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

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

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## 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.

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## 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., Szmeja 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).

(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,

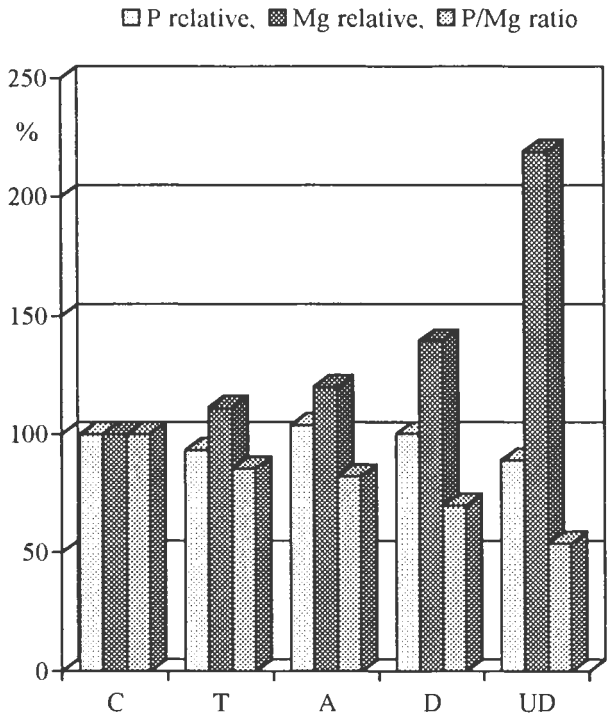


**Figure 1.** The main trends of changes in the relative P and Mg concentrations as well as the P/Mg ratios. P and Mg values ± standard error of the mean (S.E.M.) are given numerically in Table 4. P/Mg values were calculated from the individual averages (Tables 1 and 2) and group averages are shown.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

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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°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 ~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 |
|---|---|---|---|---|---|---|
| **Control thyroid tissue (C)** | | | | | | |
| 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 | |
| **Thyroiditis (T)** | | | | | | |
| 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 | |
| **Benign adenomas (A)** | | | | | | |
| 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 | |
| **Differentiated carcinomas (D)** | | | | | | |
| 1. | F | 0.0099 | 0.0136 | 0.0019 | 49 | |
| 2. | F | 0.0112 | 0.0118 | 0.0012 | 98 | 24 |
| 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 | |
| 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 | 132 |
| Group mean | | 0.0161 | 0.0167 | 0.0007 | 653 | |
| **Undifferentiated tumors (UD)** | | | | | | |
| 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.

**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 |
|---|---|---|---|---|---|---|
| **Control thyroid tissue (C)** | | | | | | |
| 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 | |
| **Thyroiditis (T)** | | | | | | |
| 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 | |
| **Benign adenomas (A)** | | | | | | |
| 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 | |
| **Differentiated carcinomas (D)** | | | | | | |
| 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 | |
| **Undifferentiated tumors (UD)** | | | | | | |
| 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 | $F_{(calc)}$ | $F_{(crit)}$ |
|---|---|---|---|---|---|
| Magnesium | | | | | |
| Among groups | 4 | 0.075405 | 0.018851 | 4.38 | |
| $F_{0.01(4,24)}$ = | | | | | 4.18 |
| Among subgroups | $25^{+}$ | 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 | | |
| Phosphorus | | | | | |
| Among groups | 4 | 7.091539 | 1.772885 | 1.94 | |
| $F_{0.10(4,26)}$ = | | | | | 2.17 |
| Among subgroups | $26^{++}$ | 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; $F_{(calc)}$ = calculated F-value; $F_{(crit)}$ = conservative critical value employed to avoid interpolation (for the probability and degrees of freedom indicated); $dF^{+}$ and $dF^{++}$ are rounded from the calculated values of 25.5699 and 26.5385, respectively; significance can be established at the level of indicated probabilities where $F_{(calc)} > F_{(crit)}$.

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 % ± 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

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

| Cell type (symbol) | Relative Mg content | ± S.E.M. (%) | Number of cell nuclei | Significance p < |
|---|---|---|---|---|
| Magnesium: | | | | |
| 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 |
| Phosphorus: | | | | |
| 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 Students 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: (a) 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. (b) 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 regressional 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.

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