Kawasaki Med. J. Vol. 6 : No. 1·2, 27-34, 1980

DETERMINATION OF IODINE AND BROMINE IN THE HUMAN THYROID GLAND BY NEUTRON ACTIVATION ANALYSIS

Yoshio MOCHIZUKI and Teruko YAMANE*

Department of Hygiene, Kawasaki Medical School, Kurashiki 701-01, Japan
**Department of Clinical Research, National Institute of Radiological Sciences, Chiba 260, Japan*

Accepted for Publication on April 18, 1980

Abstract

The measurements of iodine and bromine concentrations in the thyroid specimens and the sera, obtained from the patients with various thyroid disorders, were taken by neutron activation analysis following a rapid ion-exchange radiochemical separation of induced ^{24}Na in the irradiated samples. Among the patients, 5 cases of nodular goiter, 5 cases of thyroid carcinoma and 5 cases of thyrotoxicosis were included.

The iodine concentrations in the diseased thyroid tissues were found to be lower than those of the corresponding "normal" tissues. The iodine levels in the sera were too low to be measured accurately by the present method.

The bromine concentrations in the diseased tissues did not differ significantly from those of the "normal" tissues. The concentrations of bromine in the diseased tissues were less than the levels in the sera, while the bromine levels in the "normal" tissues were virtually equal to those of the sera.

The iodine and bromine contents per mg of phosphorus or nitrogen in the thyroid tissues were also investigated.

INTRODUCTION

The neutron activation analysis is known to be a sensitive analytical technique for the determination of most chemical elements, with high accuracy even at very low concentration levels. This technique has therefore appeared to be advantageous for the study of minor or trace elements in small biological samples.[1)]

In humans, iodine is closely involved in thyroid hormone metabolism, while the role of bromine in the thyroid physiology is still largely unknown.[2)]

The aim of the present study is to investigate the contents of iodine and bromine in the thyroid specimens, obtained from the patients with various

望月義夫，山根昭子

thyroid disorders at surgical operations.

MATERIALS AND METHODS

Samples : One or two grams of diseased and surrounding "normal" thyroid tissues were obtained at the surgical operations of the patients with various thyroid diseases at a hospital. Among them, 5 cases of papillary adenocarcinoma, 5 cases of follicular adenoma and 5 cases of primary hyperplasia (Graves' disease) were included. The diagnosis was confirmed by histopathological observations. The samples were dissected out, weighed and stored in 10 percent formalin solution for the convenience of transportation of them from the hospital to our laboratory. Blood samples were taken from the patients, just prior to surgical operations. The sera were separated by centrifugation and stored in polyethylene containers at -25°C. The thyroid specimens of approximately 0.5 g in wet weight were dried in a desiccator. The dried specimens were digested in a mixed solution of 2 ml of 2N KOH and 1 ml of 1 percent KNO_3 on a hot plate, and were evaporated to dryness by a slightly modified method of Brodie et al.[3]

The digested samples were then ashed in a muffel furnace at approximately 550° for 6 hours. The sera of 0.5 ml aliquot were treated in the same manner. The loss of iodine in the whole procedure for digestion and ashing was determined by adding a known amount of ^{131}I to the samples, and found to be negligible. The ashed samples were dissolved in 2 ml of redistilled water, then 50 μl of them were pipetted on 2×2 cm filter papers (Toyo-roshi No. 51), and dried under a infra-red rays lump. Standard iodine and bromine sources were prepared similarly from solutions of KI and NH_3Br.

Irradiations : The reactions used were ^{127}I (n, γ) ^{128}I ($T_{\frac{1}{2}}=25$min) and ^{81}Br (n, γ) ^{82}Br ($T_{\frac{1}{2}}=35.9$hr). The samples together with standards were placed in plastic containers. Filter paper blanks and formalin blanks were also prepared in the same manner. Irradiations of the samples were performed in the center experimental hole of the TRIGA II reactor in the Musashi Institute of Technology, for 1 hour at an estimated thermal neutron flux of 4.5×10^{12} n/sec. cm^2.

Chemical separation of induced ^{24}Na : Immediately after the irradiation, samples were transferred to glass beakers with approximately 3 ml of redistilled water for extraction. The extraction procedure was repeated 3 times. Approximately 10 ml of the resulting solution was passed through a resin column (1.5 cm in diameter $\times$ 10 cm in height) containing Dowex 50×8 resin (100-200 mesh, 1.5 cm in diameter $\times$ 3 cm in height) at a rate of 5 ml/min, to remove ^{24}Na and other radioactive cations.[4] The chemical yields of the separation

procedures for iodine and bromine were checked by experiments with radioactive tracers of ^{131}I and ^{82}Br, and found to be 96.0 percent and 95.0 percent respectively. The efficiency of the removal of ^{24}Na was also determined by introducing ^{24}Na as a tracer, and found that it was almost completely removed by this method.

Measurements : ^{128}I and ^{82}Br activities induced in the samples were evaluated by means of gamma-ray spectromety with a $2\frac{3}{4}\times 2\frac{3}{4}$ in. NaI(Tl)-detector connected to a 400 channel puls-height analyzer (Hitachi Type 403).

Determination of iodine : The quantitative determination of iodine was made by comparing the induced activities of ^{128}I in the irradiated thyroid samples with those of a series of iodine standards irradiated under identical conditions. The radioactivity of ^{128}I was measured by the peak-area method on the basis of the 0.45 Mev photopeak, and corrected by its decay.

The chemical determinations of iodine in the same samples were also performed by a slightly modified method of Shichijo et al[5], in order to compare the activation-analysis values with the chemical analysis values.

Determination of bromine : After a delay of 1 day to allow the decay of the 25 min ^{128}I, the same samples were counted again for the determination of ^{82}Br by counting the energy regions of the photopeaks at 0.55, 0.61 and 0.77 Mev. The activity of ^{82}Br was also corrected by the decay.

Determination of nitrogen : Several mg of dried thyroid tissues were weighed and digested, and the nitrogen contents in the specimens were determined by the Kjeldahl-Nessler method[6], as an index of protein content in the specimens.

Determination of phosphorus : Approximately 5 mg of dried thyroid tissues were digested by adding conc. H_2SO_4 and H_2O_2, then phosphorus contents in the samples were determined by a slightly modified method of Fiske-Subharow[7].

RESULTS

Total of 25 thyroid samples were analyzed by the both methods of neutron activation analysis and chemical analysis for iodine determination. A close correlation was observed between activation analysis values and chemical-analysis values ($r=0.786$, $p<0.001$), though the values for the activation analysis were found to be slightly but consistently higher than those for the chemical method by approximately 3.3 percent.

The results of the activation analytical study of iodine in the fresh tissue (I μg/tissue g), iodine-to-phosphorus ratio (I μg/P mg) and iodine-to-nitrogen ratio (I μg/N mg) in the diseased and corresponding "normal"

thyroid tissues are summarized in Table 1.

TABLE 1. Iodine concentrations in the thyroid tissues by neutron activation analysis

pathological diagnosis	No. of cases	"normal" tissues			diseased tissues		
		I μg/tissue g	I μg/P mg	I μg/N mg	I μg/tissue g	I μg/P mg	I μg/N mg
follicular adenoma	5	842.1±294.6	1948.9±590.1	31.3±9.4	264.3±179.8**	496.6±176.7**	10.1±6.6**
papillary adenocarcinoma	5	429.9± 90.5	639.0±159.8	14.7±3.1	237.2± 70.8**	199.0± 71.8**	8.7±2.9*
primary hyperplasia	5				259.9± 37.2	432.0±161.1	10.4±1.8

statistically significant **p<0.01 *p<0.05

TABLE 2. Bromine concentrations in the thyroid tissues and the sera by neutron activation analysis

pathological diagnosis	No. of cases	"normal" tissues			diseased tissues			sera
		Br μg/tissue g	Br μg/P mg	Br μg/N mg	Br μg/tissue g	Br μg/P mg	Br μg/N mg	Br μg/ml
follicular adenoma	5	5.5±2.1	12.0±4.2	0.21±0.07	4.4±1.2	8.9±2.2	0.17±0.04	7.2±1.3
papillary adenocarcinoma	5	8.1±3.7	12.3±6.1	0.27±0.11	6.6±1.7	5.3±1.1*	0.29±0.07	7.7±1.7
primary hyperplasia	5				4.7±0.8	8.6±2.3	0.20±0.05	6.9±0.7

The iodine concentrations in the thyroid tissues of adenoma, as well as carcinoma were significantly less than those of the "normal" tissues. The iodine concentrations in the tissues of hyperplasia were also significantly lower than those of the "normal" tissues for adenoma or carcinoma, though the corresponding "normal" tissues for hyperplasia were not available. The iodine contents per unit weight of phosphorus and nitrogen in the diseased tissues were significantly less than those in the "normal" tissues. It is also shown that the iodine contents per mg of phosphorus in the cancerous tissues were significantly less than those of adenoma or hyperplasia, while the iodine contents per g of fresh tissues or those per mg of nitrogen in the diseased tissues were found to be virtually of equal levels.

The iodine levels in the sera of the patients were too low to be measured accurately.

The bromine levels (μg) in fresh tissue (g), phosphorus (mg) and nitrogen (mg) basis in the diseased and the corresponding "normal" tissues are listed in Table 2. The bromine levels in the sera of the patients are also shown in the tame table.

The bromine concentrations in the tissues of adenoma, carcinoma or hyperplasia did not differ significantly from those of the "normal" tissues for adenoma or carcinoma, though the mean values for these diseased tissues were lower than those of the "normal" tissues. The bromine concentrations in the diseased tissues were less than the levels in the sera, while the bromine levels in the "normal" tissues did not differ significantly from those of the sera.

The bromine contents per mg of phosphorus in the diseased tissues were slightly less than those of the "normal" tissues, while the bromine contents per mg of nitrogen in the diseased tissues were virtually equal to those of the "normal" tissues.

No detectable amount of iodine or bromine in the filter paper extract

TABLE 3. Phosphorus and nitrogen concentrations in the thyroid tissues by chemical methods

pathological diagnosis	No. of cases	"normal" tissues		diseased tissues	
		P mg/tissue g	N mg/tissue g	P mg/tissue g	N mg/tissue g
follicular adenoma	5	0.47±0.15	25.9±5.0	0.91±0.47	26.0±3.2
papillary adenocarcinoma	5	0.72±0.53	29.6±1.9	1.27±0.37*	23.3±7.8
primary hyperplasia	5			0.71±0.38	25.9±5.0

or formalin solution was observed by the neutron activation analysis.

The contents of phosphorus and nitrogen in the thyroid specimens were also determined by chemical methods and listed in Table 3.

DISCUSSION

Neutron activation analysis is a highly sensitive and specific method of detecting elements in biological materials, however, the measurements of trace elements in biological specimens by this method is interfered with the high levels of sodium usually present.[8] The activity of ^{24}Na ($T\frac{1}{2}=15hr$) induced by neutron irradiation masks the activity of less abundunt elements, and is particularly serious when the measurements of other activities with shorter half-lives, such as ^{128}I ($T\frac{1}{2}=25min$).

In the present study, a time consuming procedure for alkali-ashing of the thyroid specimens and the sera were accomplished prior to neutron irradiation, then the measurements of ^{128}I and ^{82}Br were performed following the rapid ion-exchange radiochemical separation of ^{24}Na in irradiated samples. The removal of ^{24}Na in roughly 20 irradiated samples can be accomplished within 1 hour by this method.

Estimation of iodine in the human thyroids or the sera of healthy subjects by neutron activation analysis or conventional chemical methods have been performed by many investigators[9,10,11,12,13].

The mean iodine concentration in the "normal" thyroid tissues obtained from the patients with thyroid carcinoma was significantly lower ($p<0.05$) than that of the"normal" tissues for adenoma. The reason is unknown, however, this may reflect the influence of cancerous tissue to its surrounding "normal" tissue. The former value is in agreement with reported values for normal Japanese individuals[11], though the considerable variations in the thyroid levels of iodine was observed in the previously published values. The variations are probably, in part, due to varying iodine intake in the cases studied.

The mean values for iodine concentrations in the diseased thyroid tissues were lower than those of the corresponding "normal" tissues. Reported values for diseased thyroid tissues are very few, but Widdowson et al.[14] reported that iodine concentrations in the hyperplastic thyroid glands were reduced compared with those of the normal glands.

The bromine levels in the sera of the patients were within the range of values listed in the "Biology Data Book"[15] or other reported values.[16] No definate relation was found to exist between the bromine levels in the sera and the type of thyroid diseases. The bromine concentrations in the diseased and the "normal" thyroid tissues were found to be equal to or slightly less than

the values for the sera, although several authors[2,17] reported that there was some concentration of bromine in the thyroid gland. The varieties found in the results in the present study and other studies, may in part reflect the variations in bromine intake of the subjects studied.[14]

Little information has been available in the literatures on the bromine contents in the thyroid tissue, especially in the diseased tissues. The bromine values for the "normal" thyroid tissues in the present study were comparable to Ucko's data[18], while considerably higher values were reported by Neufeld.[17] The variations observed in the results of these studies are probably, in part, due to the difference of analytical methods employed.

The basis for the comparison of normal and diseased thyroid tissues is thought to be not the same in all kinds of thyroid disorders, since some of them, the cells and structures of diseased and normal tissues are so different that they cannot be directly compared.

In this respect, the comparison of iodine and bromine concentrations in the diseased and the "normal" thyroid tissues were made in relation to the corresponding phosphorus or nitrogen content, in addition to fresh weight basis. By examining the data presented in Table 1 and Table 2, it appears that both ratios of I μg/P mg and Br μg/P mg in the cancerous thyroid tissues are significantly less than those of the adenoma or hyperplastic thyroid tissues. This may, in part, be due to increasing amount of phosphorus in the growing cancerous tissues, as shown in Table 3. The levels of phosphorus and nitrogen in the "normal" thyroid tissues were found to be within the range of previously published values.[19]

It must be emphasized that the present investigation is of a preliminary nature and that the experimental material is not sufficiently extensive to permit a statistical evaluation of the data. Nevertheless, certain trends were observed in the results and indicated that further studies into the area might be necessary.

Acknowledgment

We are indebted to Dr. K. Ito, Director of Ito Hospital, Tokyo, for the generous cooperation in obtaining thyroid specimens, and to Dr. Y. Shinbori, Professor of Josai University, Saitama, for the helpful suggestions and technical assistance on activation analysis.

We are also grateful to Dr. T. Kimoto, Professor of Pathology, Kawasaki Medical School, for his helpful suggestions on the pathological classification of thyroid diseases.

REFERENCES

1) I. A. E. A. : Nuclear Activation Techniques in the Life Sciences, Proceedings of an I. A. E. A. Symposium, Amsterdam, 8-12 May 1967
2) Gross, J. : Iodine and Bromine. in Mineral Metabolism, vol. II, Part B, ed. by Comar,

C. I. and Bronner, F. New York and London, Academic Press. 1962, pp. 266-269
3) Brodie, B. B. and Friedman, M. M. : The determination of bromide in tissues and biological fluids. J. Biol. Chem. **124** : 511-518, 1938
4) Cottino, F., Margo, G. and Malvano, R. : Neutron activation analysis for the study of iodine balance in man. in Reference 1) pp. 419-425
5) Shichijo, K., Tanaka, S. and Nakano, M. : Kesseitanpakuketsugo-yoso oyobi sono sokuteiho ni tsuite (Proteinbound iodine and its measurement), Hormon to Rinsho **1** : 53-61, 1953 (in Japanese)
6) Lang, C. A. : Simple microdetermination of Kjeldahl nitrogen in biological materials, Anal. Chem. **30** : 1692-1694, 1958
7) Frankel, S, Reitman, S. and Sonnenwirth, A. C. ed. : Gradwohl's Clinical Laboratory Methods and Diagnosis, 7th ed. vol. 1, Saint Louis, The C. V. Mosby Co. 1970, pp. 185-186
8) Dawson, E. B., Menon, M. P., Wainerdi, R. E. and McGanty, W. J. : Activation analysis of placental trace metals, J. Nucl. Med. **9** : 160-162, 1968
9) Brune, D. and Wester, P. O. : The determination of iodine in thyroid gland with epithermal neutrons. Anal. Chim. Acta **52** : 372-373, 1970
10) Underwood, E. J. : Trace elements in human and animal nutrition, 3rd ed. New York, London, Academic Press. 1962, p. 224
11) Hayashi, K. ed. : Kaibogaku oyobi Seirigaku Keisu (Anatomical and Physiological Data). Okayama, Kaibo Seiri Keisu Kankokai. 1956, p. 428 (in Japanese)
12) ICRP : Report of the task group on reference man, ICRP Publication 23, Oxford, New York, Toronto, Sydney, Braunschweig, Pergamon Press. 1975, p. 287, p. 303
13) Tang, C. W. and Tomlinson, R. H. : Determination of proteinbound iodine by activation analysis. in Reference 1) pp. 427-435
14) Widdowson, E. M. and Dickerson, J. W. T. : Chemical composition of the body. in Mineral Metabolism, vol. II The Elements, Part A, ed. by Comar, C. L. and Bronner, F. Now York, London, Academic Press. 1964, pp. 172-173
15) Altman, P. L. and Ditter, D. S. ed. : Biology Data Book, 2nd ed. vol. III, Bethesda, Federation of American Societies for Experimental Biology. 1974, p. 1751
16) Rapaport, M. S., Mantel, M. and Nothmann, R. : Determination of bromine in blood serum by neutron activation analysis and X-ray spectrometry. Anal. Chem. **51** : 1356-1358, 1979
17) Neufeld, A. H. : Contributions to the biochemistry of bromine, II. The bromine content of human tissues. Can. J. Res., **15** B : 132-138, 1937
18) Ucko, H. : Investigations into presence and rôle of bromine in body. Biochem. J. **30** : 992-1000, 1936
19) ICRP : in Reference 12) pp. 310-311