Trace Elements and Thyroid Cancer*

Vladimir Ye. Zaichick, Anatoli F. Tsyb and Boris M. Vtyurin
Medical Radiological Research Centre, Obninsk 249020, Kaluga Region, Russia

To evaluate the importance of trace amounts of elements in thyroid cancer etiology and diagnostics, instrumental neutron activation analysis has been used to estimate Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn concentrations in malignant and benign thyroid nodules as well as in apparently intact paranodular thyroid tissue. Resected material from 135 patients was obtained from operations. Forty-five cancer cases were diagnosed and the rest were of benign nodules. The thyroid glands of 65 people, 53 male and 12 female, who died an unexpected death or committed suicide, were used as a control group. Trace element contents of the International Atomic Energy Agency reference material H-4 (animal muscle) were analysed simultaneously with the thyroid tissue in order to evaluate the accuracy of the obtained data. No dependence of trace element contents on sex and age (14–80 years) was found for normal thyroids. In paranodular tissue, the Ag, Co, Hg, I, and Rb contents were much higher for malignant and benign nodules than they were for the standard. There was also a slight deficiency of Se in the nodules compared with the standard. This result supports the hypothesis that the direct toxic heavy metal influence on thyrocytes plays a major role in thyroid cancer etiology, provided that an adequate level of the defence mechanisms is absent. Iodine concentrations are 15 times lower, on average, in malignant compared with benign nodules. It is also shown that the ratio between the iodine concentration in nodular and paranodular tissue can be used for *in vivo* thyroid cancer diagnostics.

Keywords: *Trace element; neutron activation analysis; thyroid; malignant and benign thyroid nodule; cancer etiology; diagnostics*

Introduction

During the twentieth century much knowledge has been gained about the environmental influence on the frequency of thyroid tumour origination. According to epidemological, clinical, and experimental studies, the environmental excess, deficiency, or imbalance of more than twenty chemical elements have been found to influence the development of thyroid goitres and, subsequently, cancer. Besides iodine, the additional chemical elements include: B, Ba, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, Se, Sr, Te, V, Zn. However, the information available is usually of a phenomenal nature; it does not provide concrete definitions of the mechanisms of the tumourgenic species.

In the 1970s and 1980s it was reported that thyroid tissue could accumulate both highly toxic elements (Hg, Cd) and essential elements (Se). The element concentrations were much higher than those found in the other body tissues.[1–4] The high selenium concentrations were associated with a high thyroid content of selenium-dependent glutathione peroxidase (GSH-Px), one of the defined and characterized selenoenzymes.[5] GSH-Px, as an important part of the free radical intracellular antioxidant defence system, reduces tissue concentrations of toxic free radicals, hydroperoxides, and lipoperoxides. The correct GSH-Px levels are essential for thyrocyte activity because thyrocytes have to live with the constant generation of hydroperoxide oxidizing iodide (I^-) to iodine (I^0) before its incorporation into thyroglobulin. Positive correlation between concentrations of mercury and selenium has also been found and explained by the selenium defence function.[1] Later, a selenium-containing protein with the relative molecular mass (M_r) 27800 was found. It was specific to the thyroid gland and important in acting against heavy metal toxicity.[6]

These findings show that the influence of a tumourgenic element can be a result of the direct influence of the element on the thyroid tissue. To confirm this idea the element concentrations and element interdependences in the standard thyroid gland and paranodular tissue for benign and malignant thyroid nodules have been studied.

A further aim of the present study was connected with searching the highly informative chemical markers for differential diagnostics of benign and malignant thyroid nodules. In spite of apparent progress in iodine prevention, goitre, including its nodular forms, remains one of the most widely distributed diseases. Recently, it was reported that cases of thyroid cancer have increased in the populations of Scandinavia, Israel, and the USA.[7] Usually thyroid cancer is preceded by benign nodules of euthyroid goitre.[7–9] Thus, the problem of timely differential diagnostics of benign and malignant thyroid nodules is extremely urgent. Unfortunately, the widespread method of needle biopsy and subsequent cytological findings do not provide sufficient accuracy in diagnosis.[10,11] As thyroid tissue has rather specific element composition, tumour growth is expected to be followed by considerable changes in the element contents. If there is a clear difference between the element contents and concentrations in benign and malignant nodules, this information could be applied to diagnostics.

Materials and Methods

The element concentrations were analysed in the resected material of 135 patients with different thyroid nodules being treated in the hospital of Medical Radiological Research Centre. Among them, 45 patients had undergone surgery for cancer: 31 papillary adenocarcinomas, 6 follicular adenocarcinomas, 7 medullar adenocarcinomas, 1 reticulosarcoma. For the rest of the patients, 60 had surgery for nodular goitre, 23 for adenomas, and 7 for cysts. Each diagnosis was verified by a histological study as well as clinical and dynamic findings over several years. The age of patients ranged from 15–77 years. Samples of the nodular, and apparently intact, paranodular thyroid tissue were taken in the shape of a column oriented from the periphery towards centre. It should be noted that iodine-containing drugs were not used for treatment of the operative field or when the nodular excision was made.

For a control group, 65 thyroid glands were taken during autopsies of people (53 male and 12 female) who died an

* Presented at The Fifth Nordic Symposium on Trace Elements in Human Health and Disease, Loen, Norway, June 19–22, 1994.

unexpected death or committed suicide. Each was an Obninsk resident who was relatively healthy before death. Their ages ranged from 14–80 years. The time between death and autopsy did not exceed 24 h.

The resected and autopsy material was taken using instruments made of titanium. It was weighed, then lyophilized (for 120 h) until constant mass, and then homogenized. Homogenates were used to obtain 50 mg samples, which were subsequently sealed into alcohol-washed polyethylene film and put into marked polyethylene ampoules. The iodine content of each ampoule was determined. The samples were then removed from the polyethylene film and were wrapped in pure aluminium foil for subsequent determinations of trace elements, *e.g.*, Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn.

Instrumental neutron activation analysis (INAA) using n,γ nuclear interactions was applied for iodine and trace element determination. The short-lived radionuclide ^{128}I ($t_{1/2}$ 25 min) was used for iodine analysis. The long-lived radionuclides with half-lives from tens of days to several years were used for trace element analysis.

The standard iodine samples were prepared using solutions of chemically pure NH_4I and $HIO_4 \cdot 2H_2O$. The iodine concentration of solutions was 5×10^{-4} g ml^{-1}. To estimate trace elements synthetic standards (SSB) prepared on the basis of phenol–formaldehyde resol resin in the Institute of Physics, Georgian Academy of Sciences, were used for INAA.[12] SSB were 4–6 mm diameter tablets weighing 30–50 mg. For the assessment of analytical accuracy the International Atomic Energy Agency (IAEA) standard reference material, H-4 (animal muscle), was used.[13] The standard samples and H-4 reference material were prepared for analysis in the same way as the thyroid samples.

For INAA the VVR-c nuclear reactor channels were used. The horizontal channel equipped with pneumatics and cooled with compressed air was used for iodine analysis. The neutron flux density in the channel was 1.7×10^{13} neutrons cm^{-2} s^{-1}. Ampoules with samples and standards were put into polyethylene transport containers and then irradiated separately for 20 s. Copper foils were used to determine the neutron fluency precisely. These were placed in the transport container and irradiated simultaneously with the samples and standards.

The vertical reactor channel was used to estimate trace element contents by INAA with the long-lived radionuclides. The neutron flux density was 1.2×10^{13} neutrons cm^{-2} s^{-2}. Thyroid samples, SSB tablets and IAEA H-4 reference material were wrapped in aluminium foil and placed into a nitric acid-washed quartz ampoule. Then the ampoule was sealed, placed in an aluminium transport container and irradiated for 120 h.

The 40 cm^3 Ge(Li) detector and multichannel analyser (MCA) were used for spectrometry in the 1970s. From the mid-1980s the 100 cm^3 Ge(Li) detector and on-line computer-based MCA system were used. To estimate iodine, 60 s after irradiation, samples and standards were measured for 5 min. Measurements were started 15–20 d after irradiation for INAA with the long-lived radionuclides. The duration of these measurements was 1–6 h. The INAA conditions (time of irradiation, decay and measurement as well as a sample–detector distance) were optimized for the maximum number of chemical elements with a minimum statistical error of measurement. These conditions were calculated in advance using a specially developed computer program.[14]

Results and Discussion

The results of the IAEA H-4 reference material analysis correlated well with certified values (Table 1) indicating the accuracy of our data.

The iodine and trace element concentrations of healthy adult thyroid glands do not depend on sex or age. The data obtained by Kvicala *et al.*[4] and Aaseth *et al.*[3] confirmed this conclusion for selenium and rubidium. The absence of age and sex dependence made it possible to combine results and obtain more representative data for the standard (Table 2). For Ag, Fe, I, Sb, Se, and Zn our data falls within the range of mean concentration values shown in the review of Iyengar *et al.*[15] The mean concentration values obtained for cobalt and rubidium are approximately two times lower than the lower limit of ranges given in here. The levels of chromium and mercury contents differ the most significantly. However, our data for Cr, Co, Rb, and other trace elements correlate well with the results presented in papers published in recent years.[2,4]

For mercury, according to the data of Kosta *et al.*,[1] the thyroid mercury content of people who live within the non-mercury polluted regions ranges from 2.5 to 15 ppb wet mass. Aaseth *et al.*[3] also found that mercury concentrations were lower than 20 ppb in most of the thyroid glands studied. Their data correlate well with our results in contrast to those given by Iyengar *et al.*[15]

A statistically reliable correlation ($r = 0.68$, $p < 0.004$) between the element concentrations in the standard thyroid gland is found only for selenium and zinc.

The element contents in malignant and benign thyroid nodules differ greatly from the standard (Table 3). The higher levels of mercury (3–4 times on an average) and rubidium (1.5–2 times) are more typical of nodular tissue. The cancer tissue differs also for considerably lower concentrations of iodine (15 times) and somewhat lower concentrations of selenium (20%). The concentration of zinc falls within the normal ranges. This contradicts the data of Koch and Smith[16] who found a 4–7-fold increase in the level of zinc in papillary

Table 1 INAA data on the IAEA reference material, H-4 (animal muscle), compared with certified values

	Concentration (ppm dry mass)		
	Our results		Certified values 95% Confidence
Element	Mean ± SE*	Mean	interval
Co	0.0074 ± 0.0018	0.008	—
Cr	0.071 ± 0.019	0.080	—
Fe	47 ± 2	49	47–51
Hg	0.015 ± 0.004	0.014	0.010–0.017
Rb	23.7 ± 2.0	19	17–20
Se	0.281 ± 0.028	0.280	0.250–0.320
Zn	91 ± 4	86	83–90

* SE is the standard error.

Table 2 Element concentrations in healthy adult thyroid glands

	Mean (± SE) concentrations		Range of mean values
Element	Dry mass (ppm)	Wet mass (ppm)	Iyengar *et al.*[15] wet mass (ppm)
Ag	0.131 ± 0.011	0.034 ± 0.003	0.001–0.03
Co	0.0404 ± 0.0028	0.0104 ± 0.0008	0.02–3.3
Cr	0.754 ± 0.067	0.19 ± 0.02	0.0046–0.017
Fe	237 ± 15	62 ± 4	48–93
Hg	0.029 ± 0.003	0.0076 ± 0.0009	0.086–0.85
I	1380 ± 80	348 ± 22	300–696
Rb	6.5 ± 0.3	1.7 ± 0.1	3.3–11.2
Sb	0.139 ± 0.009	0.036 ± 0.002	0.023–0.038
Sc	0.0079 ± 0.0031	0.0021 ± 0.0008	—
Se	2.61 ± 0.16	0.69 ± 0.06	0.44–1.24
Zn	87 ± 4	23 ± 1	24–37

adenocarcinoma and reticulosarcoma. Besides mercury and rubidium, the concentrations of Zn, Co, Ag, Fe, and Cr in benign nodules exceed normal levels. We found that rubidium concentrations increased regularly in benign thyroid nodules and especially in cancer tissue. This regularity correlates well with the data obtained by Kvicala *et al.*[4] according to the absolute and relative values. We showed that selenium concentration was reduced only for cancerous tissue although the above-mentioned authors found that the selenium content was statistically lower both in thyroid carcinomas and adenomas than that present in the standard.

Most important, malignant nodules differ from benign nodules by having considerably lower iodine concentrations. This comparison is also characterized by lower concentrations of Zn, Se, Ag, Co, Fe, and a higher concentration of Rb. The element concentrations in malignant and benign nodules are not interdependent. In addition, the correlation between selenium and zinc typical of the standard thyroid tissue is broken.

The element contents in paranodular tissue for malignant and benign thyroid nodules differ greatly from the standard (Table 4). Besides iodine, considerably higher concentrations of Ag, Co, Hg, and Rb are found. These changes are expressed more sharply for cancer than for benign tumours. Paranodular tissue for benign thyroid nodules is also characterized by a lower selenium content when compared with the standard.

Along with increased concentrations of such heavy metals as Ag, Co, Hg, and Rb in paranodular tissue, inter-connections between the contents of many chemical elements were found. Most of all they are shown for cancer (Table 5). Those indicated the deep deformation of chemical homeostasis mechanisms expressed by a disorder of membrane thyrocyte permeability.

The concentrations of almost all the elements studied in benign nodules are slightly higher than those in paranodular tissue (Table 6). Iodine and scandium are the only exceptions. Conversely, however, for thyroid cancer the element concentrations in tumour tissue are lower than those in paranodular tissue. The difference in iodine concentration between the two tissues is particularly clear. Thus, for thyroid cancer, the nodular iodine concentration is only 3% of paranodular iodine content, while for benign nodules it is 56%.

For normal thyroid parenchyma there are chemical homeostasis mechanisms that keep up the concentrations of heavy metals such as Hg, Co, Ag, and Rb at comparatively high, constant and independent levels that can be seen from absolute mean concentration values of these elements in the absence of any age dynamics and inter-correlations. The correlation found between concentrations of selenium and

Table 3 Element concentrations in thyroid nodules (ppm dry mass)

	Nodular thyroid tissue							
	Malignant			Benign			Comparison between M_1 and M_2	
		p*			p*		p*	
Element	M_1 Mean ± SE	w	t	M_2 Mean ± SE	w	t	w	t
Ag	0.180 ± 0.030	—	—	0.370 ± 0.056	<0.0001	<0.0002	<0.01	<0.01
Co	0.050 ± 0.005	—	—	0.068 ± 0.005	<0.0001	<0.0001	<0.02	<0.03
Cr	0.976 ± 0.192	—	—	1.113 ± 0.151	—	<0.02	—	—
Fe	249 ± 37	—	—	400 ± 55	—	<0.01	—	<0.05
Hg	0.113 ± 0.031	<0.0001	<0.01	0.144 ± 0.016	<0.0001	<0.0001	—	—
I	92 ± 15	<0.0001	<0.0001	1040 ± 260	—	—	<0.0001	<0.0001
Rb	12.48 ± 0.77	<0.0001	<0.0001	9.67 ± 0.58	<0.0001	<0.0001	<0.002	<0.005
Sb	0.148 ± 0.018	—	—	0.154 ± 0.018	—	—	—	—
Sc	0.010 ± 0.003	—	—	0.011 ± 0.002	—	—	—	—
Se	2.06 ± 0.21	—	<0.05	2.63 ± 0.16	—	—	<0.03	<0.05
Zn	71.7 ± 6.9	—	—	126.2 ± 6.7	<0.0001	<0.0001	<0.0003	<0.0001

* p, A comparison with the standard according to the Student's t-test (t) and the non-parametric Wilcocson's criterion (w).

Table 4 Element concentration in paranodular thyroid tissue (ppm dry mass)

	Paranodular thyroid tissue							
	Malignant			Benign			Comparison between M_1 and M_2	
		p*			p*		p*	
Element	M_1 Mean ± SE	w	t	M_2 Mean ± SE	w	t	w	t
Ag	0.955 ± 0.326	<0.0001	<0.0001	0.351 ± 0.064	<0.001	<0.0001	<0.05	—
Co	0.074 ± 0.011	<0.002	<0.0001	0.059 ± 0.006	<0.01	<0.002	—	—
Cr	0.588 ± 0.087	—	—	0.476 ± 0.073	<0.001	<0.01	—	—
Fe	244 ± 26	—	—	205 ± 23	<0.05	—	—	—
Hg	0.245 ± 0.044	<0.0001	<0.0001	0.131 ± 0.016	<0.0001	<0.0001	—	<0.05
I	3020 ± 280	<0.0001	<0.0001	1870 ± 190	<0.001	<0.02	<0.001	<0.002
Rb	16.6 ± 3.2	<0.0001	<0.0001	11.2 ± 1.1	<0.0001	<0.0001	—	—
Sb	0.187 ± 0.053	—	—	0.126 ± 0.031	—	—	—	—
Sc	0.018 ± 0.006	—	—	0.028 ± 0.008	<0.01	<0.01	—	—
Se	3.02 ± 0.32	—	—	1.97 ± 0.14	<0.02	<0.02	<0.004	<0.002
Zn	101.4 ± 8.6	—	—	93.3 ± 9.4	—	—	—	—

* p, A comparison with the standard according to the Student's t-test (t) and the non-parametric Wilcocson's criterion (w).

zinc can be explained by the fact that both elements play a major role in the detoxification of oxygen-derived free radicals: selenium in GSH-Px and zinc in super oxide dismutase. The physiologically dependent H_2O_2 generation by thyrocytes dictates a necessity for efficient and interconnected functioning of defence mechanisms. The influence of exogenic factors (biogeochemical residence characteristics, anthropogenic environmental pollution, occupational conditions, dietary peculiarities, bad habits [*e.g.*, smoking], taking medicine, *etc.*) and endogenic factors (consequences of acute and chronic diseases, genetically dependent disorders of body element metabolism, *etc.*) can result in heavy metal accumulation in the thyroid parenchyma with concentrations exceeding the limit of thyroid defence mechanisms. In the absence of an adequate defence mechanism reaction, these metals exert a toxic influence: they destroy thyrocytes and inhibit or pervert the process of thyroid hormone synthesis. The situation is aggravated by selenium deficiency because the amount of selenium-containing enzymes produced is found to be insufficient not only for the defence against toxic influence of heavy metals but also H_2O_2 and other physiologically dependent free oxygen radicals too. With selenium deficiency the rate of thyroid hormone T_4 convertion into more active T_3 can also be reduced.[3,17] The resultant thyroid hypo-function is a reason for an increased thyroid-stimulating hormone (TSH) level and subsequent thyroid parenchyma hyperplasia. In addition, the increased TSH level results in more intensive H_2O_2 generation in thyrocytes and then in more active iodine oxidation.[18] The active iodine oxidation is accompanied by the higher element thyroid accumulation. If new thyroid generation is subjected to the same level of toxic trace elements as the previous generation, provided the limits of thyroid defence mechanisms remain constant, the situation is not improved, and thyroid hyperplasia continues. Hyperplasia is one reason for the growth of different types of goitre. It enlarges the probability of malignant tumour origin. In addition, the toxic effect of free oxygen radicals and heavy metals has been suggested to have direct carcinogenic properties on genetic material.[19]

Table 5 Correlations between the element concentrations in paranodular tissue for thyroid cancer

Element	Element compared	Correlation coefficient	Significance level, p
Se	Hg	0.68	<0.002
	Sb	0.62	<0.008
	Co	0.59	<0.01
	Fe	0.48	<0.05
Hg	Se	0.68	<0.002
	Fe	0.67	<0.003
	Co	0.64	<0.005
	Sb	0.63	<0.006
	Rb	0.48	<0.05
Co	Fe	0.69	<0.002
	Hg	0.64	<0.005
	Sb	0.64	<0.005
	Se	0.59	<0.01
	Cr	0.47	<0.05
Fe	Sb	0.70	<0.002
	Co	0.69	<0.002
	Hg	0.67	<0.003
	Rb	0.54	<0.03
	Se	0.48	<0.05
	Zn	−0.48	<0.05
I	Zn	0.63	<0.007

Table 6 Ration (R) of mean element concentration in nodular and paranodular tissue of benign and malignant thyroid nodules

	Malignant			Benign		
		p^*			p^*	
Element	R	w	t	R	w	t
Ag	0.188	<0.0004	<0.03	1.05	—	—
Co	0.676	—	—	1.15	—	—
Cr	1.75	—	—	2.34	<0.02	<0.001
Fe	1.02	—	—	1.95	<0.01	<0.004
Hg	0.461	<0.005	<0.02	1.10	—	—
I	0.030	<0.0001	<0.0001	0.56	<0.0001	<0.0001
Rb	0.75	—	—	0.86	—	—
Sb	0.80	—	—	1.22	<0.01	—
Sc	0.56	—	—	0.39	<0.03	<0.05
Se	0.68	<0.04	<0.02	1.34	<0.002	<0.01
Zn	0.71	—	<0.02	1.35	<0.001	<0.005

* p A comparison between nodular and paranodular tissue according to the Student's t-test (t) and the non-parametric Wilcocson's criterion (w).

Thus, according to the scheme suggested here, an increase in the concentrations of heavy metals and iodine and decrease in selenium concentration in parenchyma compared with the standard samples should be expected for a situation resulting in thyroid parenchyma hyperplasia. The present authors have found that paranodular tissue characteristics corresponded well to these indications. It is also interesting to note that the results of recent investigations are convincing in demonstrating the influence of body excess amounts of elements such as Hg, Co, I, and deficiency of Se on the frequency of goitre and thyroid cancer cases.[20–25]

It is certain that the data obtained does not reveal the simple cause-and-effect relationship. It is known that malignant tumours, including thyroid tumours, usually develop very slowly. It takes up to 12 years for a clinical cancer to develop.[9,26] Hence, it is difficult to expect that the element paranodular contents reflect adequately the condition of the thyroide at the moment of tumour birth; although, such a suggestion cannot be completely discarded. Moreover, in our opinion, paranodular analysis is the only possible way for retrospective evaluation of the element activity at the central (thyroid) level. It is also obvious that the hypothesis of thyroid tumour etiology suggested here does not exclude the possible simultaneous, additional activity of selenium deficiency and heavy metal excesses at the peripheral level, inactivation of the thyroid hormone in blood, and changes in the generation process of T_3 from T_4, *etc.* However, the suggestion of possible iodine and heavy metal accumulation as well as selenium paranodular deficiency occurring as a result of thyroid nodular tumours seem to be much less likely.

The benign thyroid nodules contain larger amounts of heavy metals than those found in paranodular tissue. Additionally, the benign nodular tissue is functionally defective as can be seen from the iodine concentration levels, which are appreciably lower than the standard levels. The loss of functional specificity is more characteristic of malignantly transformed thyroid tissue: a more than 15-fold decrease of the iodine concentration testifies to this fact. The somewhat lower heavy metal concentrations found in malignant nodules than those found in paranodular tissue can be explained by this fact. However, the latter can also be partly related to the difference in blood circulation.

To estimate the diagnostic importance of the element concentrations it was shown that only iodine can be successfully applied as a marker for differential diagnostics of malignant and benign thyroid nodules. The relative native iodine concentrations (nodular/paranodular tissue) offer particular interest because their application creates the necessary prerequisites for the development of new *in vivo* diagnostic

methods, for which the iodine paranodular content is a natural standard.[27,28]

References

1 Kosta, L., Zelenko, V., Ravnik, V., Lebstek, M., Dermelj, M., and Byrne, A. K., in *Comparative Studies of Food and Environmental Contamination*, Proceedings of the Symposium FAO IAEA WHO, Otaniemi, Finland, August 27–31, 1973, International Atomic Energy Agency (IAEA-SM-175/27), Vienna, Austria, 1973, pp. 541–550.
2 Saltzman, B. E., Gross, S. B., Yeager, D. W., Meiners, B. G., and Gartside, P. S., *Environ. Res.*, 1990, **52**, 126.
3 Aaseth, J., Frey, H., Glattre, E., Norheim, G., Ringstad, J., and Thomassen, Y., *Biol. Trace Elem. Res.*, 1990, **24**, 147.
4 Kvicala, J., Havelka, J., Nemee, J., and Zeman, V., *Biol. Trace Elem. Res.*, 1992, **32**, 253.
5 Joselyn, P. C., *Biochemistry of the SH Group*, Academic Press, London, New York, 1972, p. 269.
6 Behne, D., Hilmert, H., Scheid, S., Gessner, H., and Elger, W., *Biochim. Biophys. Acta*, 1988, **966**, 12.
7 Levi, F., Franceschi, S., LaVecchia, C., Negri, E., Gulie, C., Duruz, G., and Scazziga, B., *Eur. J. Cancer*, 1991, **27**, 85.
8 Yamamoto, Y., Maeda, T., Izumi, K., and Otsuka, H., *Cancer*, 1990, **65**, 1173.
9 Dedov, V. I., Dedov, I. I., and Stepanenko, V. F., *Radiazionnaya Endokrinologiya*, Meditsina, Moscow, 1993 (in Russian).
10 Shaha, A. R., DiMaio, T., Webber, C., and Jaffe, B. M., *Surgery*, 1990, **108**, 964.
11 Cersosimo, E., Gharib, H., Suman, V. J., and Goellner, J. R., *Mayo Clin. Proc.*, 1993, **68**, 343.
12 Mosulishvili, L. M., Kolomiitsev, M. A., Dundua, V. Yu., Shonia, N. I., and Danilova, O. A., *Multielement Standards for Instrumental Neutron Activation Analysis of Biological Materials*, Institute of Physics, Georgian Academy of Sciences, Tbilisi, 1974 (in Russian).
13 Parr, R. M., *Intercomparison of Minor and Trace Elements in IAEA Animal Muscle (H-4)*, Report No. 2, International Atomic Energy Agency, Vienna, 1980.
14 Korelo, A. M., and Zaichick, V. Ye., in *Activation Analysis in Environmental Protection*, Joint Institute for Nuclear Research, Dubna, 1993, pp. 326–332 (in Russian).
15 Iyengar, G. V., Kollmer, W. E., and Bowen, H. J. M., *The Elemental Composition of Human Tissue and Body Fluids*, Verlag Chemie, Weinheim, 1978.
16 Koch, H., and Smith, E., *J. Clin. Endocrinol. Metab.*, 1956, **16**, 123.
17 Diplock, A. T., *Am. J. Clin. Nutr.*, 1993, **57**, 256S.
18 Corvilain, B., Contempré, B., Longombé, A. O., Goyens, P., Gerry-Decoster, C., Lamy, F., Vanderpas, J. B., and Dumont, J. E., *Am. J. Clin. Nutr.*, 1993, **57**, 244S.
19 Westin, T., Ahlbom, E., Johansson, E., Sandström, B., Karlberg, I., and Edström, S., *Arch. Otolaryngol. Head Neck Surg.*, 1989, **115**, 1079.
20 Sin, Y. M., and Teh, W. F., *Bull. Environ. Contam. Toxicol.*, 1992, **49**, 847.
21 LaGrutta, A., Amato, G. M., Fedele, S., and Vitaliti, S., *Minerva Pediatr.*, 1984, **36**, 691.
22 Raffin, E., Mikkelsen, S., Altman, D. G., and Christensen, J. M., *Scand. J. Work. Environ. Health*, 1988, **14**, 378.
23 Prescott, E., Netterstrom, B., Faber, J., Hegedus, L., Suadicani, P., and Christensen, J. M., *Scand. J. Work. Environ. Health*, 1992, **18**, 101.
24 Williams, E. D., Doniach, J., Bjarnason, O., and Michie, W., *Cancer*, 1977, **39**, 215.
25 Namba, H., Yamashita, S., and Kimura, H., *J. Clin. Endocrinol. Metab.*, 1993, **76**, 605.
26 Watanabe, S., *Radiology Jpn.*, 1992, **28**, 1.
27 Zaichick, V. Ye., Matveenko, Ye. G., Vtyurin, B. M., and Medvedev, V. S., *Vopr. Onkol.*, 1982, **28**, 18 (in Russian).
28 Zaichick, V. Ye., in *Nuclear Methods of the Element Analysis in Biology and Medicine*, Institute of Medical Radiology, Academy of Medical Sciences of USSR, Obninsk, 1980, pp. 88–102 (in Russian).

Paper 4/040131
Received July 4, 1994
Accepted October 12, 1994