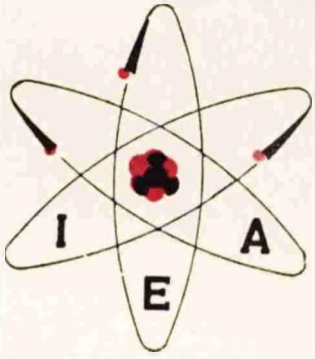

INSOLUBLE IODOPROTEIN IN THYROID NODULES

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RESUMO

Os autores estudam a distribuição do iôdo estável e radioativo em 15 indivíduos portadores de bócio, sendo: a) dez (10) eutireoidianos portadores de nódulo único, cintilogràficamente "frio"; b) dois (2) portadores de bócio hiperplástico multinodular; c) dois (2) com hipotireoidismo por dishormoneogenese; d) um (1) com adenoma folicular. Em nove casos foi encontrada, na glândula, quantidade anormal de iodoproteina particulada (13 a 54,9% do iôdo total contido na glândula). Em oito nódulos encontrou-se proporção anormalmente elevada de iodoproteina particulada, contràriamente ao verificado no tecido paranodular contralateral, no qual a distribuição de iodoproteinas pôde ser considerada normal. A iodoproteina particulada, provàvelmente, difere da tireoglobulina uma vez que apresenta atividade, por gramas de proteina, superior à encontrada na tireoglobulina e por ser mais elevada a relação iodotirosinas/iodotironinas, após hidrólise com pancreatina. A iodoproteina particulada não reage com anti-tireoglobulina humana em placas de agar e pôde ser separada da tireoglobulina em coluna de gel (Sephadex G-200). Os achados sugerem que o acúmulo de material iodoprotéico insolúvel representa uma via metabólica anômala para o iôdo intraglândular, constituindo um fator altamente significativo de perda contínua do halógeno.

RÉSUMÉ

La répartition de l'iode radioactif et stable a été etudiée sur 15 sujets atteints de goître, à savoir: a) dix (10) euthyroidiens avec un nodule froid unique; b) deux (2) avec une thyroide hyperplastique plurinodulaire; c) deux (2) avec hypothyroidisme conséquent à des troubles de l'hormonogénèse; d) un (1) avec adenocarcinone. Une quantité anormale d'iodeproteine sous forme de particules non solubles (13 à 54,9% de l'iode total contenu dans la glande) a été trouvée sur neuf sujets. Sur 8 sujets le nodule présentait une très importante proportion d'iodeproteine sous forme de particules; par contre le tissu glandulaire autour du nodule, consideré comme normal, présentait une répartition normale d'iode et de protéine. L'iodeproteine en particules diffère probablement de la thyroglobuline car le rapport iodethyrosines/iodethyronines est plus élevé et son activité, par gramme de protéine, est plus importante après hydrolise avec de la pancréatine. Elle ne réagit pas à l'action de l'anti-thyroglobuline humaine en plaque de agar et peut être separée de la thyroglobuline (filtration en gel Sephadex G-200). Ces résultats nous amenent à penser que l'accumulation de matériel iodeproteique insoluble présente une voie métabolique anormale pour l'iode intraglandulaire, constituant un facteur hautement significatif de pertes continues d'halogènes.

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Insoluble Iodoprotein in Thyroid Nodules

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Until recently it was believed that thyroglobulin constitutes the only iodoprotein of the thyroid gland. Several recent reports (1-7), however, have suggested the presence of two or more additional thyroid iodoproteins. One group seems to be more soluble than thyroglobulin in phosphate buffer, have a slower sedimentation rate in the ultracentrifuge and have electrophoretic and immunological reactions similar to those of serum albumin. The other iodoprotein is insoluble in aqueous solvents, appears to be associated with subcellular particles and has been called P-1 iodoprotein (2).

Abnormal particulate iodoprotein was first detected in transplantable rat thyroid tumors (4), but has also been identified in congenital goiter (1), in human follicular thyroid carcinoma (2), and in hyperplastic thyroid glands of congenital hypothyroidism (6).

The present report will describe the distribution of ^{131}I, ^{127}I and protein in thyroid nodules homogenates and fractions, as well as in paranodular tissue, of 15 goitrous patients and three normal controls. A large amount of iodine was found to be present in a particulate insoluble iodoprotein in 9 of these 15 goitrous patients.

MATERIAL AND METHODS

Fifteen goitrous patients, 10 females and 5 males, varying between 19 and 45 years of age, received a tracer dose of ^{131}I (200-700 μc) 2-13 days (mean: 6.5 days) before surgery. Three normal controls, 2 males and 1 female, also had their thyroid glands labeled with radioiodine (4-12 days) before surgery for parathyroid disease.

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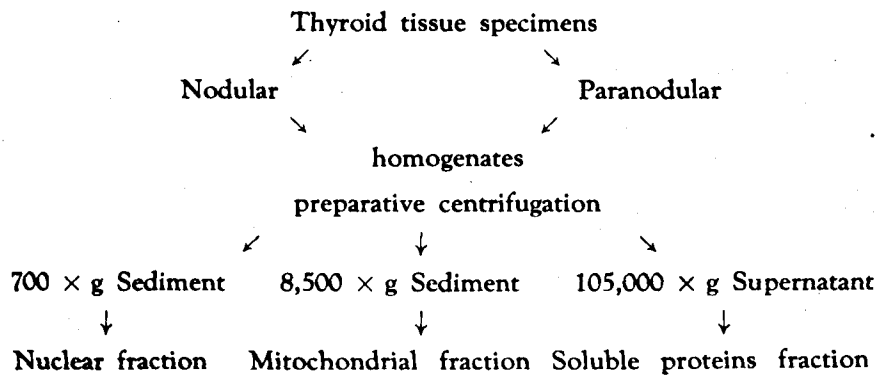
Of the goitrous patients 10 were euthyroid and have a single cold nodule, as detected by thyroid scan. Three others were euthyroid, 2 presenting a large, hyperplastic, multinodular goiter; an adenocarcinoma of thyroid was present at surgery in the other patient. The remaining 2 patients were moderately hypothyroid and were classified as belonging to the congenital goiter group with dyshormonogenesis: one a deaf-mute, with a positive perchlorate test and the other with a questionable coupling defect (Table 1).

TABLE 1

No. Cases	Clinical diagnosis	Type of Goiter	Particulate iodoprotein
10	Euthyroid	Single Cold Nodule	4
2	Euthyroid	Hyperplastic	2
1	Euthyroid	Adenocarcinoma	1
2	Hypothyroid Dyshormonogenesis	Hyperplastic with Cold Areas	2
3	Normal	Normal Thyroid Tissue	None

At surgery, besides the nodule, either a paranodular or a heterolateral fragment of what presumably seemed to be normal thyroid tissue was also removed. Both tissues were immediately collected in ice, rinsed free of blood, and homogenized, separately, at 4°C, in 0.25M sucrose, in an all glass motor-driven homogenizer. The homogenate was then submitted to preparative centrifugation, as follows:



Three main fractions were thus obtained from nodular and paranodular tissues. The iodine content (^{131}I and ^{127}I) and protein of each nodular fraction were determined and compared with paranodular and normal thyroid tissue. Protein was determined by Lowry *et al.* method (8) and stable iodine by a modified Zak's method. Pancreatin hydrolysis and chromatography were done according to Stanbury *et al.* methods (9). Others methods will be described in the text or with the tables.

Results

1. *Distribution of ^{131}I, ^{127}I and protein in nodules and paranodular tissue*: In 9 patients, particulate iodoprotein was found to be present in the 700 × g sediment from the nodular homogenate with a higher concentration than that found in the same fraction from paranodular or from thyroid of normal controls. This group of patients included 2 with hyperplastic thyroid glands, 2 with congenital goiter, and one with adenocarcinoma. Four out of the 10 patients with single cold nodules also presented high particulate iodoprotein in the nodule (Table 1).

In eight patients it was possible to compare the relative distribution of iodine and protein in the nodules to that present in the paranodular tissue (Table 2). In six patients more particulate iodoprotein was found in the nodule than in the paranodular tissue; however a high level of insol-

TABLE 2. DISTRIBUTION OF ^{131}I, ^{127}I AND PROTEIN EXPRESSED AS PERCENT OF TOTAL AMOUNT IN THE HOMOGENATE IN THE PARTICULATE FRACTION (700 × g SEDIMENT) OF NODULAR (N) AND PARANODULAR (PN) THYROID TISSUE

	Normal	CZ		CRN		JMB		FPS		JC		NGP		ERC		GR	
	Controls*	N	PN	N	PN	N	PN	N	PN	N	PN	N	PN	N	PN	N	PN
^{131}I	4.2	17.3	7.6	17.4	2.2	30.2	10.1	6.4	9.0	36.4	13.7	15.2	3.4	26.9	6.4	9.6	11.7
^{127}I	5.8	13.4	5.6	16.9	5.2	26.2	9.7	28.6	7.6	25.5	5.5	18.9	5.9	54.9	26.4	16.9	18.9
Protein	16.2	38.2	24.4	36.0	13.5	46.8	23.7	25.5	8.8	33.2	15.9	8.75	7.97	63.2	59.7	36.0	13.5

* Mean of 3 individuals.

uble iodoprotein was present in both thyroid tissues in 2 patients, one with Pendred's syndrome and the other with a multinodular goiter.

Expressing the iodine content of the particulate fraction per gram of thyroid tissue processed, it was observed that the amount of particu-

late iodoprotein was 1.2 to 11.7 times higher in the nodular than in the paranodular tissue (Table 3).

With the exception of the 2 hyperplastic thyroid glands, iodine specific activity per gram of particulate protein (700 ×g sediment) was lower than that of the soluble iodinated proteins. (105,000 ×g supernatant).

TABLE 3. RATIOS BETWEEN NODULAR AND PARANODULAR PARTICULATE IODINE (^{131}I AND ^{127}I) EXPRESSED PER GRAM OF THYROID TISSUE PROCESSED

Particulate iodine	ERC	GR	CFN	JMB	JC	FPG	MGP
$\frac{\text{Nodular } ^{131}I}{\text{Paranodular } ^{131}I}$	3.6	1.8	7.7	2.5	3.6	1.6	1.5
$\frac{\text{Nodular } ^{127}I}{\text{Paranodular } ^{127}I}$	11.7	2.4	3.6	4.8	5.3	1.2	1.7

This aspect is clearly shown by comparing soluble insoluble ratios of ^{127}I specific activities (Table 4). Note that the exception is represented by the 2 hyperplastic thyroid glands, in which more iodine is incorporated in the abnormal particulate iodoprotein than in thyroglobulin.

2. *Pathological findings*: an attempt was made to find a possible correlation between the pathological findings and the iodine and protein distribution present in the 700 ×g sediment expressed as percent of the total in the homogenate.

In Table 5 is listed the pathological diagnosis and the respective distribution of iodine and protein in the 700 ×g sediment, according to the increasing amounts of particulate iodoprotein.

As can be observed less differentiated tissues like adenomas and adenocarcinomas have relative amounts of particulate iodine higher than other pathological tissues examined. The higher concentration of particulate iodoprotein was found in the fetal adenoma where 54.9 % of the total iodine was particulate. This seems to be supported by the fact that in only 4 of the 10 adenomatous colloid goiters studied, particulate iodoprotein was found in abnormal proportions, whereas in all adenomas examined it was present in large amounts.

It can be noted that some discrepancies related to ^{131}I and ^{127}I relative distributions occurred. It is conceivable that labeling thyroid glands at different times before surgery has an important role in this situation.

TABLE 4. SPECIFIC ACTIVITY OF ^{131}I AND ^{127}I PER GRAM OF PROTEIN IN PARTICULATE FRACTION (700 × g SEDIMENT) AS COMPARED WITH THE SOLUBLE FRACTION (105,000 × g SUPERNATANT) OF HOMOGENATES OF THYROID NODULAR TISSUE

Name	CZ		ERC		CRN		GR		IB		FA		FPS		NGP		JC	
Fraction	700 × g	10^5 × g	700 × g	10^5 × g	700 × g	10^5 × g	700 × g	10^5 × g	700 × g	10^5 × g	700 × g	10^5 × g	700 × g	10^5 × g	700 × g	10^5 × g	700 × g	10^5 × g
^{131}I (%D/g)	0.034	0.097	2.1	5.9	2.35	6.5	0.44	1.6	1.92	3.93	4.3	7.3	2.7	1.4	2.7	1.5	0.09	0.085
^{127}I (mg/g)	0.97	9.9	1.58	2.03	1.10	3.10	0.40	0.71	19.5	46.3	0.71	2.0	0.77	0.68	0.9	0.37	0.012	0.018
Soluble ^{127}I / Insoluble ^{127}I	10.3		1.6		2.8		1.8		2.4		2.8		0.9		0.4		1.5	

TABLE 5. DISTRIBUTION OF ^{131}I, ^{127}I AND PROTEIN IN THE 700 × g SEDIMENT AS PERCENTAGE OF TOTAL IN THE HOMOGENATE OF PATHOLOGICAL THYROID TISSUES

No.	Pathologic diagnosis	% of the total in the 700 × g sediment		
		^{131}I	^{127}I	Protein
1	Adenomatous colloid goiter	17.3	13.4	38.2
2	Hyperplastic colloid goiter	22.7	16.2	32.4
3	Adenomatous colloid goiter	17.4	16.9	36.0
4	Adenomatous colloid goiter	11.7	18.9	23.2
5	Adenomatous hyperplastic colloid goiter	15.2	18.9	8.7
6	Follicular adenocarcinoma	36.4	25.5	33.2
7	Papilliferous adenoma	30.2	26.2	46.8
8	Papilliferous adenoma	6.4	28.6	25.5
9	Fetal adenoma	26.9	54.9	59.7

3. *Pancreatin hydrolisis*: samples of both particulate iodoprotein and samples from the 105,000 × g soluble proteins were submitted to pancreatin digestion for 48 hours. The results are shown in Table 6. Particulate iodoprotein seems to have similar amounts of iodotyrosines as compared with soluble proteins, but the latter presented a definite higher proportion of iodothyronines. Accordingly, the iodotyrosines to iodothyronines ratio was 9.01 in the particulate iodoprotein compared with 1.97 in the soluble proteins.

4. *Solubilization and characterization of the particulate iodoprotein*: Following exposure of the particulate iodoprotein to 0.4% of crystal-

TABLE 6. PANCREATIN DIGESTION AND CHROMATOGRAPHY (B.-AC.-A., DESCENDING) OF INSOLUBLE (700 × g SEDIMENT) AND SOLUBLE PROTEINS (105,000 × g SUPERNATANT) OF THYROID TISSUE FROM PATIENTS WITH HIGH PARTICULATE IODOPROTEIN. RESULTS EXPRESSED AS % OF TOTAL RADIOACTIVITY APPLIED TO THE PAPER

Patient	700 × g sediment					105,000 supernatant				
	Origin	I	MIT	DIT	T_4T_3	Origin	I	MIT	DIT	T_4T_3
ERC	6.3	45.8	16.9	24.7	4.4	7.2	43.8	9.1	18.3	15.6
CRN	17.5	18.1	32.4	17.4	6.9	12.1	21.6	26.4	13.2	18.1
FA	18.9	18.9	17.0	29.7	7.6	9.3	16.7	14.5	26.9	24.5
NGP	1.2	4.2	8.8	78.5	6.8	0.6	7.4	6.4	57.0	27.6
Iodotyrosines / Iodothyronines			9.01					1.97		

line trypsin for 10-15 minutes at room temperature, 85% of the radioactivity was found in the 105,000 × g supernatant. Soybean trypsin inhibitor was added at the end of the 10-15 minutes at the same concentration. The solubilized material was not dialyzable, and it was almost completely precipitable with 20% TCA acid.

The solubilized material failed to precipitate with goat anti-human thyroglobulin and rabbit anti-human albumin * in agar plates, following the Ouchterlony agar diffusion technique.

The soluble proteins (105,000 × g supernatant) from the same thyroid gland did react with anti-thyroglobulin (Fig. 1).

By gel-filtration, in a Sephadex G-200 column of 2.5 × 35 cm and using normal saline as eluent, it was possible to separate the solubilized material from thyroglobulin. The solubilized iodoprotein was eluted after thyroglobulin, showing a wider peak (Fig. 2).

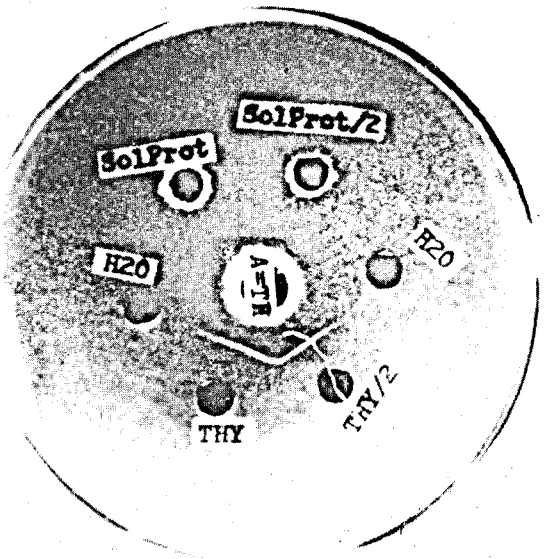


Fig. 1. Ouchterlony agar plate. A definite zone of precipitation is shown with human purified thyroglobulin. The solubilized iodoprotein did not react with the goat anti-human thyroglobulin.

* Anti-proteins obtained from Nutritional Biochemical Co., USA.

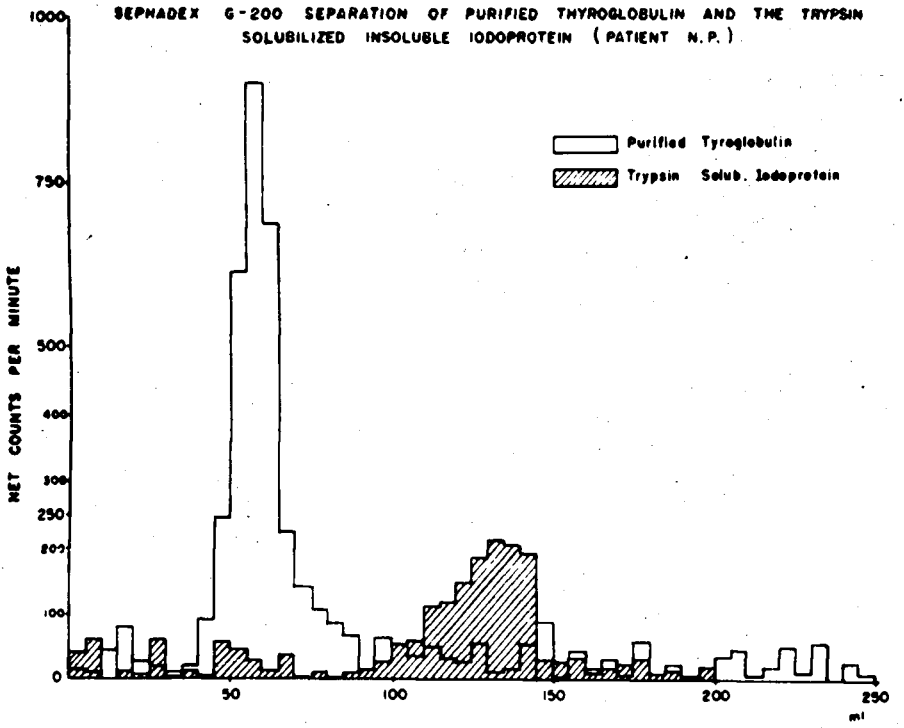


Fig. 2. Separation of the trypsin solubilized iodoprotein with the use of gel filtration (12). Note that thyroglobulin was eluted with the first 60 ml of effluent and the solubilized material came after 100 ml of normal saline.

Discussion

Practically all of the thyroid iodine is in soluble form, extractable with thyroglobulin. Usually less than 5% of the total amount sediment with cellular particulate.

Particulate and soluble proteins other than thyroglobulin have been found to be elevated in thyroid pathological tissue (1, 2, 6, 7). The present report, which shows an increased concentration of particulate iodoprotein in 9 out of 15 goitrous patients, confirms previous work. It is noteworthy to mention, however, that the present results seem to demonstrate that nodular thyroid tissue has higher amounts of particulate iodoproteins than the paranodular or normal thyroid tissue. Although no

definite conclusion could be obtained from this group of patients it seems that less differentiated tissues, like adenomas and adenocarcinomas, frequently have higher concentration of particulate iodoprotein. The presence of this abnormal iodoprotein in other pathological thyroid tissue examined suggests that loss of subcellular or follicular organization and high degree of hyperplasia may result in iodination of particulate protein.

This insoluble iodoprotein seems to be different from thyroglobulin. It did not react with goat anti-human thyroglobulin whereas a definite zone of precipitation was obtained with the soluble proteins of the same patient. Submitted to pancreatin digestion, this particulate iodoprotein presented a higher iodotyrosines iodothyronines ratio than thyroglobulin and it could be separated from thyroglobulin by gel-filtration. Similar results were reported by Smith *et al.* (11) who found that the trypsin solubilized material scarcely moves in a centrifugal field which completely sedimented thyroglobulin and, furthermore, that its amino acid composition differed from that of thyroglobulin. The present report suggests that this insoluble material may represent an abnormal pathway for iodine. It was found only in pathological tissues from thyroid nodules and not in the paranodular or in normal thyroid glands. It seems to be more frequently found and to be present in higher proportions in less differentiated pathological tissues like adenomas and adenocarcinomas. There is some evidence that it is different from thyroglobulin and there is no reason to suspect that this particulate iodoprotein is metabolically available.

However it is not clear from the present work whether the large amount of iodine that is present as insoluble iodoprotein is etiologically significant or not. A substantial subtraction of iodine from normal hormonogenesis seems to be present in some cases. In the 2 hyperplastic thyroid glands more iodine was present per gram of particulate protein than per gram of thyroglobulin and in one case as much as 54.9% of iodine was particulate. Furthermore since this protein contains less thyroxine than does thyroglobulin, its iodination represents a loss to the organism of a certain amount of thyroxine. The final result of this protein iodination disturbance could be hypothyroxinogenesis and consequent hyperplasia of thyroid tissue. Further investigations in larger groups of the population will be carried on to test if this functional attitude, characterized by sequestration of a significant amount of the available iodine from the normal pool, would be a consequence or a determinant agent in the goiter mechanism.

Abs

Conclusions—

The distribution of labeled and stable iodine was studied in 15 goitrous patients, of which 10 euthyroid with single cold nodules, 2 with hyperplastic, multinodular goiter, 2 hypothyroid with congenital goiter and one with thyroid adenocarcinoma. In 9 patients a variable (13.0-54.9% of total iodine) but definitely abnormal amount of iodine was present as an iodinated insoluble particulate iodoprotein. In 8 cases nodular tissue has a very high proportion of particulate iodoprotein whereas presumably normal paranodular tissue exhibits normal relative distribution of iodine and protein.

The particulate iodoprotein seems to be different from thyroglobulin for it has a higher iodotyrosines/iodothyronines ratio, has a lower iodine specific activity per gram of protein, does not react with goat anti-human thyroglobulin in agar plates and is eluted in front of thyroglobulin in a Sephadex G-200 column. The finding of this abnormal insoluble iodoprotein in less differentiated tissues, such as adenomas and adenocarcinomas, as well as in thyroid tissue with loss of subcellular or follicular organization may suggest that this iodoprotein represents an abnormal pathway of iodination in these thyroid tissues.

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