

# Diagnostic value of the absolute free thyroxine iodine test

## *Review of 145 cases*

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Several investigators<sup>1-5</sup> have proposed that the thyroid hormone circulates in two parts—a free unbound physiologically active part and a larger protein-bound metabolically inert part. If approximately 5.0  $\mu\text{g}/100\text{ ml}$  of thyroxine iodine is considered 100%, then the free thyroxine is approximately 0.05% of the total amount and is expressed in millimicrograms (nanograms—n). The normal mean level is approximately 3.0 n/100 ml.

Several methods<sup>6-10</sup> of measuring the amount of free thyroxine have been proposed, but the few large series<sup>7, 9, 11, 12</sup> of patients who have been studied to determine the clinical usefulness and diagnostic accuracy of the test have depended upon the protein-bound iodine (PBI) and the percentage of free thyroxine iodine and the product obtained thereof. Since March 1966, we have measured the absolute free thyroxine iodine based on total serum thyroxine iodine and have tested more than 1,000 patients who had a variety of thyroid and suspected thyroid diseases.† Measurement is similar to the free thyroxine index reported by others,<sup>13-15</sup> and our conclusions agree with theirs.

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Materials and methods

Records of 102 patients were used for the main body of this study, and tests on an additional 43 are included

in the scatter graphs (Figs. 1-3). Each diagnosis was based on clinical appraisal, standard tests and, when necessary, on results of therapy. Many

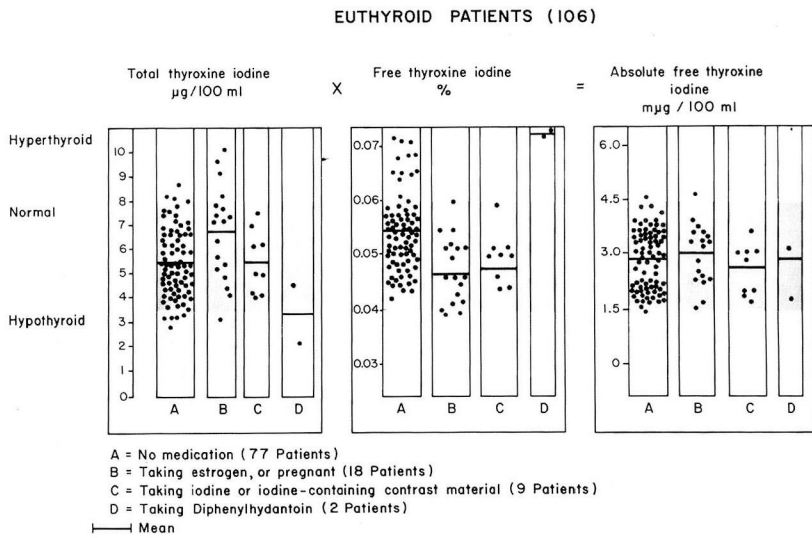


Fig. 1. Effect of exogenous drugs or materials on the absolute free thyroxine iodine levels in euthyroid patients.

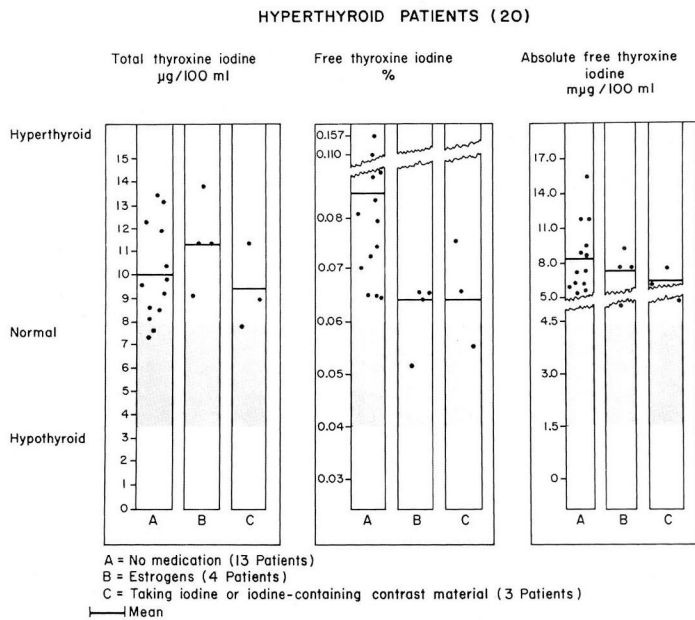
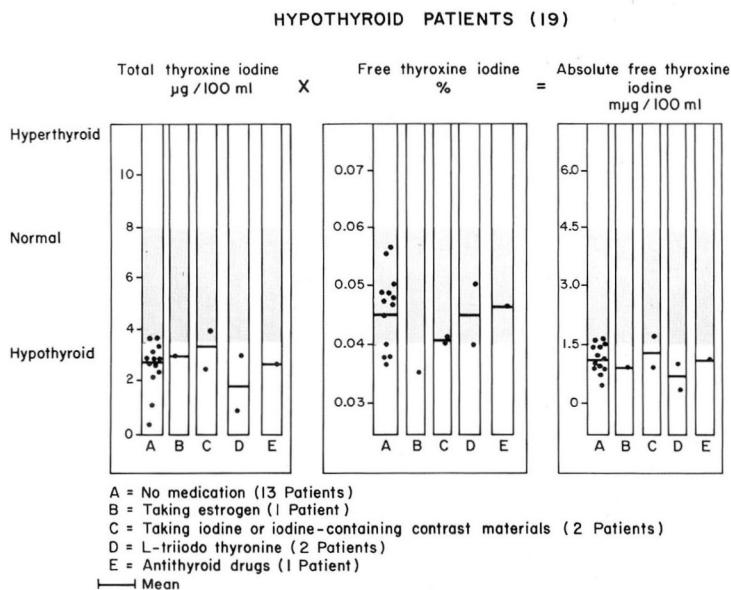


Fig. 2. Effect of exogenous drugs or materials on the absolute free thyroxine iodine levels in hyperthyroid patients.



**Fig. 3.** Effect of exogenous drugs or materials on the absolute free thyroxine iodine levels in hypothyroid patients.

tests which were previously abnormal were repeated.

The effects of iodine contamination, drugs, and estrogens were considered when making a diagnosis. The tests referred to as “standard” included BMR, PBI, and  $^{131}\text{I}$  thyroidal 24-hour uptake, and serum cholesterol determinations. The total serum thyroxine iodine ( $\text{TT}_4\text{I}$ ) and absolute free thyroxine iodine ( $\text{AFT}_4\text{I}$ ) were estimated on blood drawn on the same days as for the other tests.

The 102 patients were classified as follows:

	Patients
1. Clearly active hyperthyroidism of Graves' disease	15
2. Possible Graves' disease	12
3. Nodular goiter (toxic 2, suspected 1, nontoxic 7)	10
4. Myxedema (typical 8, suspected and confirmed 5)	13
5. Possible hypothyroidism	22
6. Euthyroid clinically and by standard tests	19
7. Euthyroid continuing various types of treatment	11

The total thyroxine iodine was measured by the method of Murphy and Pattee,<sup>16-18</sup> with minor modifications; the percentage of free thyroxine was measured by the method of Ingbar et al.<sup>7</sup> The quantity of total thyroxine iodine ( $\mu\text{g}/100 \text{ ml}$ ) was multiplied by the percentage of free thyroxine iodine to obtain a product called the absolute free thyroxine iodine. This terminology is similar to that used by Ingbar et al.,<sup>7</sup> Sterling and Brenner,<sup>9</sup> and Anderson,<sup>11</sup> except that we used total thyroxine iodine instead of protein-bound iodine, as did Arango et al.,<sup>12</sup> to avoid the difficulties inherent in the latter test, such as iodine contamination and hormone effect.

Normal values are: total thyroxine iodine, 3.5 to 8.0  $\mu\text{g}/100 \text{ ml}$ ; free thyroxine iodine, 0.04 to 0.06% absolute free thyroxine iodine, 1.5 to 4.5  $\text{n}/100 \text{ ml}$ . These values represent the mean  $\pm 2 \text{ S D}$  found in 40 medical students. Arango et al.<sup>12</sup> reported normal values for  $\text{AFT}_4\text{I}$  of 0.90 to 5.2  $\text{n}/100 \text{ ml}$  ex-

pressed as thyroxine rather than thyroxine iodine.

## Results

The results are outlined in the tables (see appendix).

*Table 1* shows the results obtained in patients with confirmed Graves' disease. In all cases the correlation of the clinical diagnosis, PBI,  $TT_4^I$ , and  $AFT_4^I$  was good. All  $TT_4^I$  tests repeated after successful therapy were normal. In case 13 therapeutic control was incomplete; as expected there was a large discrepancy between the PBI and  $TT_4^I$ . During continued treatment, however, the  $TT_4^I$  was normal but the  $AFT_4^I$  remained mildly elevated, suggesting that the latter may be a more delicate index of hyperthyroidism.

*Table 2* shows the results obtained in patients with possible Graves' disease. In all cases except case 8 the  $TT_4^I$  and the  $AFT_4^I$  correlated with the final clinical diagnosis. In case 8 the  $TT_4^I$  was elevated and did not support the final impression that the patient was euthyroid.

*Table 3* shows the results obtained in patients with nodular goiter. Patients 1 and 2 were hyperthyroid and the  $TT_4^I$  and  $AFT_4^I$  levels confirmed this. They were cured by removal of the goiter. Hyperthyroidism was suspected in case 3 but all tests were normal, and the patient was considered to be euthyroid. In the remaining 7 cases the  $AFT_4^I$  indicated the euthyroid state. However in one (case 5) the high BMR and the low  $TT_4^I$  were at variance with the final impression that the patient was euthyroid.

*Table 4* shows the results obtained in patients with confirmed hypothy-

roidism. In all instances there was good correlation between the results of the  $TT_4^I$  and  $AFT_4^I$  tests and the diagnoses. The response to therapy further confirmed the diagnosis in all instances. In all other cases there was complete correlation of the diagnosis, the  $TT_4^I$ , and the  $AFT_4^I$  except in case 1, where the  $AFT_4^I$  correlated, but the  $TT_4^I$  did not.

*Table 5* shows the results obtained in patients with possible hypothyroidism and patients in whom it was considered necessary to exclude a diagnosis of hypothyroidism.

All 22 patients were considered to be euthyroid and the standard tests were consistent with this diagnosis except in case 19 (PBI  $3.0 \mu\text{g}/100 \text{ ml}$ ). In patient 22 the  $TT_4^I$  and  $AFT_4^I$  levels were lower than normal. Whether these results are in error or whether the patient may develop hypothyroidism cannot be determined. No adequate explanation can be offered for the  $AFT_4^I$  in case 8.

*Table 6* shows the results obtained in patients judged to be euthyroid. The PBI,  $TT_4^I$ , and  $AFT_4^I$  correlate well in 17 of these 19 patients. Estrogen was apparently responsible for raising the PBI and  $TT_4^I$  in the first patient, while the  $AFT_4^I$  remained normal. In patient 13, judged to be euthyroid clinically and by standard tests, the PBI is near the lower limit of normal; the  $AFT_4^I$  and the  $TT_4^I$  are abnormally low.

*Table 7* shows the results obtained in euthyroid patients on various types of treatment. There is good correlation between the clinical diagnosis and tests in almost all instances. The PBI,  $TT_4^I$ , and  $AFT_4^I$  are depressed in case 3 by tri-iodothyronine. The low PBI in case 9 is unexplained and may

be in error. This patient was suspected of having hypothyroidism.

There appears to be a lack of correlation with the results of the  $TT_4^I$  in four cases and of the  $AFT_4^I$  in one case as compared to the final diagnosis as shown below.

Table	$TT_4^I$	$AFT_4^I$	Diagnosis
Table 3, case 8	0	+	Euthyroid
Table 4, case 3	+	0	Hypothyroidism
case 6	0	+	Hypothyroidism
Table 5, case 22	+	+	Hypothyroidism was suspected clinically, standard tests normal
Table 6, case 13	0	+	Alopecia areata
Table 7, case 1	0	+	Euthyroid

*Effect of contamination with various nonhormonal organic iodides on the free thyroxine test.* The contamination of the blood stream with a variety of iodine-containing materials had no effect on the free thyroxine test, but rendered both the PBI and  $^{131}I$  uptake abnormal. The use of anti-thyroid preparations and l-tri-iodothyronine was followed by a low level of total thyroxine iodine, leaving the percentage of free thyroxine iodine normal and thus resulting in a low level of absolute free thyroxine iodine value. Desiccated thyroid and sodium l-thyroxine had the opposite effect, raising both the total thyroxine iodine and the percentage of free thyroxine iodine, and therefore the absolute free thyroxine iodine. Iodine or iodide had no effect on the tests. The two patients

who had been taking diphenylhydantoin showed low total thyroxine with the percentage of free thyroxine iodide increased slightly over normal, leading to a normal absolute free thyroxine level. All eight patients shown in the tables who had had iodides and PBI levels over 50 had normal levels of  $TT_4^I$  varying from 1.6 to 6.6  $\mu g/100$  ml and  $AFT_4^I$  varying from 0.6 to 4.3 n/100 ml.

*Effect of various hormones and pregnancy on the free thyroxine test.* Pregnancy or the use of estrogen had no significant effect on the  $AFT_4^I$  level except in one patient, in whom it was 5.5 n/100 ml. The total thyroxine iodine was moderately increased because of increased binding, but the percentage of free thyroxine iodine was diminished, resulting in an absolute free thyroxine iodine value within normal range.<sup>19</sup>

Comment

The use of the absolute free thyroxine iodine test gives an accurate laboratory measurement of thyroid function, except possibly in hyperthyroidism due to tri-iodothyronine.

We have found good correlation between the clinical evaluation of the patient and this test, and also good correlation between it and the standard tests (PBI and  $^{131}I$  uptake), when the latter are not changed by contamination of iodine or influenced by drugs, and usually remain within the normal range during estrogen therapy and pregnancy. The absolute free thyroxine iodine test has advantages over existing tests. It is not affected by contrast materials. It is changed by the effects of antithyroid drugs and thyroid preparations, and corresponds to the clinical state.

Since this review of our earliest cases, the findings of others have confirmed the above conclusions.<sup>20</sup> However, there have been several cases in which the values of the free thyroxine did not correlate with the clinical picture or other tests. These have been due in part to laboratory error (we estimate approximately one test in a hundred) or have been completely unexplainable, since the same figures were obtained after repeating the test several times.

It is unfortunate that the determination of the total thyroxine iodine and the percentage of free thyroxine iodine is not easily done in the average clinical laboratory; however, with experience one can obtain reproducible results in most cases without difficulty. Because the tests are technically difficult and do not add clinically useful information in the majority of cases, they are not now performed routinely in our laboratories.

### Representative case reports

**Case 1.** A 37-year-old multigravida was examined in the 7th month of her fourth pregnancy because of a history and physical signs of thyrotoxicosis. She had apparently taken propylthiouracil intermittently before her initial examination. The results of tests were: BMR, +31%; PBI, 15  $\mu\text{g}/100$  ml;  $T_3$  red blood cell uptake, 15.5%; absolute free thyroxine iodine, 9.1 n/100 ml. The latter figures confirmed the initial clinical impression of hyperthyroidism, and treatment with propylthiouracil was reinstituted with subsequent clinical improvement.

**Case 2.** A 62-year-old woman appeared to be hypothyroid by history and physical examination. She had recently had a urogram. The PBI was over 50  $\mu\text{g}/100$  ml; the  $^{131}\text{I}$  uptake was less than 1%; and absolute free thyroxine iodine was 1.0 n/

100 ml, confirming the diagnosis of hypothyroidism.

**Case 3.** A 37-year-old woman was thought to be hypothyroid. She was quite anxious, was taking oral contraceptives, and recently had undergone cholecystography. The BMR was +18%; PBI 50  $\mu\text{g}/100$  ml;  $T_3$  red blood cell uptake 15%;  $^{131}\text{I}$  uptake 49%; serum cholesterol 190 mg/100 ml; and absolute free thyroxine iodine, 7.6 n/100 ml. The patient was treated with  $^{131}\text{I}$ , and within 2 months the absolute free thyroxine iodine level had fallen to 1.5  $\mu\text{g}/100$  ml and the patient appeared to be euthyroid.

**Cases 4, 5 and 6.** Three patients with possible thyroid crisis, but with confusing histories, physical findings, and laboratory data, were found to have extraordinarily high values of absolute free thyroxine iodine (from 18 to 24 n/100 ml). These results were of considerable assistance in reaching an accurate diagnosis. The patients were treated for thyroid crisis with improvement in their clinical courses as the levels of free thyroxine decreased toward normal.

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## APPENDIX

Table 1.—Results in confirmed Graves' disease

Case no.	Factors possibly affecting test results	BMR %	PBI 100 ml	<sup>131</sup> I 24-hr uptake %	Total serum TT <sub>4</sub> <sup>I</sup> μg/100 ml	AFT <sub>4</sub> <sup>I</sup> n/100 ml	Total serum TT <sub>4</sub> <sup>I</sup> μg/100 ml	AFT <sub>4</sub> <sup>I</sup> n/100 ml
							After therapy	
1	Pregnancy PTU* therapy	+31	15		13.9	9.1		
2		+45	17.1	2	14.9	19.9		
3			11.2	41	9.3	7.3	4.5	3.0
4		+19	11.4	36	13.3	8.6		
5	PTU therapy thyroid	+16	16.5		10.4	11.4	6.8	4.5
6	0		12.3	46	9.8	15.4		
7	Estrogen <sup>127</sup> I	+18	9.2	49	11.4	7.6	3.6	1.5
8	0	+53	14.0	77	13.5	11.9		
9	PTU therapy	+46			8.7	6.1		
10	Estrogen		9.9	33	10.3	6.9	4.8	2.6
11	0	+30	14.5	54	9.6	6.2	6.7	3.5
12	0	+43		59	17.4	9.3	5.0	2.7
13	Pneumonia, KI and PTU therapy when tested		17.0	39	7.6	5.5	7.2	5.0
14		+40	7.3	22	8.6	7.1	6.5	4.6
15		+49	8.3	70	12.5	12.7		

\* PTU = propylthiouracil.

Table 2.—Results in possible Graves' disease

Case no.	Factors possibly affecting test results	PBI μg/100 ml	<sup>131</sup> I uptake 24-hr	TT <sub>4</sub> <sup>I</sup> μg/100 ml	AFT <sub>4</sub> <sup>I</sup> n/100 ml	Final clinical diagnosis
1	KI	9.9	20%	5.0	3.0	Euthyroid
2		7.8	27%	7.7	3.6	Euthyroid
3	Estrogen	9.2	20%	8.1	3.7	Hyperthyroid
4	Thyroid	6.2	<1%	5.0	2.4	
5	Thyroiditis	7.5	21%	6.6	3.8	Euthyroid
6		8.1		7.1	3.9	Euthyroid
7				4.4	2.4	Euthyroid, BMR +3%
8	Estrogen	9.1		10.1	3.9	Euthyroid
9	Estrogen, Na LT <sub>4</sub> *	6.6		7.2	3.3	Euthyroid
10		>25		5.4	3.5	Euthyroid, BMR +13%
11		8.5		5.3	2.7	Euthyroid
12	Pyelogram	>25		7.5	3.7	Euthyroid

\* NaLT<sub>4</sub> = sodium levothyroxine.



**Table 3.**—Results obtained in patients with nodular goiter

Case no.	Thyroid status and factors possibly affecting test results	BMR %	PBI $\mu\text{g}/100$ ml	$^{131}\text{I}$ 24-hr uptake %	$\text{TT}_4^{\text{I}}$ $\mu\text{g}/100$ ml	$\text{AFT}_4^{\text{I}}$ n/100 ml
1	Hyperthyroid	+19	11.4	36	13.0	8.6
2	Hyperthyroid; mercury diuretic	+40	7.3*	22	8.6	7.1
3	Euthyroid		25		6.1	2.6
4	Euthyroid, estrogen	-5	14.6†		7.4	4.0
5	Euthyroid	+29	4.8		4.9	2.6
6	Euthyroid	+8		18	6.9	3.6
7	Euthyroid				7.8	4.4
8	Euthyroid		5.4		8.6	3.6
9	Euthyroid		6.5		6.6	3.9
10	Euthyroid; mesantoin		4.2		4.5	3.0

\* Possibly affected by the diuretic.

† Possibly affected by the estrogen.

**Table 4.**—Results obtained in patients with hypothyroidism

Case no.	Factors possibly affecting test results	BMR %	PBI $\mu\text{g}/100$ ml	Cholesterol mg/100 ml	$^{131}\text{I}$ 24-hr uptake	$\text{TT}_4^{\text{I}}$ $\mu\text{g}/100$ ml	$\text{AFT}_4^{\text{I}}$ n/100 ml	Follow-up
1	Pituitary neoplasm	-21	3.3		6	3.4	1.25	After treatment $\text{TT}_4^{\text{I}}$ 9.2, $\text{AFT}_4^{\text{I}}$ 0.2
2	Arteriogram; $^{60}\text{Co}$ therapy to pituitary	-22	>50*			3.2	1.5	$\text{NaLT}_4$ therapy; BMR -10%
3	0	-27		455		3.5	1.9	Tests after beginning $\text{NaLT}_4$ therapy; BMR +2%, cholesterol 340 mg/100 ml
4	0		2.0		13	0.3	1.0	Improved after therapy
5	0		1.9			2.8	1.1	Improved after therapy
6	Gall bladder dye	-21	9.8	240		3.1	1.5	Improved after therapy
7	IV pyelogram	-16	>50*	372	1	1.6	0.6	After therapy, symptom free; $\text{TT}_4^{\text{I}}$ 7.2, $\text{AFT}_4^{\text{I}}$ 3.9
8	Struma lymphomatosa	-4	3.2	310		3.6	1.2	Thyroid antibod. 1:2500 after therapy, PBI 4.5

\* Affected by injected dye.

**Table 5.**—Results obtained in patients with possible hypothyroidism

Case no.	Factors possibly affecting test results	BMR %	PBI $\mu\text{g}/100$ ml	Cholesterol mg/100 ml	$^{131}\text{I}$ 24-hr uptake %	Total serum $\text{TT}_4^{\text{I}}$ $\mu\text{g}/100$ ml	$\text{AFT}_4^{\text{I}}$ n/100 ml	Follow-up
1	None	-1		255		4.6	2.5	
2	None	-14	3.7	160		5.9	3.5	
3	Off $\text{TT}_4^{\text{I}}$ , 1 mon.	-2	7.3	226		7.7	2.2	
4	None	+3	5.0		25	4.6	2.2	
5	Depressed	-10		430		6.2	3.1	
	IV pyelogram							
6	Dilantin	+7				4.5	3.3	All patients considered euthyroid
7	None	+17	4.4	375	7	4.5	2.9	
8	$^{90}\text{Y}$ implant	+7	25		19	6.7	5.5	
9	Estrogen	-5	11.7	295		6.3	2.6	
10	None	+5	4.9			5.2	3.6	
11	None			170		5.4	3.0	
12	None		6.1		35	7.4	3.4	
13	Iodine	-14		270		4.2	2.1	
14	None	-10	7.8	130		6.2	3.1	
15	Estrogen	-13		205		7.3	3.4	
16	None		5.4			4.6	2.3	
17	None		9.4			5.2	2.7	
18	None		3.6			4.4	2.2	
19	None		3.0	150		4.2	2.3	
20	None			205		4.0	2.1	
21	None	-9	6.7	250		6.0	3.4	
22	None	-3	8.9	190		3.2	1.0	

**Table 6.**—Results obtained in patients judged to be euthyroid

Case no.	Clinical impression	Drugs	BMR %	PBI $\mu\text{g}/100$ ml	Cholesterol mg/100 ml	$^{131}\text{I}$ 24-hr uptake %	Total serum $\text{TT}_4^{\text{I}}$ $\mu\text{g}/100$ ml	$\text{AFT}_4^{\text{I}}$ n/100 ml
1	Hyperthyroidism to be excluded	Estrogen		9.5			9.6	3.7
2	Euthyroid	Previous tapazole	+7	7.1		39	6.8	2.8
3	Goiter	0	+2	9.6		20	5.0	2.9
4	Goiter	0		6.4	285		6.6	3.9
5	Thyroiditis	Iodine estrogen	-8	13.9	240		4.0	1.9
6	$^{90}\text{Y}$ implant	0	+22	5.5		43	4.9	3.5
7	Treated Graves' disease	PTU T3	-13	6.8	210	6	4.0	2.2
8	Treated Graves' disease	Previous tapazole Lugol's solution		5.5		10 (4 hr)	3.8	1.9
9	Euthyroid	Estrogen	-19	10.3	255		5.4	2.5
10	Euthyroid	0	-6	6.9	255		6.2	3.1
11	ATS euthyroid	Estrogen				29	5.7	3.4
12	Euthyroid	0				26	5.9	3.2
13	Alopecia	0	-3	4.2	225		3.3	1.7
14	ATS		-2	7.3	220		7.7	4.2
15	Exophthalmos	0	+14	5.5	170	30	5.5	3.3
16	ATS	0	-29	4.3			3.8	2.5
17	Goiter	0	+2	9.6		20	5.0	2.8
18	ATS	Estrogen	-6	13.9			4.0	1.9
19	Post $^{131}\text{I}$ therapy	0	+7	8.5			0.8	0.3

Table 7.—Results obtained in euthyroid patients receiving various types of treatment

Case no.	Clinical impression	Drugs	BMR %	PBI $\mu$ g/100 ml	Choles- terol mg/100 ml	<sup>131</sup> I 24-hr uptake %	Total serum T <sub>4</sub> <sup>I</sup> $\mu$ g/100 ml	AFT <sub>4</sub> <sup>I</sup> n/100 ml	Follow up
1	Myxedema	NaLT <sub>4</sub>	2				3.5	1.9	Symptom free
2	Euthyroid	None now post <sup>131</sup> I PTU	-13	6.8 7.4		6	4.0	2.2	
3	Thyroid carcinoma	T <sub>3</sub>	-2, +6	1.8, 0.9		1, 2	3.0	1.2	No advance 4 yr
4	Goiter (postoperative)	<sup>125</sup> I estrogen	+8		185	8	4.4	4.3	
5	<sup>90</sup> Y implant	T <sub>3</sub> 1mm	+11	6.5			4.1	1.9	
6	Mental retardation	Thy. Gr. II/day Urogram	-5	4.9	292	3	6.5	3.1 pre Rx 3.3 during Rx	
7	skeletal retardation	Thy. Gr. II/day		12.5					
8	Treated Graves' dis- ease	NaLT <sub>4</sub>	+14	5.5	170	30	5.5	3.3	BMR -3, Chol. 150, PBI 5.4
9	Treated Graves' dis- ease	NaLT <sub>4</sub>		5.4			4.0	1.9	
10	Hypothyroid	Desic. thy.	-4		250	15	6.0	3.4	
11	Myxedema NaLT <sub>4</sub> 0.3 mg/dy	<sup>131</sup> I 150 mCi	-2	7.1			6.2	3.7	
12	Mild hyperthyroid	Tapazole	+18	7.1	220	39	7.7	3.2	
13							6.8	2.8	