PS
<b>Control thyroid tissue (C)</b>						
1.	F	0.9827	0.1910	0.0171	125	
2.	F	0.8017	0.1789	0.0231	60	
3.	F	0.8577	0.1862	0.0302	38	
4.	F	0.9219	0.1270	0.0232	30	
5.	F	0.8407	0.1294	0.0167	60	
6.	F	0.9426	0.1427	0.0159	81	
7.	F	0.8318	0.1627	0.0257	40	
8.	F	0.9275	0.1916	0.0333	33	
9.	M	1.0130	0.1398	0.0184	58	
10.	M	0.9160	0.1871	0.0419	20	
Group mean		0.9155	0.1792	0.0077	545	
<b>Thyroiditis (T)</b>						
1.	F	0.8594	0.1836	0.0227	65	
2.	F	0.8168	0.0780	0.0108	52	
3.	F	0.8900	0.1393	0.0201	48	
Group mean		0.8549	0.1464	0.0114	165	
<b>Benign adenomas (A)</b>						
1.	F	0.9854	0.2271	0.0331	47	
2.	F	0.9332	0.2265	0.0253	80	
3.	F	0.9611	0.1589	0.0161	97	
4.	F	0.8722	0.1310	0.0163	65	
5.	F	1.0377	0.2004	0.0164	149	
6.	M	0.8369	0.1116	0.0123	82	
Group mean		0.9502	0.1943	0.0085	520	
<b>Differentiated carcinomas (D)</b>						
1.	F	0.7918	0.1577	0.0225	49	
2.	F	0.7132	0.0924	0.0093	98	24
3.	F	0.9146	0.1195	0.0121	98	
4.	F	0.9633	0.1945	0.0159	149	
5.	F	1.0095	0.2186	0.0220	99	
6.	F	0.9277	0.2325	0.0355	43	
7.	M	1.0234	0.1798	0.0220	67	
8.	M	0.9788	0.1313	0.0186	50	132
Group mean		0.9176	0.1993	0.0078	653	
<b>Undifferentiated tumors (UD)</b>						
1.	F	0.7269	0.1044	0.0146	51	2
2.	F	0.9625	0.1461	0.0145	101	12
3.	F	0.7692	0.0989	0.0066	223	0
4.	M	0.7647	0.1462	0.0087	285	1
5.	M	1.0152	0.1238	0.0149	69	48
Group mean		0.8145	0.1595	0.0059	729	

Notes: F = female, M = male; S.D. = standard deviation; S.E.M. = standard error of the mean; PS = postoperative survival in months (if no figure is given, the patient is alive at the moment of the closure of this manuscript). Group means are calculated from the total cell pool of the group, i.e., not as the average of the individual means.



**Table 3.** ANOVA table of the nested (hierarchical) analysis of variance for the relative magnesium and phosphorus contents of cell nuclei on the 5 groups and 32 subgroups described in Tables 1 and 2.

Source of variation	dF	SS	MS	F <sub>(calc)</sub>	F <sub>(crit)</sub>
<b>Magnesium</b>					
Among groups	4	0.075405	0.018851	4.38	
$F_{0.01(4,24)} =$					4.18
Among subgroups	25 <sup>+</sup>	0.091185	0.004305	11.24	
$F_{0.001(4,\infty)} =$					2.13
Within subgroups	2580	0.988205	0.000383		
Total	2609	1.154796	0.000442		
<b>Phosphorus</b>					
Among groups	4	7.091539	1.772885	1.94	
$F_{0.10(4,26)} =$					2.17
Among subgroups	26 <sup>++</sup>	19.026850	0.915229	35.81	
$F_{0.001(24,\infty)} =$					2.13
Within subgroups	2580	65.938440	0.025558		
Total	2610	92.056830	0.035257		

Notes: dF = degree of freedom; SS = sum of squares; MS = mean square; F<sub>(calc)</sub> = calculated F-value; F<sub>(crit)</sub> = conservative critical value employed to avoid interpolation (for the probability and degrees of freedom indicated); dF<sup>+</sup> and dF<sup>++</sup> are rounded from the calculated values of 25.5699 and 26.5385, respectively; significance can be established at the level of indicated probabilities where F<sub>(calc)</sub> > F<sub>(crit)</sub>.

The design of the statistical evaluation was a two-level nested ANOVA variance analysis with unequal sample sizes {see for details: Chapter 10 (pp. 294-301) of Sokal and Rohlf, 1981} performed for Mg and P, as follows:

(1) The histopathological diagnoses of the tissue samples were considered as the main groups (C, T, A, D and UD).

(2) The cell nuclei measured within a sample of a given diagnosis deriving from the same individual, were taken as the subgroups (altogether 32). In each subgroup, various numbers of cell nuclei were measured, totaling 2611 (Tables 1 and 2).

The computerized statistical analysis gave the nested ANOVA results summarized in Table 3. Tests of significance were completely justified in terms of the mathematical requirements (Sokal and Rohlf, 1981, p. 298).

It is evident from the F-values shown in Table 3 that the intranuclear Mg contents of the 5 thyroid tissue groups differ significantly from each other at a probability level of  $p < 0.01$ , since the obtained  $F = 4.38$  is larger than the critical value  $F_{0.01(4,25)} = 4.18$  (Sokal and Rohlf, 1981). At the same time, the differences in the P-contents of the same groups are statistically not significant ( $p > 0.10$ ).

Table 3 also demonstrates that the variances between subgroups for both Mg and P contents are significantly

larger than the intra-subgroup mean square (MS) values, i.e., the individual scatter of these data is considerable between various persons. In other words, this means that we have to measure cells from several control people and several from each diagnostic groups, in order to get a realistic view of the situation. It is important to stress that the among group differences of the Mg contents remained statistically significant above the individual variations of this parameter, whereas in case of P contents, the among group differences disappeared when a sufficient number of individuals were involved in the data collection (Table 3).

The F-values obtained for "among subgroups" were larger than those of the "among groups" (Table 3). This fact suggest that pooling together all the cell nuclei within the same group cannot result in any misleading, or "false" significance values. On the contrary, pooling together all data in the same group can offer a more realistic judgement of observed differences among groups.

Table 4 expresses our group averages in relative terms, taking the average of control cases as 100 %  $\pm$  standard error of the mean (S.E.M.), and expressing all other group averages in relation to this level. It is interesting to note that the average increase of Mg contents correlates with the known malignancy of the thyroid tumors: This increase is moderate (19.8%) in the benign adenomas, larger (39.3%) in the differentiated

## Internuclear Mg and P in human thyroid tumors

**Table 4.** Relative magnesium and phosphorus contents in the nuclei of intact and tumorous thyroid cells.

Cell type (symbol)	Relative Mg content	$\pm$ S.E.M. (%)	Number of cell nuclei	Significance $p <$
<b>Magnesium:</b>				
Control (C)	100.00	5.51	545	
Thyroiditis (T)	111.12	10.20	165	(C/T) N.S.
Adenoma (A)	119.81	5.76	520	(C/A) 0.01
Differentiated carcinoma (D)	139.32	5.67	653	(C/D) 0.001 (D/UD) 0.001
Undifferentiated tumors (UD)	219.01	9.29	729	(C/UD) 0.001 (A/UD) 0.001
<b>Phosphorus:</b>				
Control (C)	100.00	0.84	545	
Thyroiditis (T)	93.38	1.25	165	(C/T) 0.01
Adenoma (A)	103.79	0.93	520	(C/A) 0.01
Differentiated carcinoma (D)	100.23	0.85	653	(C/D) N.S. (D/UD) 0.001
Undifferentiated tumors (UD)	88.97	0.65	729	(C/UD) 0.001 (A/UD) 0.001

Notes: N.S. = not significant; comparisons are indicated by the relevant group symbols in the last column, performed by the Student's *t*-test.

carcinomas, and extremely large (119%) in the undifferentiated tumors. Comparisons to the C group by the Student's *t* test are highly significant for Mg contents in any pair of groups, except the C/T comparison (Table 4).

The malignancy of the tumors investigated has been confirmed also by the clinical course of the disease: Tables 1 and 2 give the postoperative survival times of the patients. Where no figure is indicated, the patient is still alive at the closure of this manuscript. In Group D, we had two deaths out of 8, with a relatively long postoperative survival, whereas from the Group UD nobody is actually surviving.

As regards the P-contents, no clear correlation can be seen with the increasing malignancy of the tumors. As a matter of fact, although some group-comparisons proved to be statistically significant by the Student's *t*-test (Table 4), the tendencies were contradictory: a decrease was observed in the T and UD groups, no change was seen in the D group and an increase was recorded in the A group (Table 4).

It should be noted that the actual cell counts given in Tables 1 and 2 differ from those published in our previous papers (Lukács *et al.*, 1983; Zs.-Nagy *et al.*, 1983). The explanation for these differences is twofold:

- In the case of the controls, the benign adenomas and differentiated carcinomas, the measurements were continued after the communications mentioned had been sent to press, thus, here we can present a larger cell count.
- In one case of undifferentiated carcinoma, the histopathological diagnosis had to be revised on the basis of later examinations. This tumor, first diagnosed as an anaplastic carcinoma, was later reclassified as medullary carcinoma. This revision had to be carried out since in the course of the clinical follow-up, an elevated calcitonin serum level was found in the patient, which raised suspicion of medullary carcinoma. This suspicion was later verified by the positive result of the Congo-red test performed on the original operative material, which suggested the presence of amyloid. This is why the Mg concentrations found in this material are also included in the group of differentiated carcinomas.

Attention should be called to another fact. In our original studies, we established the parameters of measurements suitable for monovalent electrolytes (Na, K and Cl), the intracellular concentration of which is higher than that of Mg. An analysis time of 40 seconds was quite sufficient even for Na, however, this measuring time cannot be regarded as optimal for Mg. The result of this situation was that in certain cell nuclei the X-ray



spectra gave a zero value for the Mg peak, when the Mg-impulses, relatively smaller in number, did not significantly exceed the background intensity. The values presented now also contain these 0 measurements, although we can be sure that Mg is contained in all nuclei, so the 0 value can only be considered an error in the measurement. However, since to arbitrarily neglect these 0 values would have resulted in an upward correction of the P/B ratios, we did not omit them. This fact does not impair the comparability of the measured results, it only causes an increase of the standard deviation (S.D.). As a matter of fact, the S.D. values for Mg may be larger than the individual or group averages, as shown in Tables 1 and 2.

### Discussion

It is generally known that in the sera of tumor patients lower Mg levels are found than in those of healthy persons (Anghileri *et al.*, 1981; Collery *et al.*, 1981; Durlach, 1985; Holtmeier, 1988). Our own observations with thyroid tumor patients are similar, as we found in them an average of  $1.37 \pm 0.15$  (S.D.) mEq/l Mg, whereas in sera of healthy individuals the mean reached  $1.89 \pm 0.11$  mEq/l (Uray *et al.*, 1978). It was the generally encountered hypomagnesemia that suggested the possibility of an attempt at treatment of tumor patients with Mg supplementation (Blondell, 1980). However, it has so far not been proven that the growth of tumors can actually be diminished by Mg administration (Holtmeier, 1988); in fact, some data suggest that Mg depletion has a regression effect on the growth of the tumor in terminal-stage patients (Parsons *et al.*, 1974), therefore, the question is worth serious consideration. On the other hand, according to Durlach *et al.* (1986), Mg administration for several lymphoreticular tumors appears to have an anticancer effect, whereas in solid tumors, very often, Mg rather stimulates tumor growth. On this basis, these latter authors recommend Mg therapy only for the previously mentioned types of tumors as adjuvant treatment, and in the solid tumors, they consider it as contraindicated.

The increase of the intranuclear Mg concentration observed by us in positive correlation with the increasing malignancy of the thyroid tumors seems to support the findings reviewed by Cameron and Smith (1989a), according to which the more rapidly dividing cells contain more intracellular Mg. The non-parallel changes observed in the P contents suggest that one can reject the hypothesis according to which the Mg-content would have increased simply because of a greater P (i.e., phosphate ion) content. On the other hand, our data are also in agreement with those of Durlach *et al.* (1986), who described a mitogenic effect of increased Mg content in

the cell nucleus. In this respect, the onset of hypomagnesemia observed in the presence of thyroid tumors can probably be interpreted so that the changes in the permeability conditions of the cell membrane accompanying (or perhaps causing) mitogenic activity induce the tumor tissue to increase considerably its Mg uptake, and the serum-level regulating mechanisms are unable to compensate for this process. The consequent hypomagnesemia, very naturally, may involve unpleasant symptoms such as muscular weakness, tremor, and tetanic spasms. (Wester and Dyckner, 1982; Berkelhammer and Bear, 1985; Holtmeier, 1988). The treatment of these symptoms is possible and Mg-supplementation may be recommended. Nevertheless, we must not forget that excessive administration of Mg may further increase the Mg uptake of the existing tumor cells, and may promote the progression of the tumor.

Another aspect of hypomagnesemia is the problem that certain antitumor drugs, such as cisplatin and adriamycin, while they inhibit tumor cell growth, cause hypomagnesemia, too (Durlach *et al.*, 1986; Araszkiewicz *et al.*, 1987).

All these data and results suggest that knowledge of the Mg level in the serum alone is not enough to judge the metabolic changes of this element in tumor patients. The method used by us for the measurement of intracellular Mg content is not a routine technique, however, since it is the only possibility of gaining such data, it may be necessary in places where clinical practice and research capacity are close to each other. The aim to deplete high intracellular Mg content by certain pharmacological interference does not seem impossible, and by this means, we can hinder tumor growth in a target-oriented way. There have been animal experiments to this effect: e.g., the growth of C3HBA breast adenocarcinoma has been considerably inhibited by the oral administration of gallium-chloride, which resulted in a significant reduction both in the weight of the tumor and the Mg content of the tumorous tissue, whereas the Mg content of the kidneys remained unchanged (Collery *et al.*, 1986). We do not want to exaggerate the significance of the results so far achieved, but wish to emphasize the importance of the extension of these investigations in this direction, all the more so as surgical treatment for rapidly growing, highly malignant tumors has so far hardly been successful, thus one cannot overestimate the importance of a target-oriented, successful chemotherapeutical procedure.

### Acknowledgements

The authors acknowledge the valuable help of Dr. Ildikó Kéri-Fülöp, scientific co-worker of the Laboratory of Informatics, University Medical School (Debrecen) in

the supervision of the method of statistical analysis.

## References

- Anghileri LJ, Collery P, Condoux P, Durlach J (1981) Experimental relationship between magnesium and cancer. *Magn Bull* 3, 1-5.
- Araszkiewicz Z, Derentowicz P, Pirozynski M, Radomsky A (1987) Effect of vincristine, adriamycin and cyclophosphamide on selected indices of magnesium metabolism. *Magn Bull* 9, 40-43.
- Berkelhammer Ch, Bear RA (1985) A clinical approach to common electrolyte problems: Hypo-magnemia. *Canad Med Assoc J* 132, 360-368.
- Blondell JM (1980) The anticarcinogenic effect of Mg. *Med Hypotheses* 6, 863-871.
- Cameron IL, Smith NKR (1989a) Cellular concentration of magnesium and other ions in relation to protein synthesis, cell proliferation and cancer. *Magnesium* 8, 31-44.
- Cameron IL, Smith NKR (1989b) Applications of electron probe X-ray microanalysis to the study of ionic regulation of growth in normal and cancer cells. In: *Microprobe Analysis in Medicine*. Ingram P, Shelburne JD, Roggli VI (eds.). Hemisphere Publishing Co., New York. pp. 291-302.
- Cameron IL, Pool TB, Smith NKR (1979a) An X-ray microanalysis survey of the concentration of elements in the cytoplasm of different mammalian cell types. *J Cell Physiol* 101, 493-502.
- Cameron IL, Smith NKR, Pool TB (1979b) Element concentration changes in mitotically active and postmitotic enterocytes. An X-ray microanalysis study. *J Cell Biol* 80, 444-450.
- Cameron IL, Smith NKR, Pool TB, Sparks RL (1980) Intracellular concentration of sodium and other elements as related to mitogenesis and oncogenesis *in vivo*. *Cancer Res* 40, 1493-1500.
- Cameron IL, Hardman WE, Hunter KE, Haskin C, Smith NKR, Fullerton GD (1990) Evidence that a major portion of cellular potassium is "bound". *Scanning Microscopy* 4, 89-102.
- Collery P, Anghileri LJ, Condoux P, Durlach J (1981) Magnesium et cancer: Données cliniques. (Magnesium and cancer: Clinical data). *Magn Bull* 3, 11-20.
- Collery P, Millart H, Pluot M, Anghileri LJ (1986) Effect of gallium chloride oral administration on transplanted C3HBA mammary adenocarcinoma: Ga, Mg, Ca and Fe concentration and anatomopathological characteristics. *Anticancer Res* 6, 1085-1088.
- Dolev E, Deuster PA, Solomon B, Trostmann UH, Wartofsky L, Burman KD (1988) Alterations in magnesium and zinc metabolism in thyroid disease. *Metabolism* 37, 61-67.
- Durlach J (1985) *Le Magnésium en Pratique Clinique*. (Magnesium in Clinical Practice). Baillière JB EMI (ed.). Paris, France. p. 412.
- Durlach J, Bara M, Guet-Bara A, Collery P (1986) Relationship between magnesium, cancer and carcinogenic or anti-cancer metals. *Anticancer Res*, 6, 1353-1362.
- Elin RJ, Hosseini JM (1985) Magnesium content of mononuclear blood cells. *Clin Chem* 31, 377-380.
- Hall TA, Clarke-Anderson M, Appleton T (1973) The use of thin specimens for X-ray microanalysis in biology. *J Microsc* 99, 177-182.
- Heeley DM, Warner GT, Mayer ALR (1974) Magnesium in malignant disease. *The Lancet* 1, 634-635.
- Holtmeier HJ (1988) *Das Magnesiummangel-Syndrom* (The Magnesium Deficiency Syndrome). Hippokrates Verlag, Stuttgart, Germany. pp. 11-13, 90-103 and 147-148.
- Lukács GL, Zs.-Nagy I, Lustyik Gy, Balázs Gy (1983) Microfluorimetric and X-ray microanalytic studies on the DNA content and  $\text{Na}^+:\text{K}^+$  ratios of the cell nuclei in various thyroid tumors. *J Cancer Res Clin Oncol* 105, 280-284.
- Parsons FM, Edwards GF, Anderson CK, Allmad S, Clark PB, Hetherington C, Young GA (1974) Regression of malignant tumours in magnesium and potassium depletion induced by diet and haemodialysis. *The Lancet* 1, 243-244.
- Rubin AH, Terasaki M, Sanui H (1979) Major intracellular cations and growth control: Correspondence among magnesium content, protein synthesis and the onset of DNA synthesis in BALB/c 3T3 cells. *Proc Natl Acad Sci USA* 76, 3917-3921.
- Smith NKR, Sparks RL, Pool TB, Cameron IL (1978) Differences in the intracellular concentration of elements in normal and cancerous liver cells as determined by X-ray microanalysis. *Cancer Res* 38, 1952-1959.
- Sokal RR, Rohlf FJ (1981) *Biometry. The Principles and Practice of Statistics in Biological Research*. 2nd edn. Freeman and Co., New York. pp. 271-320.
- Szmeja Z, Konciewska H (1986) Der Magnesiumgehalt bei larynxprekanzerosen und larynxkarzinomen. (The magnesium content in precancerous larynx and laryngeal carcinoma). *HNO* 34, 85-87.
- Terasaki M, Rubin H (1985) Evidence that intracellular magnesium is present in cells at a regulatory concentrations for protein synthesis. *Proc Natl Acad Sci USA* 82, 7324-7326.
- Uray É, Ország I, Félegyházi Á, Makár V, Arday G, Szabó É, Lukács G, Nagy Z (1978) Serum magnesium, zink- und kupferkonzentrationen bei erkrankungen der schilddrüse. (Serum magnesium, zinc and copper concentrations in thyroid diseases). *Magyar Seb* 31, 225-

231.

Walker GM (1986) Magnesium and cell cycle control: An update. *Magnesium* **5**, 9-23.

Wester PO, Dyckner T (1982) The importance of the magnesium ion. Magnesium deficiency - symptomatology and occurrence. *Acta Med Scand (Suppl.)* **661**, 3-4.

Whang R, Ryder KW (1990) Frequency of hypomagnesemia and hypermagnesemia. *J Am Med Assoc* **263**, 2063-2064.

Wroblewski R (1994) Effect of delay on cryofixation on the elemental composition of biopsies and post mortem specimens of the thyroid gland. *Histochemistry* **101**, 405-412.

Wroblewski R, Wroblewski J (1994) X-ray microanalysis of endocrine, exocrine and intestinal cells and organs in culture: technical and physiological aspects. *Scanning Microscopy* **8** (Suppl), 149-161.

Wroblewski R, Jalnäs M, Grimelius L (1991) Distribution of sodium, magnesium, phosphorus, sulphur, chlorine, potassium, calcium and iodine in mammalian thyroid gland. *J Submicr Cytol Pathol* **23**, 539-549.

Zamecnik P (1960) Historical and current aspects of the problem of protein synthesis. *Harvey Lect* **54**, 256-281.

Zar JH (1984) *Biosatistical Analysis*. 2nd edn. Prentice-Hall, Inc. Englewood Cliffs, NJ. pp. 253-260.

Zs.-Nagy I (1983) Energy dispersive X-ray microanalysis of biological bulk specimens: A review on the method and its application to experimental gerontology and cancer research. *Scanning Electron Microscopy* **1983**;III, 1255-1268.

Zs.-Nagy I (1989) A review on the use of bulk specimen X-ray microanalysis in cancer research. *Scanning Microscopy* **3**, 473-482.

Zs.-Nagy I, Casoli T (1990) A review on the extension of Hall's method of quantification to bulk specimen X-ray microanalysis. *Scanning Microscopy* **4**, 419-428.

Zs.-Nagy I, Pieri C, Giuli C, Bertoni-Freddari C, Zs.-Nagy V (1977) Energy dispersive X-ray microanalysis of the electrolytes in biological bulk specimen. I. Specimen preparation, beam preparation and quantitative analysis. *J Ultrastruct Res* **58**, 22-33.

Zs.-Nagy I, Lustyik Gy, Lukács G, Zs.-Nagy V, Balázs Gy (1983) Correlation of malignancy with the intracellular  $\text{Na}^+:\text{K}^+$  ratio in human thyroid tumors. *Cancer Res* **43**, 5395-5402.

**Editor's Note:** All of the reviewer's concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.