STUDIES ON THE CONCENTRATION OF PARTICULATE IODOPROTEIN, RNA, AND DNA IN NORMAL AND ENDEMIC GOITER GLANDS

GERALDO A. MEDEIROS NETO, W. NICOLAU, and A. B. ULHÔA CINTRA

PUBLICAÇÃO IEA N.º 216
Junho — 1970

INSTITUTO DE ENERGIA ATÔMICA
Caixa Postal 11049 (Pinheiros)
CIDADE UNIVERSITÁRIA "ARMANDO DE SALLES OLIVEIRA"
SÃO PAULO — BRASIL

STUDIES ON THE CONCENTRATION OF PARTICULATE IODOPROTEIN, RNA, AND DNA IN NORMAL AND ENDEMIC GOITER GLANDS*

Geraldo A. Medeiros-Neto, W. Nicolau,
and A.B. Ulhôa Cintra

Divisão de Radiobiologia
Instituto de Energia Atômica
São Paulo - Brasil

Publicação IEA Nº 216
Junho - 1970

---

*Report of the Meeting of the PAHO Scientific Group on Research in Endemic Goiter held in Puebla, México, 27 to 29 June 1968, Chapter 16 - pg. 183-193, 1969.

Comissão Nacional de Energia Nuclear

Presidente: Prof.Dr. Hervásio Guimarães de Carvalho

Universidade de São Paulo

Reitor: Prof.Dr. Miguel Reale

Instituto de Energia Atômica

Diretor: Prof.Dr. Rômulo Ribeiro Pieroni

Conselho Técnico-Científico do IEA

Prof.Dr. José Moura Gonçalves Prof.Dr. José Augusto Martins Prof.Dr. Rui Ribeiro Franco	pela USP
Prof.Dr. Theodoreto H.I. de Arruda Souto	pela CNEN

Divisões Didático-Científicas

Divisão de Física Nuclear -
Chefe: Prof.Dr. José Goldenberg

Divisão de Radioquímica -
Chefe: Prof.Dr. Fausto Walter de Lima

Divisão de Radiobiologia -
Chefe: Prof.Dr. Rômulo Ribeiro Pieroni

Divisão de Metalurgia Nuclear -
Chefe: Prof.Dr. Tharcísio D.S. Santos

Divisão de Engenharia Química -
Chefe: Lic. Alcídio Abrão

Divisão de Engenharia Nuclear -
Chefe: Engº Pedro Bento de Camargo

Divisão de Operação e Manutenção de Reatores -
Chefe: Engº Azor Camargo Penteado Filho

Divisão de Física de Reatores -
Chefe: Prof.Dr. Paulo Saraiva de Toledo

Divisão de Ensino e Formação -
Chefe: Prof.Dr. Rui Ribeiro Franco

STUDIES ON THE CONCENTRATION OF PARTICULATE IODOPROTEIN, RNA, AND DNA IN NORMAL AND ENDEMIC GOITER GLANDS

Geraldo A. Medeiros-Neto, W. Nicolau, and A.B. Ulhôa Cintra[1]

SUMMARY

Thyroid glands were obtained from three normal subjects at the time of parathyroid surgery and from five patients with endemic goiter. These specimens were examined for distribution of iodine and of labeled iodine among the various particulate and soluble fractions and for iodinated protein content.

Approximately one-fifth of the total labeled and stable iodine was easily sedimentable in the goitrous glands, whereas only a small fraction was sedimentable in the homogenates of the normal thyroids.

The iodine concentration was smaller in the goitrous gland. Turnover of iodine was slow in the insoluble and easily sedimentable iodoprotein. There was more DNA-phosphorus and RNA per gram in the goitrous glands than in the normal specimens.

The easily sedimentable particulate iodoprotein had a higher ratio of iodotyrosines to iodothyronines than did the soluble iodoproteins. When solubilized it did not react with antihuman thyroglobulin.

The physiological significance of the easily sedimentable particulate thyroidal iodoprotein with sluggish turnover is unknown. Evidently it is not metabolically available and represents a considerable sequestration of iodine in the thyroid in endemic goiter.

The thyroid gland is unique in that much of its protein-synthetic machinery is concerned with the formation of a large, complex iodinated glyco-protein, thyroglobulin. This protein is a required intermediate in the production of the chemically less complex hormonal iodine. It may comprise 70 or more per cent of the dry weight of the gland and serves as a reservoir for storage of thyroid hormones and their precursors in order that iodine may be conserved and buffered against changes in iodine supply from external sources. It is quite clear that when synthesis of thyro-

1/ Medical Clinic, Clinical Hospital of the School of Medicine of the University of São Paulo, São Paulo, Brazil, and the Institute of Atomic Energy, São Paulo, Brazil.

globulin is blocked or modified owing to disorders of the thyroid gland, this buffering action may be seriously impaired.

Efforts of many investigators (13-15, 17, 19, 20, 21) have yielded an impressive amount of information on changes that occur in thyroid disease in the relative proportion of thyroglobulin and other iodinated proteins which are found somewhat variably in small amounts in normal thyroid tissue. One of these, an iodinated albumin-like protein may comprise as much as 1.3 per cent of the total iodine (19). Its sedimentation constant is approximately 4S. The fraction of the total iodoprotein which is 4S may be sharply increased in thyroid disease (17, 19, 21). The other is a dense particulate iodoprotein which is readly solubilized by trypsin and is present in large relative and absolute amounts in congenital goiter (14, 15), thyroid tumors (20), and adenomas (13, 14). The physiological role, if any, of this moiety of the thyroid iodoproteins is entirely unknown.

The present study is concerned with the occurrence, nature an properties of the particulate iodoprotein found in enlarged thyroid glands from an endemic goiter region. Included are the results of DNA and RNA analyses on the same specimen and on normal glands. We have tried to establish a relation ship between cellularity, protein synthesis or turnover, and the presence of particulate iodoprotein.

MATERIAL AND METHODS

Three normal glands were obtained from euthyroid patients at the time of neck surgery for parathyroid adenomas (patients S.N. and A.S.) and from the opposite lobe of a thyroid gland in which there was a solitary thyroid adenoma (patient A.C.). Microscopic examination disclosed a normal morphological picture.

The five patients with endemic goiter (with one exception

S.G.) came from São Paulo State, Brazil, which is within the endemic goiter area delineated by the Brazilian Government. They had large hyperplastic glands that had been present for many years without significant changes in size or position. Only one of these patients had received some iodine drops as treatment (patient A. M.B.) but discontinued the medication more than one year before the present study.

Clinical and laboratory information on the endemic goiter patients appears in Table 1. These data included a high uptake of RAI, a negative TRC agglutination test, and a normal PBI. A pathological diagnosis of colloid goiter was rendered on four specimens; the fifth was an adenomatous goiter (patient S.G.). The total weight of the gland was estimated from the thyroid scintiscan.

TABLE 1 Clinical and laboratory data on goitrous patients

Name	Age	Sex	PBI (µg/100ml)	RAI Uptake		Tanned red cell agglutination	Estimated weight of the gland (g*)	Histologic picture
				2 hrs.	24 hrs.			
M.R.	48	F	8.6	13.0%	36.0%	Neg.	147	Colloid goiter
A.M.B.	37	F	6.7	23.0%	56.0%	1:80	137	Colloid goiter
M.D.T	39	F	7.8	34.5%	75.5%	1:40	96	Colloid goiter
S.G.	29	F	6.7	24.0%	68.0%	Neg.	72	Adenomatous colloid goiter
M.G.	48	F	6.2	19.0%	46.0%	Neg.	112	Colloid goiter

* Based on thyroid scintiscan.

The eight patients received a tracer dose of ^{125}I at 35 to 47 days before surgery and to two patients (M.R. and M.G.) another tracer dose of ^{131}I was given 24 hours before the operation. The glands were collected at the time of surgery in cracked ice, rinsed free of blood, and dissected to remove fibrous tissue from glandular tissue. In every case of endemic goiter two specimens were processed independently. The tissues were homogenized

at 4°C in 0.26 m sucrose solution in an all-glass motor-driven homogenizer. The homogenates were made up to four times their original weight with 0.26M sucrose and an aliquot was separated for chemical determinations (^{127}I, protein, RNA, and DNA-phosphorus), and then centrifuged for 10 minutes at 700xg in a refrigerated centrifuge. The supernatant was removed, the sediment was resuspended, centrifugation was repeated, and the supernatants were pooled. The final sediment (nuclear fraction) was made to 50 per cent of the volume of the homogenate. The combined supernatant fractions were centrifuged at 105,00xg for 60 minutes at 0°C in the Spinco preparative ultracentrifuge and the fluid above the pellet was removed by aspiration to constitute the soluble protein fraction. The pellet was consideres to be a misture of mitochondria and microsomes and separated for a mitochondrial fraction. Aliquots were taken for ^{127}I, ^{125}I, and protein determination as indicated.

Analytical Methods

Chromatography was done in Dowex resin columns, according to the Blanquet-Meyniel technique (3). Stable iodine determinations were made by the Benotti and Benotti (2) modification of the Zak method. Protein was determined by the method of Lowry et al. (10). Pancreatin and pronase hydrolusis of the thyroid proteins was performed with the sample brough to pH 8.4 it the presence of 10^{-3}M propylthiouracil and a few drops of toluene. The samples were stored at 37 C for 4 hours and then chromatographed. Separation of thyroid proteins was made on Sephadex G-200 column eluted with 0.15M NaCl according to Perelmutter et al. (16). Solubilization of particulate iodoprotein was attempted by a brief treatment with 0.4 per cent trypsin (15 min) at room temperature, followed by soybean trypsin inhibitor. The samples were centrifuged and the solubile fraction applied to Sephadex columns for gel filtration. RNA and DNA-phosphorus content of the thyroid tissue were determined following the method of Munro, as modified by Goldberg and his

colleagues (7). These investigators demonstrated the unsuitability of colorimetric methods for nucleic acid estimation when applied to the human thyroid gland. When colloid storage is poor, as in thyrotoxicosis, interference with RNA and DNA estimations is less than that found in normal glands. It is likely that carbohydrates associated with thyroglobulin contribute to this interference. Therefore RNA was estimated by ultraviolet spectrophotometry and DNA content of the lipid extracted fraction was estimated by its content of phosphorus (7).

RESULTS

1. Relative Proportion of Labeled Iodine, Stable Iodine, and Protein in Goitrous and Normal Glands

In the goitrous glands close to one-fifth of the total labeled and stable iodine was present in the 700xg fraction. In the normal glands less than 10 per cent of the total iodine was present in this fraction. In most goitrous glands the proportion of stable iodine in the nuclear fraction was higher than the labeled iodine percentage. This was not the case for the normal tissue. The relative proportion of protein was also significantly higher in the goitrous tissue as compared with normal glands (Table 2).

2. Concentration of ^{127}I and Protein per Gram of Wet Weight in the Nuclear Fraction

In bot goitrous and normal glands the absolute amount of iodine and protein was measured in the nuclear fraction and corrected to the total wet weight of each specimem. As can be seen in Table 3 the concentration of protein in the nuclear cut of the abnormal glands is considerably higher as compared to the same determination in the normal tissue (espectively 44.71 ± 9.55 and 14.72 ± 4.58). This was significant at a level of $p<0.01$. The

amount of stable iodine in the goitrous tissue, however, in the same fraction, was not significantly different as compared with the normal. Respectively 11.8 ± 6.53 mcg/g and 14.06 ± 3.48 were obtained for goitrous and normal glands. The ratio of stable iodine per mg of protein is significantly lower in the abnormal tissue .. (0.288 ± 0.155) as compared with the normal tissue (1.36 ± 0.393).

TABLE 2 Relative proportion of labeled iodine, stable iodine, and protein in goitrous (patients 1-5) and normal glands (patients 6-8). Results in % of the total in the whole homogenate

Patient	Labeled iodine (^{125}I)	Stable iodine (^{127}I)	Protein
1 - M.R.	17.1	22.80	25.0
2 - A.M.B.	14.3	9.26	33.3
3 - M.D.T.	12.4	17.47	29.9
4 - S.G.	26.2	28.72	27.6
5 - M.G.	12.5	18.40	18.6
Mean	16.53	19.33	26.8
S.D.	±7.33	±9.37	±6.5
6 - S.N.	7.3	9.3	13.2
7 - A.S.	10.7	7.8	20.4
8 - A.C.	9.7	8.4	10.8
Mean	9.23	9.50	14.8
S.D.	±1.83	±1.2	±4.7

When the same determinations were applied to the whole homogenate the results were quite similar. The goitrous glands had only 65.6 ± 16.8 µg of ^{127}I per g of wet weight, whereas the normal tissue had 165.0 ± 12.3 µg. The protein content of abnormal glands was significantly higher than in the normal tissue, and the ratio of stable iodine per mg of protein was significantly lower in the abnormal tissue as compared with the normal glands (Table 4).

TABLE 3 Concentration of protein and ^{127}I in the 700 xg sediment ("nuclear" fraction) in goitrous (patients 1-5) and normal glands (patients 6-8). Results in mg per g of wet weight.

Patient	Protein mg/g of wet weight	^{127}Iodine µg/g of wet weight	µg of ^{127}I/mg of protein
1 - M.R.	36.46	16.13	0.458
2 - A.M.B.	48.54	8.13	0.167
3 - M.D.T.	59.65	6.02	0.109
4 - S.G.	38.08	10.48	0.275
5 - M.G.	40.81	17.66	0.433
Mean	44.71	11.80	0.288
S.D.	±9.55	±6.53	±0.155
6 - S.N.	12.58	15.57	1.237
7 - A.S.	19.99	11.60	0.580
8 - A.C.	11.61	15.01	1.292
Mean	14.72	14.06	1.036
S.D.	±4.58	±3.48	±0.393
P	<0.001	>0.05	< 0.01

TABLE 4 Concentration of ^{127}I and protein per gram of glandular tissue (wet weight) in goitrous (patients 1-5) and normal glands (patients 6-8)

Patient	µg of ^{127}I/g	µg of protein/g	µg ^{127}I/mg protein
1 - M.R.	73.4	145.8	0.503
2 - A.M.B.	87.8	145.8	0.602
3 - M.O.	34.5	199.5	0.172
4 - S.G.	36.5	138.0	0.261
5 - M.G.	96.0	219.4	0.437
Mean	65.6	169.7	0.395
S.D.	±16.8	±37.1	± 0.178
6 - S.N.	167.4	95.3	1.756
7 - A.S.	148.8	98.0	1.517
8 - A.C.	178.8	107.5	1.662
Mean	165.0	100.3	1.645
S.D.	±12.3	±6.41	± 0.119
P	< 0.001	<0.05	< 0.001

Thus the goitrous gland has three times less iodine in the gland per unit of weight, but in the nuclear cut the same amount of iodine as the normal tissue. The ratio of stable iodine to protein is quite low in both the nuclear fraction and in the

whole homogenate of the goitrous glands.

3. Ratio of Labeled Iodine (^{125}I and ^{131}I) in Particulate and Soluble Proteins

In two patients (M.R. and M.G.) it was possible to measure the ratio of both ^{125}I and ^{131}I as related to protein. By the double-labeling technique we intended to measure the rate of turnover of labeled iodine in both particulate (700xg fraction) and soluble protein fractions. The ^{125}I was administered at least four weeks before surgery and ^{131}I was given 24 hours before the gland was removed. In both patients the particulate iodoprotein had a higher ratio four weeks after the tracer dose (^{125}I) than at 24 hours (^{131}I). Particulate iodine was six to ten times higher in iodine after four weeks than at 24 hours after administration. This suggests a slow turnover of iodine in this insoluble iodoprotein (Table 5). For the soluble protein fraction the ratio at 24 hours was higher than at four weeks. This suggests a more active incorporation and release of iodine in the iodoproteins of the soluble fraction.

TABLE 5 Ratio of ^{125}I and ^{131}I to particulate (700 x g fraction) and soluble protein fraction (105.000 x g fraction). ^{131}I was administered 24 hours and ^{125}I four weeks before surgery. Results expressed as % of the administered dose per gram of protein

Patient	Isotope	Particulate iodoprotein	Soluble iodoproteins
M.R.	^{131}I	0.056	1.096
	^{125}I	0.123	0.908
M.G.	^{131}I	0.048	0.985
	^{125}I	0.076	0.738

4. Concentration of Protein, RNA, and DNA-Phosphorus

There was an increased proportion of protein, RNA, and DNA-phosphorus per gram of wet weight of tissue in the five specimens of goitrous glands (Table 6). These values were compared with the same obtained for the normal glands and were found to be statistically different ($p < 0.05$). Thus the goitrous glands have more DNA-phosphorus and RNA per gram of tissue than the normal specimens. As an approximation to the content of the individual cell the use of DNA as a reference parameter is widely accepted. For this reason the other tissue constituents (protein and RNA) were expressed relative to the DNA content of the sample and the results given in Table 7.

TABLE 6 Concentration of protein, RNA, and DNA-phosphorus in goitrous and normal glands (number of specimens in parentheses). Results as mean ± SD in mg/g of wet weight. Statistical comparison by Student's t test

	Normal glands (3)	P	Goitrous glands (5)
Protein	100.3 ± 6.41	<0.05	169.71 ± 37.11
RNA	0.917 ± 0.110	<0.05	1.988 ± 0.617
DNA-phosphorus	0.264 ± 0.126	<0.05	0.414 ± 0.128

TABLE 7 Concentration of protein and RNA in goitrous and normal glands in relation to the total concentration of DNA-phosphorus (number of specimens in parentheses). Statistical comparison by Student's t test).

	Normal glands (3)	P	Goitrous glands
Protein	440.64 ± 59.50	>0.05	587.94 ± 11.32
RNA	3.91 ± 1.33	<0.05	6.27 ± 1.95

The goitrous glands had a significant increase in RNA per mg of DNA-phosphorus but not in protein per mg of DNA, as compared with normal tissue. The increase in the RNA/DNA ratio

is compatible with the increased protein synthesis or turnover occuring in the abnormal tissue.

Both the DNA-phosphorus content of the tissue and the RNA/DNA ratio were related to the absolute amount of particulate protein in each specimen of the goitrous glands (Table 8). Thus there seems to be no correlation between the amount of particulate protein and the abnormal proportion of RNA and DNA-phosphorus in these goitrous glands ($\alpha = 0.05$).

TABLE 8 Correlation between particulate protein, RNA/DNA ratio, and DNA-phosphorus in the goitrous glands.

Patient	Particulate protein mg/g of wet weight	DNA-phosphorus mg/g of wet weight	RNA/DNA ratio
M.R.	36.46	0.220	8.76
A.M.B.	48.54	0.439	7.36
M.D.T.	59.65	0.462	4.16
S.G.	38.08	0.566	4.45
M.G.	40.81	0.383	6.62
Correlation coeficient	-	r = 0.11*	r = 0.35*

* There is no significant coefficient of correlation between particulate protein versus DNA-phosphorus and RNA/DNA ratio ($\alpha = 0.05$)

5. Properties of the Solubilized Iodoprotein

The solubilized iodoprotein of the goitrous glands was applied to a column of Sephadex (G-200) fel and eluted in 3 ml fractions with 0.15M NaCl and collected by an automatic fraction collector. A peak was observed after 150 ml of effluent which was distinctly different from normal human thyroglobulin. This component had an ultracentrifugal sedimentation coefficient between 3.1 and 6.8S (18).

Both the particulate and the soluble proteins were hydrolyzed with pronase and submitted to column chromatography. The results appear in Figure 1. The particulate protein had a higher iodotyrosine/

/iodothyronine ratio as compared with soluble proteins and a significantly lower content of T_3/T_4. The solubilized iodoprotein did not react with goat anti-human thyroglobulin in Ouchterlony agar plates; both normal and trypsin-treated thyroglobulin exhibits

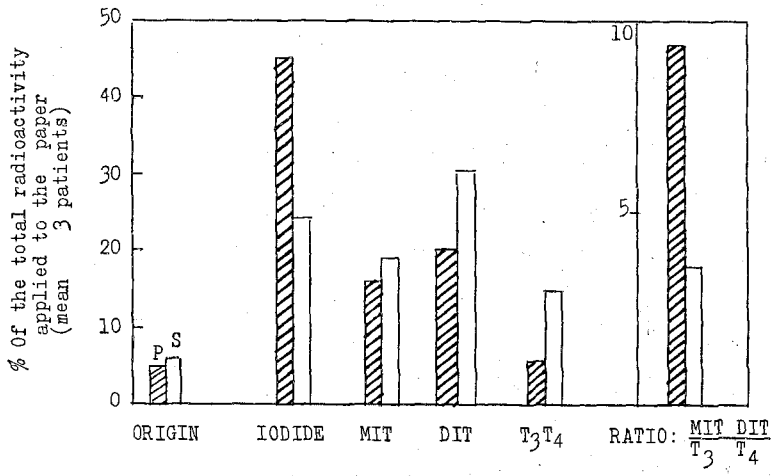

Figure 1. Column chromatography of hydrolyzed particulate (P) and soluble protein fraction (S). The results are expressed as per cent of the total radioactivity applied to the column and are the mean of three specimens. Note a lower relative proportion of T_3T_4 for the particulate protein and a higher iodotyrosine to iodothyronine ratio.

a definable line of precipitation in the same agar plate. Thus the solubilized iodoprotein behaved distinctly differently from normal human thyroglobulin in both chemical and immunological tests.

DISCUSSION

In the normal thyroid gland amost all the iodine is in soluble form as part of the thyroglobulin molecule. A small fraction sediments along with the cell nuclei and tissue debris at 700xg for 10 minutes. One of us (G.M.N.) has studied this component in five normal glands, and in these tissues less than

10 per cent was found in the nuclear cut (12).

In some abnormal tissue as much as 50 per cent of the total iodine may be found in this form (12). This component was first detected in a rat transplantable thyroid tumor by Robbins et al. (20) and later found in congenital goiter (13, 14, 20), thyroid adenomas (13, 14), and human thyroid tumor (13). In endemic goiter glands several abnormalities in protein biosynthesis have been reported. An appreciable increase of 4S protein was observed both in hyperplastic endemic goiters and in endemic cretinism. This soluble protein has electrophoretic mobility and immunological reactions similar to those observed with serum albumin (9). An increased MIT/DIT ratio was also observed (1). A similar finding was observed in hyperplastic goiters produced in hamsters by iodine-deficient diets but not in colloid goiters (6). As judged by these studies chronic iodine-deficiency may induce an abnormal pattern in the distribution of iodine in the soluble proteins, characterized by an increase in an iodinated albumin-like protein.

Few studies have been devoted to insoluble proteins in these abnormal tissues. Beckers and De Visscher (1) reported their findings in four specimens of goitrous glands from the Uele region. These patients received labeled iodine three to seven days before surgery and, with one exception, iodine prophylaxis for one year before the study was begun. In these glands 6.94 to 12.13 per cent of the total labeled iodine was particulate. Stable iodine concentrations in the particulate fractions were not reported. When these results are compared to those reported here it should be recalled that the time of labelling is important when particulate labeled iodoprotein is analized. We labeled the gland at least four weeks before surgery and this may be an explanation for somewhat different proportions of particulate protein in the tissue.

Westra et al. (22) observed in rats that the particulate labeled iodine rose sharphy with time and that the percentage of

thyroidal insoluble iodoprotein, which is approximately 10 in rats on an iodine-deficient diet, was lowered to 2.6 in the iodine-rich glands. These studies have also shown that in rats there is a fraction of particulate labeled iodoprotein which is relatively slowly labeled with the isotope and which turns over slowly. It appears that the percentage of thyroidal stable iodine in particulate form must be influenced by factors related to the cellularity and iodine content of the gland. In our patients there was a low iodine content of the thyroid tissue, both in goitrous and in normal glands. In nine normal individuals who underwent neck surgery Ermans et al.(5) reported a mean of 620 ± 66 µg of ^{127}I per gram of fresh thyroid tissue. This is almost four times higher as compared with the value we have obtained in our normal specimens (165,0 ± 12.3) and almost ten times higher than the value found in goitrous glands. It should be stressed that our patients were on a chronic iodine-deficient diet and this could be the main reason for the low iodine content in the gland. If the conclusions of Westra et al. (22) are valid for the human thyroid gland, then the higher absolute and relative proportion of particulate protein found in our goitrous glands is related to chronic iodide deficiency. It is also possible that in these hyperplastic thyroids the percentage of particulate (i.e., intracellular) iodine is actually elevated because of the preponderance of cells.

The data obtained from analysis of the goitrous glands for RNA and DNA phosphorus have shown that there is actually an increase in both parameters in the abnormal tissue as compared with the normal specimens. It has previously been shown in animal experiments that an increase in RNA content, but not in DNA content, follows thyrotropin injection, whereas the former falls after hypophysectomy, without change in the latter (8, 11). Thus the data relative to DNA are in accord with an increased cellularity in the hyperplastic glands, but no correlation was found between the increased DNA content and the absolute or relative proportion of particulate iodo-

protein in the goitrous glands. Two reservations must be made regarding these findings. First no account has been taken of polyploidy, which almost certainly occurs in these abnormal glands. The second is the problem of lymphocytes and plasma cells that may be found infiltrating the tissue. Goldberg et al. (7) demonstrated a higher proportion of RNA and DNA-phosphorus in chronic thyroiditis and attributed this to the large proportion of lymphocytes infiltrating the thyroid tissue. Our data on normal glands fall within the range of the normal group of Goldberg et al. (7). Their specimens were removed within a short time of death from patients without thyroid disease. We have also compared our data on goitrous glands to those published by Goldberg and his colleagues for thyrotoxic glands. They are quite similar except for an increase in protein content in the latter as compared with the former. This suggests that the endemic goiter glands are also highly stimulated to increased cellularity and protein synthesis similarly to thyrotoxic glands. Increased protein synthesis or turnover in the goitrous glands is suggested by the higher RNA/ /DNA ratio as compared with normal thyroid tissue.

As judged by our data and from previously reported findings (1, 9), the increased protein synthesis in the endemic goiter glands seems to be related to the production of abnormal iodoproteins. Furthermore, double labeling of the gland demonstrated that the ratio of labeled iodine to particulate protein is higher at four weeks than at 24 hours of a tracer dose. This suggests that this abnormal component has a slow turnover and that it is gradually labeled to a considerably higher content of iodine because of a slower loss of label, as compared with the soluble proteins. Moreover, the chromatographic distribution of labeled amino acids seems to be quite different as compared with soluble proteins.

The physiological significance of particulate thyroidal

iodoprotein with sluggish turnover rate is unknown. They may constitute "an adventitious concomitant of thyroid hormone production" (4). The particulate iodoprotein apparently is not metabolically available and thus a considerable amount of iodine is sequestered into this abnormal component. If one considers that these glands have a very low content of iodine available for normal hormone synthesis, the production of this iodoprotein constitutes such a drain on the iodine economy of the gland that growth of the gland is required in order to provide sufficient normal hormone for daily needs. It is tempting to relate the presence of large amounts of particulate iodoprotein in endemic goiter to a basic pathologic process that aggravates chronic iodine deficiency.

ACKNOWLEDGMENT

This investigation was supported by the Research Development Foundation of the State of São Paulo and by the Research Fund of the Clinical Hospital.

RESUMO

Glândulas tireóide foram obtidas de 3 indivíduos normais (na ocasião de cirurgia exploradora das glândulas paratireoides) e de 5 pacientes com bócio endêmico. Os espécimes foram analisados em seu conteúdo de iôdo estável e distribuição de iôdo radioativo nas frações solúveis e particuladas, bem como no conteúdo total de proteínas iodadas.

Aproximadamente 20% do iôdo total, tanto estável como radioativo sedimentava-se em baixa velocidade (700 x g x 10 minutos) nos tecidos provenientes de bócio endêmico, enquanto apenas uma fração diminuta de iôdo sedimentou-se, à mesma velocidade para espécimes de tireóide normal.

A concentração de iôdo era menor no caso de glândulas de bócio endêmico e a velocidade de metabolização dêste halogênio na proteina insolúvel e fàcilmente sedimentável foi considerada muito lenta. Encontrou-se mais DNA-fósforo e RNA por grama de tecido nas glândulas patológicas do que nas normais.

A proteína insolúvel e fàcilmente sedimentável exibia uma razão elevada de iodotirosinas em relação a iodotironinas comparativamente as proteínas solúveis. Quando a proteína insolúvel foi levada à solubilização com tripsina não reagiu com anti-tireoglobulina em placa de agar.

A significação fisiológica da proteína insolúvel e fàcilmente sedimentável é ainda desconhecida. Evidentemente parece não ser metabòlicamente acessível à fisiologia normal e representa uma sequestração ponderável do iôdo fisiológico do ciclo hormonal normal da glândula tireóide em casos de bócio endêmico

REFERENCES

(1) Beckers, C. and M. De Visscher. Metabolism 10:695, 1961.

(2) Benotti, J. and N. Benotti. Clin. Chem. 9: 408, 1963.

(3) Blanquet, P.G., G. Meyniel, and J. Savoie. C. R. Acad. Sciences (Paris) 250: 217, 1960.

(4) Boat, T.F. and N.S. Halmi. Endocrinology 77: 537, 1965.

(5) Ermans, A.M., J. Kinthaert, C. Delacroix, and J. Collard. J. Clin. Endocrinol. 28: 169, 1968.

(6) Follis, R.M. Laboratory Invest. 12: 943, 1963.

(7) Goldberg, D.M., R.B. Gondie, and H.A. Ayre. J. Clin. Endocrinol. 28: 41, 1968.

(8) Lindsay, R.M. and P.P. Cohen. Endocrinology 76: 737, 1965.

(9) Lobo, L.C.G., M.M. da Silva, F.B. Hargreaves, and A.M. Couceiro. J. Clin. Endocrinol. 24: 285, 1964.

(10) Lowry, D.M., W.J. Rosebrough, A.L. Farr, and R.J. Randall. J. Biol. Chem. 193: 265, 1951.

(11) Matovinovic, J. and A.L. Vickery. Endocrinology 64: 145, 1959.

(12) Medeiros-Neto, G.A. Rev. Hosp. Clin. 22: 55, 1967.

(13) Medeiros-Neto, G.A., W. Nicolau, J. Kieffer, and R.R. Pieroni. In CURRENT TOPICS IN HORMONE RESEARCH, ed. by Cassano, C. and M. Andreoli, Academic Press, New York, 1965, p. 910-919.

(14) Medeiros-Neto, G.A. and J.B. Stanbury, J. Clin. Endocrinol. 26: 23, 1966.

(15) Michel, R., J.E. Rall, J. Roche, and M. Tubiana. J. Clin. Endocrinol. 24: 352, 1964.

(16) Perelmutter, L., W. Devlin, and N.R. Stephenson. Can. J. Bioch. and Phys. 41: 2493, 1963.

(17) Rall, J.E., J. Robbins, and H. Edelhoch. Ann. N.Y. Acad. Sci. 86: 373, 1960.

(18) Ramagopal, E. and J.B. Stanbury, J. Clin. Endocrinol. 25: 526, 1965.

(19) Robbins, J. and J.E. Rall. Physiol. Rev. 40: 415, 1960.

(20) Robbins, J., J.E. Rall, and R.W. Rawson. J. Clin. Endocrinol. 15: 1315, 1955.

(21) Stanbury, J.B. Arq. Bras. Endocrin. Metab. 15: 145, 1966.

(22) Westra, J.P., R.E. Polly, and N.S. Halmi. Endocrinology 79: 197, 1966.