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Research Article

EARLY EFFECTS OF THYROTROPIN ON PROTEIN-BOUND IODINE^{131} AND PLASMA IODIDE^{131} *

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The mechanism by which the pituitary thyroid-stimulating hormone (TSH) causes the thyroid gland to concentrate iodide and to synthesize and release hormone has not been clearly defined. Whether it acts by selectively stimulating one of the steps in thyroidal iodine metabolism, following which there is a secondary increase in the other reactions, or acts by affecting all stages of iodine metabolism simultaneously through some basic general stimulus to the entire cell is still a matter of speculation. Some of the evidence favoring the latter thesis rests on the demonstration that TSH affects such fundamental biochemical processes of thyroid tissue as oxygen consumption (2) and phospholipid synthesis (3, 4) and on the well known fact that TSH is required for normal growth and maintenance of the thyroid gland. One of the major obstacles to the universal acceptance of this thesis has been the apparent temporal dissociation of the effects of TSH on thyroid hormone release and iodide uptake by the gland. Although some workers have noted that stimulus to uptake occurred (after an eight hour delay) without any significant hormone release (5), perhaps the majority of data favor the view that in the normal gland hormone release occurs before iodide uptake is stimulated (6, 7).

The present study was designed in an attempt to re-evaluate these time relationships of TSH effects simultaneously in man by following protein-bound iodine^{131} (PBI^{131}) and plasma iodide^{131} disappearance time as measures of hormone release and thyroidal uptake of iodide, respectively. In the course of these studies observations were made that may explain previous discrepancies in the time relationships of these TSH effects.

MATERIAL AND METHODS

Oral tracer doses (200 to 600 μc.) of carrier-free sodium iodide^{131} were given to the euthyroid patients. These subjects were free of endocrine disease but in the case of the patients receiving the larger doses, did have some longevity-threatening illness. The previously untreated hyperthyroid subjects were given similar doses of I^{131} prior to treatment with radioiodine. Samples of heparinized venous blood were drawn at varying intervals throughout the remainder of the study. A single intramuscular injection of a thyrotropic hormone preparation (Armour Laboratory, Thytropar®) was given to each subject either at 12 or 24 hours after the original dose of I^{131}. To some subjects a second oral dose of I^{131} was given to maintain measurable levels of iodide^{131} in the blood for a total of 72 hours.

The blood samples were centrifuged and a 3 ml. aliquot of plasma from each sample was placed in a vial and

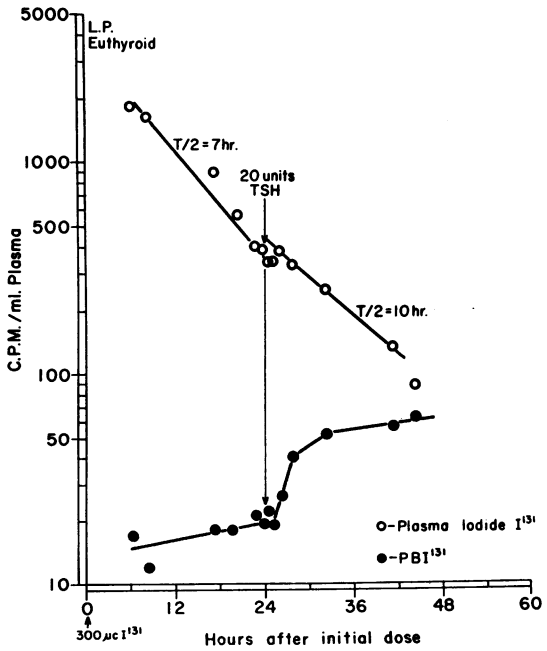

FIG. 1. PBI^{131} RELEASE AND SLOWING OF PLASMA IODIDE^{131} DISAPPEARANCE AFTER TSH ADMINISTRATION IN A NORMAL SUBJECT

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counted in a well-type scintillation counter equipped with a scaler and a pulse height analyzer. Background counts averaged 24 to 28 counts per minute (cpm). PBI^{131} was prepared by the ion exchange resin method of Fields and co-workers (8) and counted in the same scintillation well system. We have confirmed the validity of this resin method by recovering from the column 98 to 103 per cent of radiothyroxine (rechromatographed Abbott Laboratory preparation) added to plasma. Essentially all of the radioiodide added to serum is extracted by this resin. Values for PBI^{131} by this method were also in good agreement with those determined by trichloroacetic acid precipitation and by dialysis of plasma against physiological saline in all of the subjects' plasma so tested. All radioactivity measurements were corrected for physical decay to the time of the original dose of radioiodine. PBI^{131} was expressed as cpm per ml. The total plasma radioactivity minus the PBI^{131} was considered $iodide^{131}$.

RESULTS

Effects of TSH on plasma iodide^{131} and PBI^{131} in euthyroid subjects

In Figures 1, 2 and 3 typical examples of simultaneous effects of TSH upon hormone release and plasma iodide are shown. The increase of PBI^{131} following TSH was prompt in each of these euthyroid subjects, occurring within 2 to 4 hours. In Figure 1 after a 6 hour equilibration period the control plasma $iodide^{131}$ had a disappearance half-time (T/2) of 7 hours. During the first 18 hours after 20 units of TSH intramuscularly the plasma iodide disappearance "slowed" to a T/2 of 10 hours. The lower plasma $iodide^{131}$ at 44 hours may be the first evidence of the speed-up in the thyroidal uptake seen in the subsequent figures. Figure 2 again shows the slowing of plasma $iodide^{131}$ disappearance from the control T/2 of 8.5 hours to 10.5 hours following TSH. In this study a second 50 μc. dose of NaI^{131} was given at 49 hours to increase the statistical significance of the radioactivity counts. Following 6 hours of equilibration the subsequent disappearance of the $iodide^{131}$ had increased to a T/2 of 7.0 hours which represents a slightly more rapid rate of disappearance than during the control period. A similar

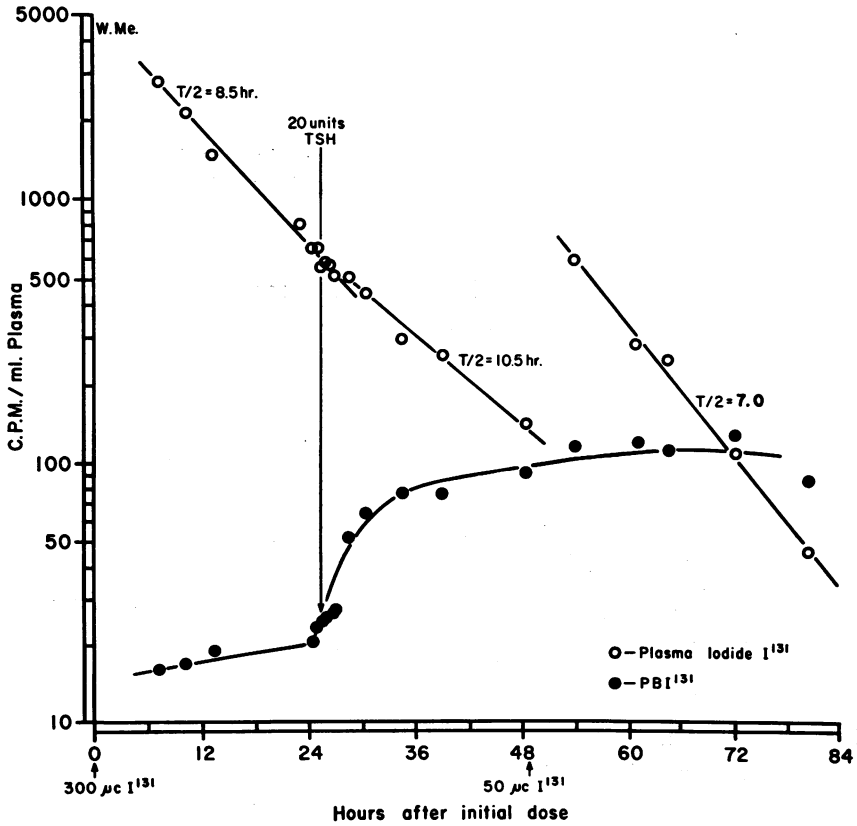

FIG. 2. PBI^{131} RELEASE AND CHANGES IN PLASMA $IODIDE^{131}$ AFTER TSH ADMINISTRATION IN A NORMAL SUBJECT

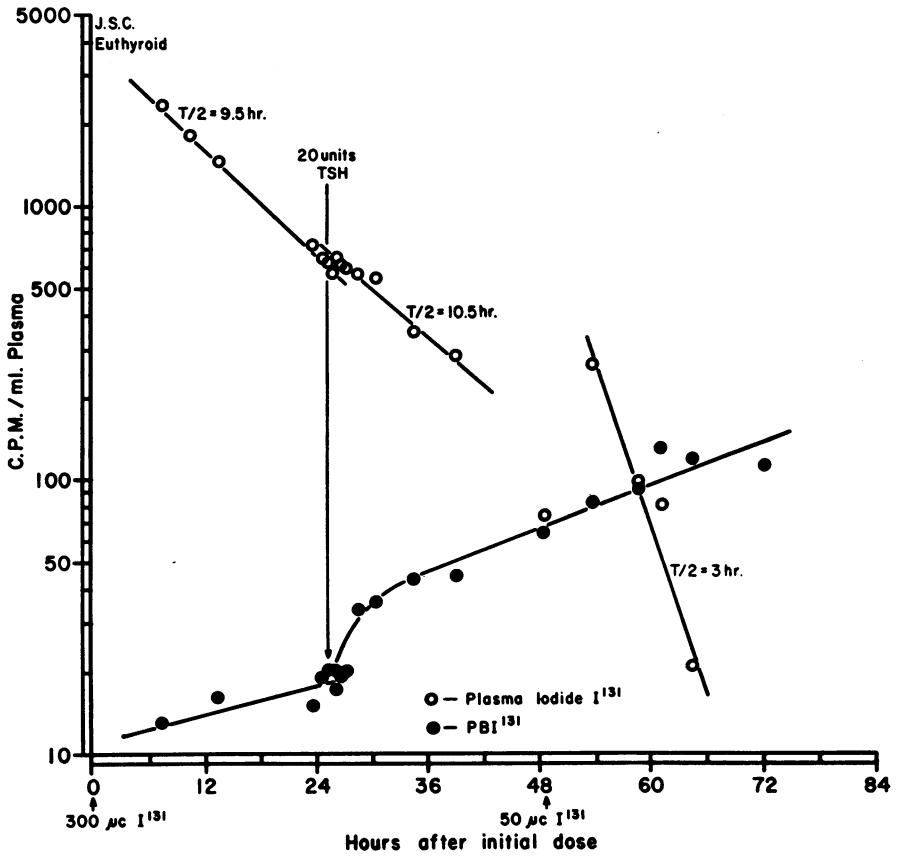

FIG. 3. INITIAL DECREASE AND SUBSEQUENT INCREASE IN PLASMA IODIDE^{131} DISAPPEARANCE AFTER TSH ADMINISTRATION IN A NORMAL SUBJECT

study is illustrated in Figure 3 where the plasma iodide^{131} disappearance slowed after TSH. By 48 hours (24 hours after the TSH) the plasma iodide^{131} content had begun to fall (note the single point at 48 hours just before the second dose of I^{131} was given). The very rapid disappearance of iodide thereafter is striking.

Table I is a summary of the results of comparable studies in the group of 17 euthyroid subjects. In all of these the PBI^{131} release by TSH was evident within 6 hours. During the first 12 to 24 hours after the TSH the plasma iodide disappearance was slowed from the mean control T/2 of 7.9 to 11.2 hours. It is quite significant that in four subjects there was either no further decrease or an actual increase in plasma iodide^{131}. During the period beginning 24 hours after the TSH administration the mean T/2 of the plasma iodide^{131} disappearance of 5.4 hours indicates that during this period the thyroidal uptake of plasma iodide had again accelerated.

When four additional subjects were given single 5 unit injections of TSH this slowing effect was not observed. Three of these patients showed a rise in PBI^{131} but no real change in plasma iodide^{131} disappearance was noted in 18 hours. This would lead to the conclusion that to observe this latter effect one must use a relatively large dose of TSH.

Effects of TSH on plasma iodide^{131} and PBI^{131} in hyperthyroid patients

Five patients with hyperthyroidism were studied in a similar fashion. Two of these studies are shown in Figures 4 and 5. In Figure 4 the control plasma iodide^{131} disappearance was rapid (T/2, 4 hours) as compared with the normal subjects, in keeping with the more rapid thyroidal clearance in such patients. Following 10 units

TABLE I

Effect of thyroid-stimulating hormone (TSH) on plasma iodide^{131} disappearance and protein-bound iodine (PBI)^{131} release in euthyroid subjects

No.	Patient	Dose 1	Control T/2 plasma iodide^{131} before TSH	TSH	T/2 plasma iodide^{131} during first 12–24 hours after TSH	Dose 2	T/2 plasma iodide^{131} from 24–48 hours after TSH	Time of definite stimulus after TSH on: PBI^{131} release	Time of definite stimulus after TSH on: Plasma iodide^{131} disappearance
		μc. I^{131}	*hours*	*units*	*hours*	*μc. I^{131}*	*hours*	*hours*	*hours*
1	J. W.	300	9.5	20	9.5			5	>18
2	E. A.	500	10.5	20	*		9.5	3	> 6
3	E. K.	500	8.0	20	8.0			3	>24
4	J. K.	600	9.0	20	9.0			1	>24
5	L. P.	300	7.0	20	10.0			4	>18
6	R. W.	300	6.5	20	11.0			2	>18
7	B. B.	300	8.0	20	21.0			4	10
8	R. V.	300	6.0	20	*			6	>18
9	L. W.	300	6.0	20	10.0	50	4.5	2	24
10	A. W.	300	10.5	20	10.5	50	4.0	2	33
11	H. C.	300	12.0	20	9.5	50	8.0	3	12
12	J. C.	200	7.0	20	14.0	50	4.0	6	31
13	J. P.	300	5.5	15	11.5	50	4.5	2	34
14	J. S. C.	300	9.5	20	10.5	50	3.0	3	23
15	W. Mc.	300	6.5	20	*	50	5.5	4	29
16	W. Me.	300	8.5	20	10.5	50	7.0	4	29
17	C. W.	300	5.0	20	*	50	4.0	2	10
Mean values			7.9		11.2		5.4		
p values					<0.01†		<0.01†		

* In these subjects there was either no further decrease or there was an increase in plasma iodide^{131} concentration.
† Values represent the difference between the means of paired data. Each of the periods was compared with the control period.

of TSH there was a distinct increase in plasma iodide^{131} lasting about 7 hours. On the day following the TSH plasma iodide was disappearing at a faster rate (T/2, 2.5 hours) than during the control period. It is difficult to say whether there was any increase in PBI^{131}. A very remarkable increase in plasma iodide^{131} followed 20 units of TSH given to the patient in Figure 5. In this subject as in several of the euthyroid group the plasma samples containing the increased plasma iodide^{131} after TSH were subjected to further tests to affirm the identity of this radioactivity as iodide. The same amounts of radioactivity (within experimental error) that remained on the resin column also were not precipitated by trichloroacetic acid and were dialyzable when the plasma samples were dialyzed against physiological saline. Butanol extracts of the plasma were chromatographed in butanol-ammonia and in collidine-water (9) and only thyroxine, triiodothyronine and iodide were found; *i.e.*, no iodotyrosines. Of the other three patients with hyperthyroidism one showed a response to the 20 unit dose very similar to that in Figure 5. One other subject showed some increase in iodide^{131} after 10 units of TSH followed by a marked slowing of iodide^{131} disappearance from a control T/2 value of 4 hours to a T/2 of 12 hours which persisted for 24 hours. These four patients all received their injections of TSH at 12 hours after the original dose of radioiodine for two reasons. The first of these was one of expedience since the plasma iodide^{131} falls so fast as a function of rapid thyroidal clearance that statistically significant counts would be difficult to obtain after 24 hours following a radioiodine dose in the tracer range. The second reason was that, since the intrathyroidal turnover of the isotope is greatly accelerated, the labeling of the thyroglobulin of the hyperthyroid patients at 12 hours would be more closely comparable to that in the euthyroid subjects at 12 hours. The remaining hyperthyroid patient was, however, given an injection of 20 units of TSH at 24 hours. While an increase of PBI^{131} was noted promptly, a very rapid increase in plasma iodide^{131} disappearance was noted.

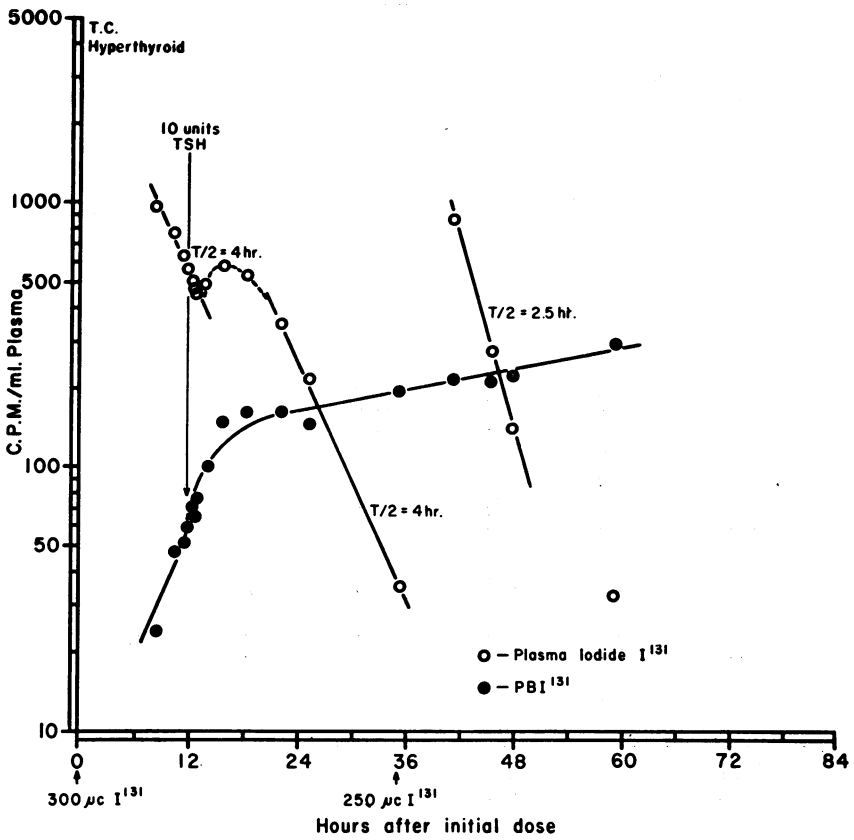

FIG. 4. TSH EFFECTS ON PBI^{131} AND PLASMA IODIDE^{131} IN A HYPERTHYROID SUBJECT

To test the possibility that the more marked effect of plasma iodide in the hyperthyroid patients was due to giving the TSH at 12 rather than at 24 hours after the dose of radioiodine two more euthyroid subjects were given 20 units of TSH at 12 hours after the I^{131}. In one there was no response of either the PBI^{131} or the plasma iodide^{131}. In the other, however, a modest increase of PBI^{131} and a slight slowing of plasma iodide^{131} disappearance were noted. This would indicate that the timing of the TSH administration alone is not sufficient explanation for the more marked response in the hyperthyroid patients.

DISCUSSION

The rapid increase in PBI^{131} following these doses of TSH was expected but prolongation of plasma iodide^{131} disappearance time and, in some experiments, actual increase in plasma iodide^{131} is, of course, not what one would anticipate if TSH were causing early stimulation to the trapping mechanism with an increase in thyroidal clearance rate of plasma iodide. Before attempting an explanation of these changes in plasma iodide produced by TSH, it is appropriate to inspect the validity of the plasma iodide^{131} disappearance time as a measure of thyroidal uptake and utilization under the conditions of these studies.

After equilibration the disappearance of plasma iodide^{131} is a function of both renal and thyroidal clearances. Therefore to use the plasma iodide^{131} disappearance time as a measure of thyroidal uptake following TSH we must assume that any early effect on the plasma iodide^{131} is a result of thyrotropin activity on its primary target, the thyroid, rather than upon renal clearance, extrarenal extrathyroidal iodide space, or peripheral degradation of endogenously labeled hormonal iodine. That these are reasonable assumptions can be supported in part. The early effect of TSH on renal clearance of iodide^{131} (using endogenous creatinine for glomerular filtration rate) was negligible when

calculated in two normal subjects given 20 units of TSH 24 hours after the dose of radioiodine. That the early effects of TSH on plasma iodide^{131} cannot be attributed to alterations in renal clearance was further shown when these same effects, *i.e.*, actual increase in plasma iodide^{131}, were produced in a patient with acute renal failure whose urine output was only 100 ml. per day. The only experimental evidence we have that the effects are not due to some alteration of extrarenal, extrathyroidal iodide space was the failure to detect any change in plasma iodide^{131} with TSH in a patient given sufficient inorganic iodide to block thyroidal uptake of the tracer dose of radioiodide. That increase in peripheral degradation of endogenously labeled thyroxine by TSH cannot account for the early changes in plasma iodide^{131} is evident from the fact that repeated doses of TSH (5 units every 24 hours for a three day period) given to five patients whose thyroids were blocked by potassium iodide did not alter the biologic decay of radiothyroxine administered before the TSH. Even though the fractional rate of peripheral degradation of hormone remains constant in spite of wide variations in circulating hormone (10) it is conceivable that the increase in specific activity of the endogenously labeled hormone released by TSH would lead to an increase in plasma iodide^{131} arising from peripheral deiodination of this hormone. This iodide^{131} would then invalidate the use of the plasma iodide^{131} disappearance time as a measure of thyroidal uptake. It is not likely that this is a problem during these studies since in many patients the plasma iodide^{131} increased as much as did the PBI^{131} in the first three to four hours after TSH. This would be far in excess of the usually quoted figure of 10 per cent degradation per 24 hours. To check this possibility further, however, two normal subjects were given high specific activity radiothyroxine (that had been added to their plasma *in vitro* and dialyzed against physiological saline to remove any iodide^{131}) intravenously in amounts sufficient to simulate the PBI^{131} curves after TSH. Plasma samples were drawn thereafter and analyzed as in the TSH studies. There was essentially no evidence of "deiodination" iodide^{131} in any of the samples during the three hours of these studies. Comparable studies with radiotriiodothyronine showed less than 10 per cent of the total plasma

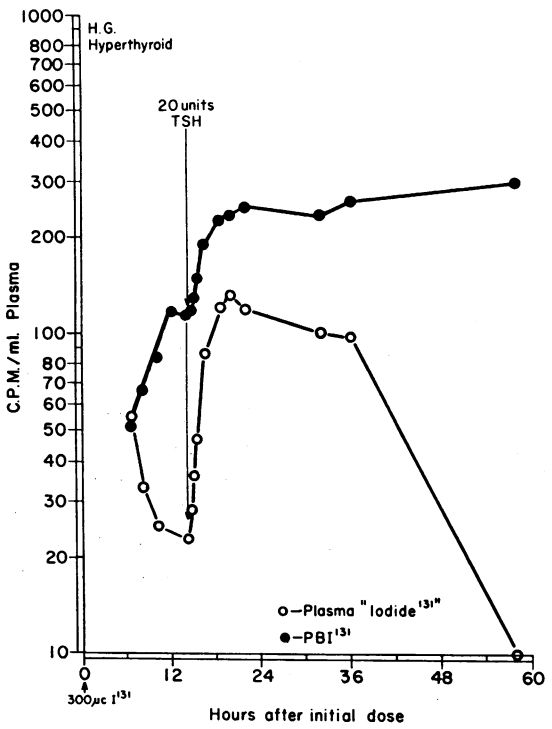

FIG. 5. MARKED INCREASE IN PLASMA IODIDE^{131} AFTER TSH ADMINISTRATION IN A HYPERTHYROID SUBJECT

counts at any time as iodide^{131}. The PBI^{131} in the labeled triiodothyronine studies was determined by dialysis of plasma samples since this hormone is less firmly bound to protein and adheres to the anion exchange column usually used. One further possibility to account for the increase in plasma iodide^{131} is that iodotyrosines were released by TSH and deiodinated sufficiently rapidly (11) to contribute to plasma iodide. Though this cannot be absolutely ruled out we were never able to detect increase in iodotyrosine in plasma chromatographs.

It is a reasonable assumption then that the early slowing of the plasma iodide^{131} disappearance time and in some instances the increase in plasma iodide^{131} results from the effect of thyrotropin on the thyroid and only after this phase is it possible to detect the increased trapping of plasma iodide by these methods. These observations suggest that some product of the TSH-increased proteolysis of thyroglobulin temporarily acts as an inhibitor of further utilization of iodide, *i.e.*, an intrathyroidal feedback. This inhibition may affect the iodide being recycyled from the deiodinase reaction within

the gland as well, producing in some patients the actual increase in plasma iodide^{131} from leakage of this iodide to the plasma. This loss of intrathyroidal "deiodinase" iodide is compatible with the data of Nadler and Leblond (12) who find that not all the trapped iodide is oxidized and that some of the recycled iodide from the deiodinase reaction also is exposed to re-entry to the plasma.

This interpretation of our data in the human is also consistent with experiments of Halmi (13) who found in rats an initial depression of the thyroid to serum (T/S) ratio of iodide^{131} following TSH before the increase in T/S was noted. This same interpretation fits equally well with data of others (14, 15) showing an actual depression of *in vitro* uptake of iodide^{131} by isolated thyroids of normal rats treated with TSH whereas hypophysectomized rats (presumably thyroglobulin-depleted) similarly treated showed increased uptake. These observations may also explain the differences in rate of iodide uptake as compared with hormone release noted in the literature (5, 16) for if the thyroglobulin deficient gland is stimulated by TSH one would anticipate less early inhibition and consequently the uptake of plasma iodide would be greater. Conversely, the more adequately thyroglobulin-stocked the gland, the more delayed the trapping of the administered iodide. Halmi has described in animals an intrathyroidal "depressor" mechanism operating to control uptake of iodide (13). Our data can be interpreted indirectly to support the existence of such a mechanism in man.

Unfortunately, it is not possible to say whether or not the biochemical mechanism responsible for trapping is stimulated as promptly as is the hormone secretion, for it is still possible that it is simultaneously stimulated but temporarily blocked by the postulated product of thyroglobulin proteolysis, or flooded with deiodinase iodide. The latter then must either be reutilized or released before the effect of the stimulation can become manifest as increased trapping of plasma iodide. It does seem, however, that we should beware of emphasizing, as a strong point in favor of the view that TSH primarily affects secretion, the observation that increased hormone release precedes stimulated uptake of the administered dose of iodide, since study of the trapping mechanism soon after TSH will be confused by the release of intrathyroidal iodide.

The striking increase in plasma iodide^{131} following TSH in three of the five hyperthyroid patients would seem to indicate that in these patients the loss of intrathyroidal iodide is greater after TSH than in normal subjects. Regarding the possible positive (17) or negative (18) role of TSH in the etiology of Grave's disease, all that can be said from our data is that it can be shown that TSH can produce in some euthyroid subjects the excessive loss of intrathyroidal iodide to the plasma that is reported (19, 20) to occur in untreated patients with Grave's disease.

SUMMARY AND CONCLUSIONS

Following the administration of radioiodine to 17 euthyroid subjects plasma iodide^{131} disappearance and protein-bound iodine^{131} were determined as measures of thyroidal uptake of iodine and thyroid hormone release, respectively. Following a single injection of thyrotropin prompt release of protein-bound iodine^{131} was noted before six hours in all subjects. During the first 12 to 24 hours after thyrotropin there was a significant increase in plasma iodide^{131} disappearance time in 12 subjects. In 4 of these there was a period during which plasma iodide^{131} either did not decrease further or actually increased. It was only after this period of slowed iodide disappearance that the more rapid iodide^{131} disappearance was observed.

These changes in plasma iodide^{131} are believed to reflect an intrathyroidal feedback mechanism activated by some product of the thyrotropin-induced increase in thyroglobulin proteolysis or by increased deiodinase activity leading to a period of decreased plasma iodide disappearance and/or to leakage of intrathyroidal iodide^{131} to the plasma. These indirect effects of thyrotropin upon the thyroid trap make interpretation of a possible direct effect of this tropic hormone on iodide trapping difficult. These observations should lead, however, to a cautious approach in assigning the primary locus of thyrotropin activity to thyroglobulin proteolysis simply because hormone release can usually be detected before increased uptake of iodide in normal subjects.

Similar studies were made on five hyperthyroid subjects. In one of these a prompt increase in

iodide^{131} disappearance was noted. In one patient a marked decrease was seen and in the other three there was a pronounced loss in intrathyroidal iodide^{131} into the plasma. The possible implication of these observations is briefly discussed.

REFERENCES

1. Deiss, W. P., Jr., O'Shaughnessy, P. J., and Wynn, J. O. Early effects of thyrotropin on PBI^{131} release and on plasma iodide^{131} disappearance. Amer. J. Med. 1958, **25**, 117.
2. Vander Laan, J. E., Vander Laan, W. P., and Logan, M. A. Effect of administering thyrotropic hormone with and without iodine on thyroid tissue metabolism. Endocrinology 1941, **29**, 93.
3. Morton, M. E., and Schwartz, J. R. The stimulation in vitro of phospholipid synthesis in thyroid tissue by thyrotrophic hormone. Science 1953, **117**, 103.
4. Freinkel, N. Pathways of thyroidal phosphorus metabolism: The effect of pituitary thyrotropin upon the phospholipids of the sheep thyroid gland. Endocrinology 1957, **61**, 448.
5. Stanley, M. M., and Astwood, E. B. The response of the thyroid gland in normal human subjects to the administration of thyrotropin as shown by studies with I^{131}. Endocrinology 1949, **44**, 49.
6. Keating, F. R., Jr., Rawson, R. W., Peacock, W., and Evans, R. D. The collection and loss of radioactive iodine compared with the anatomic changes induced in the thyroid of the chick by the injection of thyrotropic hormone. Endocrinology 1945, **36**, 137.
7. Wahlberg, P. Percentage of colloid and behaviour of radioactive iodine in the chick thyroid after stimulation with TSH. Acta endocr. (Kbh.) 1955, **20**, 240.
8. Fields, T., Kinnory, D. S., Kaplan, E., Oester, Y. T., and Bowser, E. N. The determination of protein-bound iodine^{131} with an anion exchange resin column. J. Lab. clin. Med. 1956, **47**, 333.
9. Albright, E. C., Larson, F. C., and Deiss, W. P. Single dimension chromatographic separation of thyroxin and triiodothyronine. Proc. Soc. exp. Biol. (N. Y.) 1953, **84**, 240.
10. Sterling, K., and Chodos, R. B. Radiothyroxine turnover studies in myxedema, thyrotoxicosis, and hypermetabolism without endocrine disease. J. clin. Invest. 1956, **35**, 806.
11. Stanbury, J. B., Kassenaar, A. A. H., and Meijer, J. W. A. The metabolism of iodotyrosines. I. The fate of mono- and di-iodotyrosine in normal subjects and in patients with various diseases. J. clin. Endocr. 1956, **16**, 735.
12. Nadler, N. J., and Leblond, C. P. Rates of passage of iodine into and out of the thyroid gland of the rat under various conditions of dietary iodine intake and body weight. Endocrinology 1958, **62**, 768.
13. Halmi, N. S. Factors influencing the thyroidal iodide pump *in* Ciba Foundation Colloquia on Endocrinology. Boston, Little, Brown and Co., 1957, vol. 10, p. 79.
14. Botkin, A. L., Eskelson, C. D., Firsheim, M. S., and Jensen, H. In vitro studies on the intact thyroid gland. J. clin. Endocr. 1954, **14**, 1219.
15. Eskelson, C. D., Firschein, H. E., and Jensen, H. Effect of thyrotropin administration on the in vitro uptake and conversion of I^{131} and release of organic-bound I^{131} by the isolated thyroid gland. Endocrinology 1955, **57**, 168.
16. Vander Laan, W. P., and Caplan, R. Observations on a relationship between total thyroid iodine content and the iodide-concentrating mechanism of the thyroid gland of the rat. Endocrinology 1954, **54**, 437.
17. Greer, M. A., and Shull, H. F. A quantitative study of the effect of thyrotropin upon the thyroidal secretion rate in euthyroid and thyrotoxic subjects. J. clin. Endocr. 1957, **17**, 1030.
18. Werner, S. C., Spooner, M., and Hamilton, H. Further evidence that hyperthyroidism (Graves' disease) is not hyperpituitarism: Effects of triiodothyronine and sodium iodide. J. clin. Endocr. 1955, **15**, 715.
19. Slingerland, D. W., and Burrows, B. A. The estimation of non-thyroxine iodine released by the thyroid gland in hyperthyroidism (abstract). J. clin. Invest. 1957, **36**, 930.
20. Slingerland, D. W., Dell, E. S., Graham, D. E., Trakas, A. P., and Burrows, B. A. Thyroid secretion of nonthyroxine iodine (abstract). J. clin. Invest. 1958, **37**, 932.