Alterations of Iodine Metabolism in Asymptomatic Thyroiditis

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Iodine metabolism has been studied in patients affected with asymptomatic auto-immune thyroiditis, as indicated by a significant titre of serum thyroglobulin antibodies, in the absence of overt thyroid disease. When matched with adequate controls, these subjects fail to show significant changes of ^{131}I thyroid uptake, which was, however, markedly depressed by triiodothyronine and potassium iodide. The plasma $PB^{125}I$ was increased. After a single injection of TSH, the iodine trapping was normally enhanced, but after 24 hours, the radioactive content of the thyroid was lower than in control subjects. It was shown that this phenomenon was related to the discharge from the thyroid of PBI with abnormally high specific activity. Kinetic studies indicated a 2 to 1 reduction of the thyroid exchangeable organic iodine, as well as of the thyroid ^{131}I half life. The fraction of plasma PBI, extracted by butanol, was 73 ± 15 per cent in the thyroiditis patients, contrasting with a value of 93 ± 6 per cent in the controls. The iodine metabolism anomalies observed in asymptomatic thyroiditis may be explained by the reduction of the exchangeable iodine in the thyroid and its increased turnover. However, the interference of an underlying congenital metabolic defect cannot be excluded. (Metabolism 17: No. 12, December, 1064–1072, 1968)

SEROLOGICAL and anatomo-pathological studies have demonstrated the existence of a variant of auto-immune thyroiditis, for which the term "asymptomatic atrophic thyroiditis" has been proposed.[1,2]

In contrast to the characteristics of Hashimoto's lymphoadenoid goiter, the female/male sex ratio is 3 to 1, the age peak lies after 50 years and there is no enlargement of the thyroid gland. Focal or diffuse asymptomatic thyroiditis has been diagnosed in 16 per cent of the female and in 15 per cent of the male patients admitted to this hospital for various medical disorders. Extensive serological pathological confrontation have shown that lesions are detectable when thyroglobulin antibodies reach a title of at least 1/125, which is measured by the Boyden tanned red cell test. In other series of patients with asymptomatic thyroiditis, the complement fixation test has been used.

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Received for publication March 29, 1968.

Supported by the "Fonds de la Recherche Scientifique Médicale," by the "Fondation Tournay Solvay," Belgium, and by the contract Euratom/Universities of Pisa and Brussels, BIAC 026-63-4.

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Recent findings[6] have confirmed the concept[1,5,6] that this condition is the usual forerunner of clinical myxoedema. Moreover, although in most patients the condition is non-progressive and remains symptomless, biochemical signs of latent hypothyroidism may be detected. Evidence of increased serum TSH levels together with plasma PBI and high cholesterol suggests that in these patients a state of euthyroidism is only maintained thanks to increased thyroid stimulation by the pituitary.

So far, only two studies[4,8] have investigated iodine metabolism in subjects affected with this condition. Both have shown a marked reduction in the exchangeable thyroid iodine. According to Buchanan et al.,[4] an increased fraction of plasma PB^{131}I corresponds to the presence of abnormal iodoproteins. These authors consider that the above-mentioned alterations, which are similar to those found in Hashimoto's thyroiditis, substantiate the evidence of defective iodine utilization. However, since increased TSH secretion has been demonstrated in a number of cases, the question arises whether the observed anomalies are indeed signs of some basic inborn abnormality or whether they are the result of a reduced iodine pool.

The purpose of the present study is to reevaluate iodine metabolism in asymptomatic autoimmune thyroiditis, with special reference to: (1) the responsiveness of the thyroid to increased TSH stimulation and (2) the detection of abnormal iodoproteins in the serum.

Patients Studied and Methods

The patients investigated were adults of both sexes, admitted to the department of medicine for various internal diseases. Subjects who had known thyroid disorders, who were acutely ill, who had been given iodine or who were affected with a malignant growth were excluded from the study.

For each investigation, approximately equal numbers of subjects were selected from patients without detectable thyroglobulin antibodies in the serum and from patients with circulating thyroglobulin antibodies at a title of at least 1/125.

Standard procedures included the measurement of thyroid radioiodine uptake, 6 and 24 hours after oral administration of 3 μC of ^{131}I, and the measurement of the plasma PB^{127}I and PB^{125}I, the latter being estimated 24 hours after the oral administration of 50 μC of ^{125}I.

Inhibition and stimulation tests. Inhibition of thyroid uptake was achieved by using the Werner method of daily administration of 75 μg. of T3 for six days.[9] Inhibition was also obtained by giving 2 mg. of potassium iodide as indicated by Boyle et al.[10] Both tests were performed two weeks after a conventional radioiodine uptake test; the difference between the two results was expressed as a percentage of the initial test uptake value.

For the TSH stimulation test, a single intramuscular injection of 10 units (U.S.P.) of TSH (Ambinon) was given 24 hours before the administration of a second radioiodine tracer; the difference was expressed as a percentage of the initial uptake value.

The perchlorate discharge test was performed according to the method of Stewart and Murray.[11] The thyroid radioiodine content at 60, 90 and 120 minutes after the intravenous injection of 10–15 μC of ^{131}I was derived from the measured neck radioactivity; potassium perchlorate (400 mg.) was administered orally at 63 minutes. The test was considered positive when the iodine content was lower at 130 than at 60 minutes.[11]

Iodine kinetics were studied in 14 control subjects (11 men and 3 women aged from 20 to 67 years (mean age: 48) and in 12 patients with circulating thyroglobulin antibodies (2 men and 10 women aged from 36 to 70 years; mean age: 48). The thyroid uptake was measured 2, 4, 6 and 24 hours and 4 and 7 days after the simultaneous oral administration

of 10 μC ^{131}I and 50 μC ^{125}I. The plasma $PB^{125}I$ was measured 24 hours, 4 days and 7 days after the administration of the radioiodine. Between the 4th and 7th days the daily excretion of ^{125}I and ^{127}I was measured in the urine. The following parameters were derived from the data:

The thyroidal clearance rate of iodide before and after the administration of TSH. This rate was calculated from ^{131}I uptake measurements using the formula proposed by Kutka and Licko.[12]

The "apparent release rate" of thyroidal iodine (K'_4) using the formula proposed by Stanbury et al.[13] $K'_4 = \frac{E^*}{Q^*}$ where E^* and Q^* are respectively the daily fraction of the dose excreted in the urine and that remaining in the gland at the 7th day after the administration of radioiodine. The half-life of thyroid radioiodine is estimated according to the formula $0.693/K'_4$

The specific activity of the iodine discharged after a single TSH stimulation expressed in per cent of the dose per μg. of iodine, was calculated from the formula:

$$\frac{PB^{125}I \text{ (after TSH)} - PB^{125}I \text{ (before TSH)}}{PB^{127}I \text{ (after TSH)} - PB^{127}I \text{ (before TSH)}}$$

The exchangeable organic iodine (Qg) in the thyroid was calculated using the method described by Berson and Yalow[14] modified by Ermans and Camus.[15] Qg was derived from the quotient of the thyroid radioiodine content at the 7th day (Qg^*) divided by the specific activity of PBI discharged in the circulation 24 hours after the administration of TSH

$$Qg = \frac{QC^*}{\text{SA of discharged PBI}}$$

Techniques

The radioiodine uptakes in the thyroid gland were measured at a distance of 30 cm. from the neck by means of a scintillation counter connected with an analysor and a scaler (Philips, Belgium) using a conical collimator (Philips type XL 6004).

Labeled plasma organic iodine was measured after discarding iodide by means of an anion-exchange resin (Iobeads inorganic iodine resin AR 150-65 Technicon) using 100 mg. of resin per ml. of serum. The mixture was shaken for 3 minutes at room temperature; 1 ml. of supernatant was then measured in a dual channel well-type scintillation counter. Recovery of labeled PBI was in the range of 97 per cent; 100 per cent of radioiodide was eliminated even in the presence of stable iodide of about 50 μg./100 ml.

The chemical assay of plasma $PB^{127}I$ was performed in accordance with the method of Barker et al.[16] adapted by Ermans et al.[17] to the Auto-analyzer Technicon (Technicon Instrument Corporation Chaunçay, New York). Measurements of stable and radioactive PBI were made in duplicate. All measurements concerning the same patients were made at the same time.

For plasma $PB^{127}I$, the accuracy of the method was 0.1 μg. per 100 ml. Urinary ^{127}I was measured using the same technique, care being taken to heat the urine samples to 37° C in order to avoid crystallization.

Estimation of Butanol Extractable Iodine in Serum

An original method has been evolved for estimating butanol extractable iodine (BEI) by a simplified technique adapted to the Autotechnicon Analyzer. The serum was first treated with Iobeads resin, as described above, in order to eliminate iodide. One ml. of serum was then brought to pH 3 with 0.1 M H_2SO_4. and extracted 3 times with 2 ml. n butanol saturated with 10 per cent HCl and twice with pure butanol.

The pooled butanolic extracts were evaporated under vacuum at 40° C. The dry residue was dissolved with 1 ml. of 0.1 M NaOH and put into sample cups. Testing for iodine was carried out in the same way as described for $PB^{127}I$. Amounts of iodine recovered from butanolic extracts were expressed in μg. per 100 ml. of serum and as percentages of the

Table 1.

	Units	Patients with Negative TGA Test	Patients with Positive TGA Test
I^{131} Thyroid Uptake (24 hrs.)	% dose	46 ± 16* (35)†	46 ± 19 (92)
Plasma PBI^{127}	μg. %	5.9 ± 1.4 (54)	5.6 ± 1.7 (79)
Plasma PBI^{125} (24 hrs.)	% dose/I.	.06 ± .04 (27)	.10 ± .06 (31)

*Standard deviation.
†Number of subjects.

corresponding $PB^{127}I$ values. Recovery of various iodinated aminoacids was checked after dissolving known amounts of T4, T3, MIT and DIT in a 1 per cent serum albumine solution. Recovery percentage was 95 ± 4 for T4 and 93 ± 2 for T3, respectively, for concentrations of 5.7 and 4.3 μg. per 100 ml. For MIT and DIT, at a concentration of about 12 μg. per 100 ml., recovery was less than 5 per cent.

Reproducibility of the method was checked for 10 samples of the same pool of serum and was 2 per cent. Extreme values of $BE^{127}I$ obtained by this method in 18 normal subjects were 3.5 and 6.9 μg. per 100 ml. of plasma. These data agree with the results reported by Dreyer and Mann[18] and by Benotti and Quinaux.[19]

Results

Table 1 shows the results of the routine tests performed on patients with circulating thyroglobulin antibodies and on the control patients. The mean values of radioiodine thyroid uptake and plasma $PB^{127}I$ over 24 hours are similar in both groups, although amongst the positive cases a large number of subjects have values below 4 μg./100 ml. ($\chi 2 = p < 0.05$). The values of 24 hours plasma $PB^{125}I$ and $PB^{131}I$ expressed as per cent of dose/I., are significantly higher in the patients affected with thyroiditis ($p < 0.05$).

Results of inhibition and stimulation tests are reported in Table 2.

The administration of T3 in thyroiditis patients produces a marked drop in the thyroid uptake of ^{131}I, equal to the drop observed in normal subjects.[9]

In these patients ingestion of potassium iodide slows down the initial radioiodine uptake almost twice as much as in the controls ($p < 0.05$). The perchlorate test induces an abnormal discharge in 5 out of the 9 patients studied. On the other hand, the increase of radioiodine uptake, normally observed after TSH administration, is strikingly reduced; in most cases, the values obtained at the 24th hour are lower after TSH administration than before the test ($P < 0.05$).

Table 2.—*Thyroid ^{131}I Uptake: Inhibition or Stimulation Tests Expressed as Per Cent of Control 24-hr. Value*

	Patients with Negative TGA Test	Patients with Positive TGA Test
Triiodothyronine (75 μg. for 6 days)	−50*	−70 ± 22 (8)†
IK (2 mg., single oral dose)	−32 ± 8 (5)	−64 ± 20 (6)
TSH (15 USP units, I.M.)	+53 ± 64 (7)	+28 ± 48 (17)

*Werner[9].
†Number of cases.

Table 3.

Parameters	Units	Patients (14) with Negative TGA Test	Patients (12) with Positive TGA Test	Significance of Difference
^{131}I thyroid uptake (24 hrs.)	% dose	45 ± 11	44 ± 16	P > .50
Plasma $PB^{127}I$	μg./100 ml.	6.1 ± 1.6	5.4 ± 1.9	P > .20
Urinary ^{127}I excretion	μg./24 hrs.	54 ± 25	45 ± 17	P > .40
Plasma $PB^{125}I$ (24 hrs.)	% dose./I.	.02 ± .01	.10 ± .10	P < .025
" (4 days)		.10 ± .04	.27 ± .23	P < .05
" (7 days)		.14 ± .03	.38 ± .24	P < .05
^{125}I Urinary excretion from 4th to 7th days	% dose/ 24 hrs.	.170 ± .070	.460 ± .270	P < .10*
"Apparent" release rate of thyroidal ^{131}I	%/24 hrs.	.46 ± .25	1.12 ± .93	P < .025*
Half life of thyroidal radioiodine	days	209 ± 131	106 ± 66	P < .025
Exchangeable thyroidal organic iodine	mg.	8.1 ± 4.6	3.3 ± 3.5	P < .050
S.A. of discharged PBI 24 hours after TSH	$.10^{-3}$ % dose/μg.	6.5 ± 4.5	26.6 ± 21.4	P < .025

*Estimations only obtained for six patients of each group.

Kinetics of Iodine Metabolism

The kinetics of iodine metabolism were studied in more detail in 26 subjects: 12 patients with a significant titre of circulating thyroglobulin antibodies and 14 controls. The results of the measurements of radioiodine uptake, of $PB^{127}I$ and of the labeled PBI, at 24 hours, 4 and 7 days after the administration, are indicated in Table 3. These values are comparable to those reported in Table 1 for the larger groups. The relative increase of the plasma protein-bound radioiodine observed in the thyroiditis patients 24 hours after ^{131}I (Table 1) is found again between 4 and 7 days after the administration of ^{125}I (Fig. 1). Parallel to the plasma PBI radioiodine is the urinary excretion of radioactive iodine between the 4th and the 7th days (Table 3). In the thyroiditis patients, the half-life of the intrathyroidal radioiodine is reduced to half its normal value. The exchangeable thyroidal organic iodine pool is 8.1 mg. in the normal group and is reduced to 3.3 mg. in the patients with circulating thyroglobulin antibodies.

Study of Response to Exogenous TSH

The thyroid iodide clearance rate per hour, measured before TSH administration, is 10.6 ± 3.4 in the control subjects and 9.6 ± 13.4 in the thyroiditis patients. Twenty-four hours after TSH administration, this coefficient rises in the first group to 26 ± 16 and in the latter group to 26 ± 15. Whereas in the thyroiditis patients the increase in plasma radioactive PBI expressed as per cent of dose/L. is definitely larger than in the normal subjects, the increase in $PB^{127}I$ is smaller than in the controls. This is related to the release by the thyroid glands of thyroiditis patients of organic iodine with greatly increased specific activity: 26.6 in the thyroiditis patients and 6.5 10^{-3} of dose/μg. iodine, in the controls (Table 3 and Fig. 1).

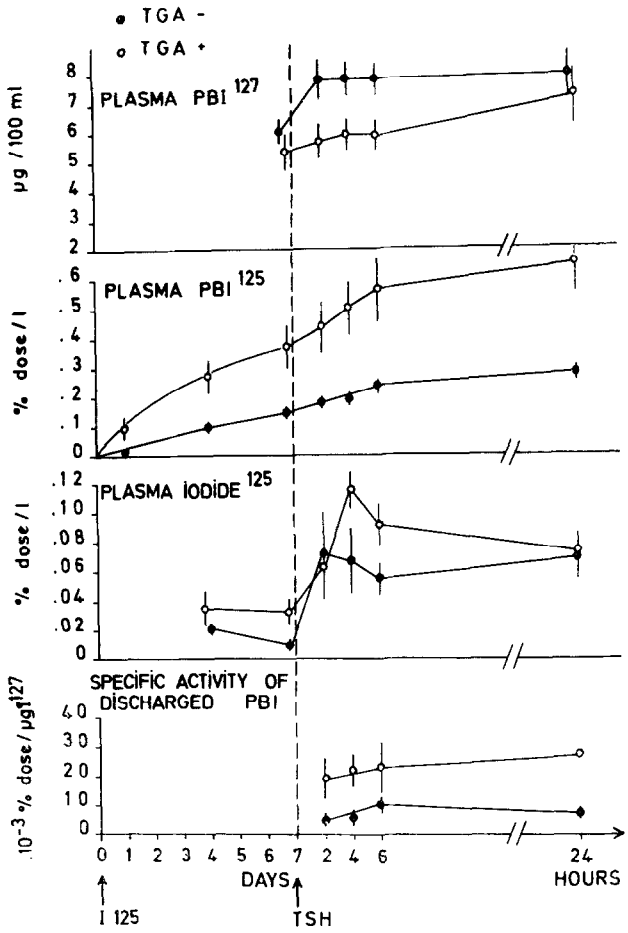

Fig. 1.—Changes in plasma $PB^{127}I$, $PB^{125}I$, ^{125}iodide, and specific activity of discharge PBI induced by single injection of TSH, seven days after ^{125}I administration. Vertical bars indicate standard deviations of means.

Estimation of Butanol Extractable Iodine

Stable butanol extractable iodine was measured in a separate group of patients comprising 18 controls (mean plasma $PB^{127}I$: 6.3 ± 1.4 µg. per 100 ml.) and 17 subjects with circulating TGA (mean plasma $PB^{127}I$: 7.0 ± 2.3 µg./100 ml.). The mean values of plasma $BE^{127}I$ in the two groups were respectively 5.6 ± 1.5 and 5.1 ± 1.8 µg./100 ml. The fraction of the plasma $PB^{127}I$ extracted by butanol was 73 ± 15 per cent in the thyroiditis patients, significantly lower ($P < .05$) than that found in the controls (93 ± 6 per cent).

As indicated in Fig. 2, the butanol extractable fraction rises with the increased values of plasma $PB^{127}I$; no such relationship is observed in the controls.

Discussion

This study confirms that subjects who are free from clinical thyroid disease but in whom latent thyroiditis is detected by the presence in the serum of circulating antithyroid auto-antibodies may have marked anomalies in their iodine metabolism. These alterations may not be apparent in the classical

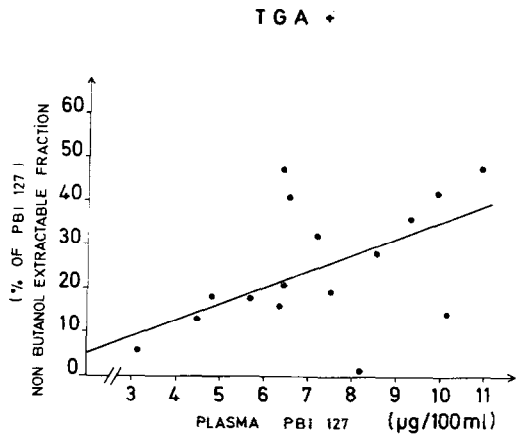

Fig. 2.—Relationship between NBEI and PBI in plasma of 15 patients with asymptomatic thyroiditis.

routine tests such as radioiodine thyroid uptake or plasma $PB^{127}I$. In the present series of patients with circulating TGA, an abnormally high plasma $PB^{131}I$ level is often observed although the increase is smaller than that found by Buchanan et al.[4] in their patients showing positive results to the complement fixation test.

The major alteration of iodine metabolism which is present in this condition is a marked reduction of the exchangeable iodine pool. Measurements indicating that this reduction is about 50 per cent are confirmed by the findings that the half-life of the intrathyroidal organic iodine and the level of plasma labeled PBI are also altered by a 2/1 ratio.

The thyroid gland affected with asymptomatic auto-immune thyroiditis retains its ability trapping more iodine in response to exogenous TSH stimulation. However after TSH administration, the radioiodine content measured at 24 hrs. is usually paradoxically lower than before TSH administration; this is probably due to the rapid discharge of the accumulated iodine. Indeed the response of the thyroid gland is mainly characterized by the mobilization of intrathyroidal organic iodine with a very high specific activity, whereas the actual output of hormone, in terms of stable iodine, is lower than normal. This situation reflects the lesser dilution of the tracer by the limited intrathyroidal iodine and thus appears as a direct consequence of the reduced exchangeable iodine pool.

The findings of circulating butanol insoluble iodoproteins and in some cases, of an abnormal perchlorate discharge test, have already been mentioned by Buchanan et al.[4] and are similar to those described in Hashiomoto's disease.[20] The same disorders have been described as inborn metabolic defects[21,22] in hereditary thyroid diseases. According to Steward and Murray,[11] the perchlorate discharge test appears specific for demonstrating defective binding of iodine by the thyroid gland. Buchanan et al. found the test positive in 12 out of 27 patients with Hashimoto's disease[20] and in 9 out of 28 patients with asymptomatic thyroiditis.[14] The discharge phenomenon induced by the iodine inhibition test, which is also observed in Hashimoto's disease,[23] was found in all our patients. It is reminiscent of the discharge of ^{131}I which occurs after

$KClO_4$ or KSCN administration to patients with faulty organification of iodine. Thus, each alteration in iodine metabolism observed in thyroiditis patients has also been observed in congenital thyroid diseases. The possibility of a slight congenital defect, increasing with age, cannot be ruled out as the underlying cause of the process which leads to asymptomatic thyroiditis.

However, according to recent data from Boyle et al.,[10] all conditions in which the iodine discharge test is positive are characterized by a reduction of the thyroidal iodine pool. Such are the post-thyroidectomy state (non-toxic or toxic goiter) and thyrotoxicosis treated with radioiodine or antithyroid drugs. Stanbury[24] has suggested that the organic binding of iodine may indeed be the factor limiting the rate of thyroid iodine uptake and has postulated that in hyperplastic glands there might be a build-up in the quantity of iodide that can be discharged with perchlorate. Abnormal iodine discharge induced by perchlorate or potassium iodide might thus merely represent non-specific phenomena resulting from thyroid hyperplasia. The same theoretical considerations apply to the presence of non-butanol extractable iodo-proteins in the serum of thyroiditis patients. This theory would be in agreement with the findings of Nilsson and Berne,[25] who point out that abnormal perchlorate reactions in children with auto-immune thyroiditis are more marked in the presence of high plasma $PB^{131}I$ and symptoms suggesting thyroid failure.

Thus far the present studies cannot answer the critical question of whether the observed iodine metabolism alterations are responsible for the thyroid stimulation (and perhaps for the thyroiditis process), or whether they are merely the consequence of this process.

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