Elevated Serum Thyroglobulin

A MARKER OF METASTASES IN DIFFERENTIATED THYROID CARCINOMAS

ANDRE J. VAN HERLE and ROBERT P. ULLER

From the Department of Medicine, Division of Endocrinology, Center for the Health Sciences, University of California at Los Angeles, School of Medicine, Los Angeles, California 90024

ABSTRACT The presence of human thyroglobulin (HTg) in serum of patients with differentiated thyroid carcinoma was studied. The thyroglobulin detected in the serum of such patients was identical by immunological criteria to the serum standard used in the radioimmunoassay. The serum thyroglobulin levels in untreated patients with differentiated thyroid carcinoma ranged from 22.0 to 445.0 ng/ml with a mean of 144.3± 46.5 ng/ml (SEM) ($n = 10$). The mean serum thyroglobulin measured postoperatively in seven of these patients was 6.4±1.5 ng/ml, not statistically different from the mean level of 5.1±0.49 ng/ml (range 0–20.7 ng/ml) observed in 71 out of 95 control subjects with detectable HTg levels. By contrast serum HTg levels were normal or undetectable in subjects with medullary carcinoma of the thyroid. HTg levels were within normal limits in sera of patients who had previously undergone successful therapy for a differentiated thyroid carcinoma and in whom no metastases could be documented. The mean level for this group was 4.9±0.51 ng/ml ($n = 43$). In contrast, patients with documented metastases had a mean serum thyroglobulin level of 464.9±155.6 ng/ml ($n = 6$). The data support the thesis that in differentiated thyroid carcinoma serum thyroglobulin levels are elevated when metastases develop after initial treatment. It is proposed that the measurement of thyroglobulin in the serum represents a simple and valuable adjunct in the posttreatment follow-up of patients with differentiated thyroid cancer.

Presented in part at the 66th Annual Meeting of the American Federation for Clinical Research, 1974, Atlantic City, N. J. and the 56th Annual Meeting of the Endocrine Society, 1974, Atlanta, Ga.

Received for publication 16 October 1974 and in revised form 24 March 1975.

INTRODUCTION

Several previous investigations cast serious doubts on the theory that thyroglobulin is a secluded antigen confined to the thyroid gland in man (1–3). In recent years data showing the presence of thyroglobulin in blood were extended to studies in patients with various pathologic states of the thyroid gland (4, 5). Although the mechanism of the release of this thyroidal protein into the circulation is presently unknown, profound architectural and biological changes of the thyroid gland, which accompany neoplastic growth, could be responsible.

After the administration of ^{131}I in patients with thyroid cancer, several authors have described the release of labeled iodinated proteins, among them thyroglobulin (6–9). The recent development of a specific and sensitive radioimmunoassay for the measurement of serum thyroglobulin permitted us to investigate the presence of nonradioactive thyroglobulin in patients with thyroid carcinoma, thus avoiding the administration of radioisotopes in tracer or therapeutic amounts. The present study was undertaken to investigate human thyroglobulin (HTg)[1] levels in the serum of patients with differentiated thyroid carcinomas and those with medullary carcinoma of the thyroid.

METHODS

Radioimmunoassay. Serum thyroglobulin was determined in a double antibody radioimmunoassay, previously described, using a standard reference serum GA (40,000 ng/ml) which was appropriately diluted for the assay (4). All samples were analyzed in duplicate, and, before introduction into the assay, antithyroglobulin antibodies were determined using a

[1] *Abbreviation used in this paper:* HTg, Human thyroglobulin.

tanned red cell agglutination technique (Burroughs and Wellcome, Greenville, N. C.).

Serum samples with an antibody titer of 1:5 or larger were rejected for analysis; this amounted to 13.2% of the cases. The serum thyroglobulin levels for 95 healthy subjects ranged from 0 to 20.7 ng/ml with a mean detectable HTg level of 5.1±0.49 ng/ml (SEM). All samples exceeding 200 ng/ml were appropriately diluted before retesting in goat serum (1:10) in phosphate-buffered saline.

Study subjects. In a retrospective study, serum was obtained from 43 subjects in whom a differentiated thyroid carcinoma had been present and had been removed from 2 mo to 20 yr before the sampling. The mean time from initial treatment to follow-up study was 7 yr. The patients had either undergone lobectomies ($n=8$), subtotal thyroidectomies ($n=9$), or total thyroidectomies ($n=26$). The histologic diagnosis was verified in all cases; papillary carcinoma was present in 19 cases, follicular carcinoma in 11 cases, and mixed papillary-follicular in 13 cases. Of the 43 patients studied, 23 had received one or more therapeutic doses of ^{131}I at various times before sampling, 16 received no ^{131}I therapy at any time, and information was not obtained for four subjects. All patients were evaluated clinically, and in most instances a postoperative total body scan with ^{131}I was performed while they were off thyroid supplementation. Also, serum HTg was measured in six additional patients with differentiated thyroid carcinomas, all of whom had documented metastases. Five of the six patients had been treated with ^{131}I in the past.

Serum of six patients with histologically proved medullary carcinoma of the thyroid were also analyzed for their thyroglobulin content. No sera of patients with anaplastic thyroid carcinoma were evaluated.

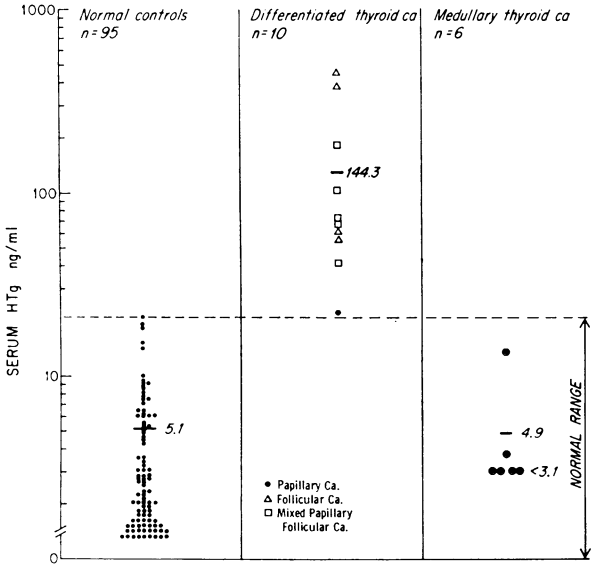

FIGURE 1 Serum HTg concentrations in patients with thyroid malignancies. Serum HTg levels in a normal population are shown in the left panel. The middle panel represents serum HTg concentrations in patients with differentiated thyroid carcinoma. The right panel represents serum HTg values in patients with medullary thyroid carcinoma. The mean for each group is indicated by a solid horizontal line.

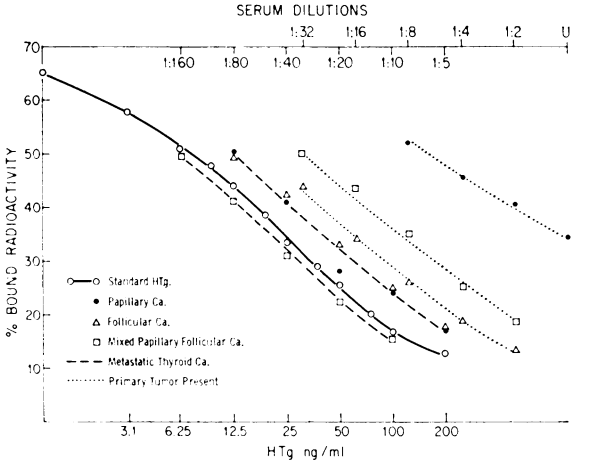

FIGURE 2 Serum dilution curves of patients with elevated HTg levels attributable to a differentiated thyroid carcinoma or metastatic involvement by such a tumor are shown to be parallel with the standard HTg preparation.

Prospective studies included the following: (*a*) 10 patients who were proved subsequently to have differentiated thyroid carcinomas were sampled before tumor removal. In seven of these patients postoperative samples were obtained at various intervals after surgery and ^{131}I therapy with a mean postsurgical interval of 3.3 mo. (*b*) Serum HTg was determined in 11 patients with nonthyroidal malignancies. (*c*) HTg levels were determined in pleural effusions of six subjects; four of these had nonthyroidal malignancies, one (R. F.) had a medullary carcinoma of the thyroid, and one (J. S.) had a mixed papillary-follicular carcinoma.

RESULTS

Serum thyroglobulin levels. Fig. 1 represents the level of serum thyroglobulin in normal subjects, in patients with differentiated thyroid carcinoma before surgery, and in patients with medullary carcinoma of the thyroid during the active phase of the disease. The mean serum thyroglobulin level in these groups were, respectively, 5.1±0.49 ng/ml (95 controls), 144.3±46.6 ng/ml (10 differentiated carcinomas), and 4.9±1.6 ng/ml (6 medullary carcinomas). The mean for the differentiated types of carcinoma was statistically significantly different from the normal control group ($P < 0.005$), whereas the serum HTg level in the medullary carcinoma group was not.

Immunologic characteristics of serum, pleural, and tumor HTg. Dilution curves of serum of patients with all three types of differentiated thyroid carcinoma were analyzed for parallelism with the standard HTg preparation. Parallelism was observed in all instances which was also the case for the dilution curves carried out with sera of patients with metastases from a differentiated carcinoma (Fig. 2). Similar studies with tumor extract, pleural fluid, and serum of one single patient

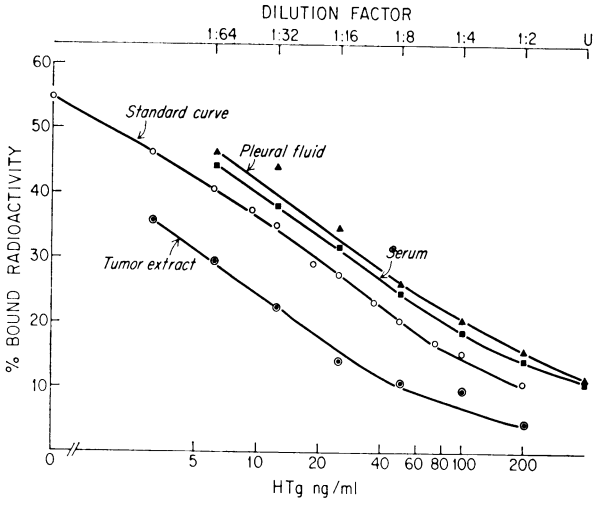

FIGURE 3 Dilution curves of several biological fluids in a single patient (J. S.) with a mixed papillary-follicular carcinoma of the thyroid. Dilution curves of serum, thyroid extract, and pleural fluid are shown to be parallel with the standard preparation used.

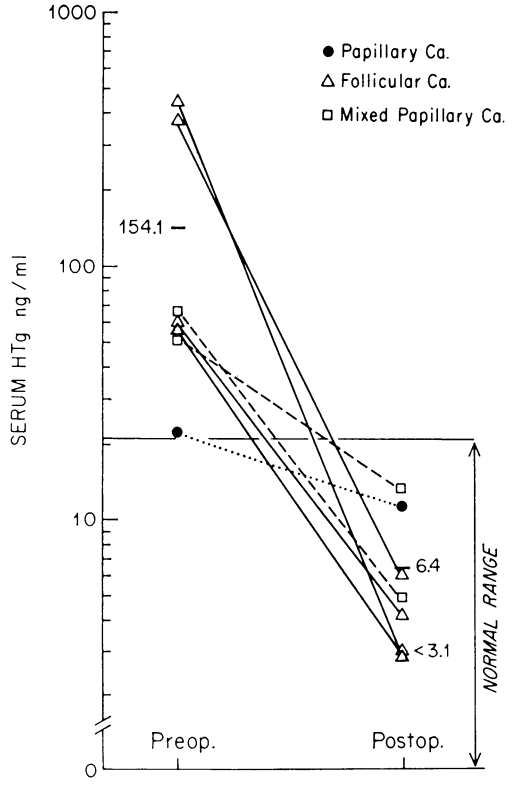

FIGURE 4 Serum HTg concentrations before and after therapy in seven patients with differentiated thyroid carcinoma are shown. Preoperative serum HTg levels are connected with postoperative concentrations for each subject. The horizontal short solid line represents the mean for the group before and after surgery.

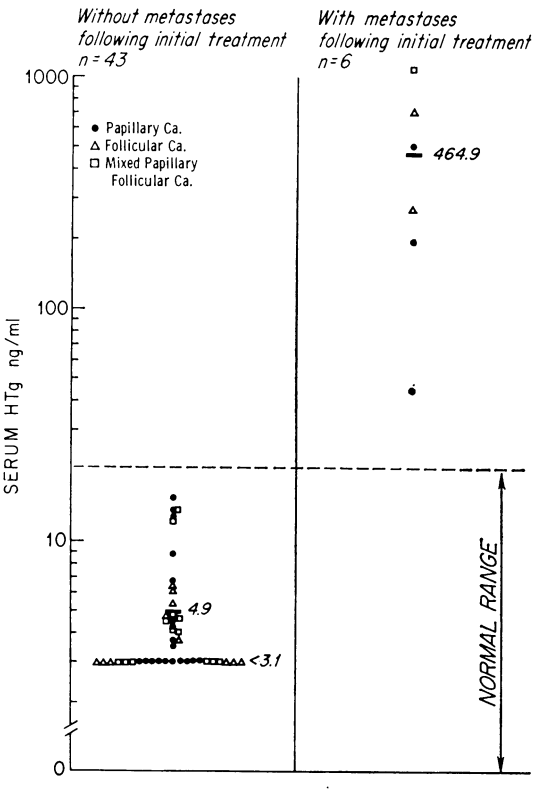

FIGURE 5 Serum HTg levels in patients with differentiated thyroid carcinoma after therapy is shown. The mean serum HTg concentration for patients without evidence of metastases (left panel) and with evidence of metastases (right panel) are indicated by the horizontal solid line. The upper limit of the normal range is indicated by the interrupted horizontal line.

(J. S.) displayed the same parallelism with the standard HTg preparation (Fig. 3).

Effect of therapy. In the prospective study data on seven patients in whom thyroglobulin levels were obtained before and after surgery and ^{131}I therapy are shown in Fig. 4. The mean preoperative serum thyroglobulin level was 154.1±66.7 ng/ml falling to a mean postoperative level of 6.4±1.5 ng/ml. The latter was obtained at a mean interval of 3.3 mo postsurgery.

Results of the retrospective study are shown in Fig. 5 which indicates the long-term postoperative results of therapy on HTg levels in patients with differentiated thyroid carcinoma. The mean level in patients free of metastatic disease, as defined previously, was 4.9±0.51 ng/ml ($n = 43$). This value was in sharp contrast with the mean level of 464.9±155.6 ng/ml observed in six patients with documented metastases from differentiated thyroid malignancies. Detectable HTg levels found with no evidence of metastatic disease were thought to be attributable to residual normal tissue in patients who had undergone a lobectomy or subtotal thyroidectomy.

To substantiate this view, data are presented from two patients who had had thyroid surgery in the past and who subsequently developed clinical symptoms suggestive of tumor recurrence (for example, voice changes in subject R. M. and development of a cervical lymph node in J. T.). At the time that these clinical symptoms developed, serum HTg levels were normal or undetectable and consequently did not suggest tumor recurrence. Both patients, however, underwent a second surgical procedure, and the following observations were made: (*a*). Subject R. M. had a serum HTg level of 4.7 ng/ml 2 yr after a right lobectomy had been performed for a follicular carcinoma of the thyroid. Subsequent extensive exploration of the neck did not reveal any malignant tissue or pathologic lymph nodes. The remaining thyroid lobe did not contain residual malignant tissue on serial sections. (*b*). Subject J. T. underwent a total thyroidectomy for a papillary carcinoma of the thyroid. She presented 19 yr later with cervical lymphadenopathy. Serum HTg levels remained undetectable at the time the lymph nodes were observed. The subsequent resected lymph nodes were free of metastatic disease.

HTg levels in serum of patients with nonthyroidal malignancies and in pleural fluid. Serum HTg levels in 12 patients with nonthyroidal malignancies are shown in Table I. Four patients have a moderately elevated serum HTg level, two of whom carried the diagnosis of a lymphoma (M. E. and L. G.). The mean serum HTg level for the total group was 19.8±6.5 ng/ml ($n = 12$) and was significantly different from the normal control group ($P < 0.05$).

TABLE I

*Serum HTg Levels in Patients with Various Nonthyroidal Malignancies**

Patient	Primary tumor	Serum HTg level
		ng/ml
M. E.	Histiocytic lymphoma	33.0
E. F.	Hodgkin's disease	7.4
L. E.	Breast carcinoma	<3.1
E. L.	Breast carcinoma	6.7
G. V.	Epidermoid carcinoma of the lung	29.0
H. C.	Spindle cell sarcoma	5.4
O. S.	Lung carcinoma	15.7
M. F.	Alveolar cell carcinoma of the lung	<3.1
L. G.	Breast carcinoma and lymphoma	74.0
N. P.	Breast carcinoma	<3.1
M. R.	Kaposi's sarcoma	6.4
E. C.	Epidermoid carcinoma of the larynx	50.5

* The mean serum HTg level for the total group ($n = 12$) was 19.8±6.5 ng/ml (SEM); this was statistically significantly different from the normal control group ($P < 0.05$). A value of 3.1 ng/ml was assigned to the sera with nondetectable levels (for interpretation of elevated serum thyroglobulin levels, see Discussion Section).

TABLE II

Pleural Fluid HTg Levels in Patients with Malignant Effusions

Patient	Primary tumor	Pleural fluid HTg
		ng/ml
B. B.	Bronchogenic carcinoma	<3.1
P. R.	Oat cell carcinoma of the lung	<3.1
P. T.	Bronchogenic carcinoma	4.5
M. R.	Kaposi's sarcoma	9.3
R. F.	Medullary carcinoma of the thyroid	<3.1
J. S.	Mixed papillary follicular carcinoma of the thyroid	1,744.0

The results of pleural fluid analysis in six patients are shown in Table II. Only two (J. S. and R. F.) with a documented malignant thyroid tumor and a pleural effusion were studied. HTg concentration in the pleural effusion of patient J. S. was 1,744 ng/ml. The original tumor in this patient was a mixed papillary-follicular carcinoma of the thyroid. In contrast, patient R. F., who had a metastatic pleural effusion from a medullary carcinoma of the thyroid, had no detectable HTg levels in the pleural fluid; however, a high thyrocalcitonin level was detected (17.0 ng/ml). The HTg levels in pleural effusions due to nonthyroidal malignancies were either undetectable or in the range for serum levels of normal subjects.

DISCUSSION

The present study investigates the levels of thyroglobulin in the circulation of patients with a differentiated thyroid carcinoma and metastases thereof.

Earlier studies measuring ^{131}I HTg in serum after ^{131}I administration suggested thyroglobulin release into the circulation (6–9). However, quantitation of this protein in the circulation was not feasible at that time. Torrigiani, Doniach, and Roitt (5), studying unlabeled thyroglobulin in the serum, reported normal levels in the circulation of a limited number of patients with papillary thyroid carcinoma. In contrast, the present study discloses an elevated serum thyroglobulin level in patients with documented differentiated thyroid carcinoma before therapy and in patients with recurrence of such tumors.

The immunological characteristics of thyroglobulin in the serum of patients with differentiated thyroid carcinoma was identical with the standard preparation used. This is not surprising since thyroglobulin of carcinomatous tissue has been shown to have the same physicochemical characteristics and immunologic prop-

erties as thyroglobulin derived from normal tissue (10). The immunologic similarity of HTg in serum, tumor extract, and pleural fluid of patient J. S. provides additional evidence for this thesis.

Inasmuch as previous studies indicated that serum thyroglobulin levels are equally elevated in patients with Graves' disease and subacute thyroiditis (4, 11), the test in its present form cannot be regarded as a specific one for the detection of thyroid cancer. This measurement, however, can be usefully applied in patients with histologically established thyroid carcinoma to assess the effective therapeutic manipulation of tumor mass if return of serum thyroglobulin levels into the normal range reflects total removal of the tumor mass. This is indeed the case since prospective and retrospective studies revealed that reduction of the HTg level toward normal or undetectable levels was associated with apparent complete tumor removal and the absence of clinically or isotopically demonstrable metastases.

The amount of thyroid tumor-cell mass necessary to generate excessive circulating thyroglobulin levels (> 20.7 ng/ml) is unknown. The data obtained from two subjects (R. M. and J. T.), where reintervention was performed notwithstanding normal serum levels, support the thesis that undetectable or normal thyroglobulin levels in a patient previously treated for thyroid cancer reflect the absence of detectable malignant tissue. Conversely, the finding of elevated serum thyroglobulin levels in our patients who were operated on previously invariably lead to the discovery of residual tumor or metastases. These observations emphasize the value of HTg measurements in the follow-up studies of patients with differentiated thyroid carcinoma.

The mechanism controlling the release of thyroglobulin into the circulation in both physiologic and pathologic conditions of the thyroid gland is unknown but is of considerable interest. Three different mechanisms could be invoked to explain the presence of elevated serum HTg levels in patients with thyroid cancer and/or metastases thereof. These include: (*a*) the HTg is released from normal thyroid tissue by a thyroid-stimulating substance in the circulation of patients with thyroid carcinoma; (*b*) the excessive levels of HTg in the circulation are due to the release from the tumor tissue itself; or (*c*) the release of HTg is a result of the invasion of normal thyroid tissue by the maligant tumor.

The first (*a*) of the mechanisms proposed hinges entirely on the presence of a thyroid-stimulating substance in the serum of patients with thyroid cancer. The presence of such a factor was suggested (12) but later denied by one group of investigators (13). As to the second possible mechanism of HTg release (*b*), the fact that serum HTg levels are increased in patients with differentiated thyroid carcinoma who underwent a total thyroidectomy and have only metastatic tissue would indeed favor this hypothesis. The observation that patients with medullary carcinoma of the thyroid (C-cell tumor) have normal serum thyroglobulin levels reinforces this view.

Finally, evidence in favor of the third hypothesis (*c*) results from the observation that invasion of the thyroid by an epidermoid carcinoma of the larynx was accompanied by an elevated serum HTg level in patient E. C. (Table I). If this third mechanism is operative, one would expect that any malignant process involving the thyroid gland could cause excessive thyroglobulin release. Previous studies by Shimaoka, Sokal, and Pickren (14) indicated that 10.9% of the patients with malignant lymphomas had involvement of the thyroid gland at autopsy. These investigators further indicated that non-Hodgkin's lymphomas had more frequent thyroidal involvement than Hodgkin's disease patients. Consistent with these pathologic studies are the findings of elevated serum thyroglobulin levels in patients M. E. and L. G., both with non-Hodgkin's lymphoma; this contrasted with the normal serum thyroglobulin level in patient E. F. with Hodgkin's disease. The elevated serum thyroglobulin level in patient G. V., who suffered from an epidermoid carcinoma of the lung, remains unexplained. Although clinical evidence of thyroid involvement was absent in these patients, confirmatory evidence by thyroid biopsy material would be required to render these observations meaningful.

Clearly the present study leaves inconclusive evidence of the mechanisms responsible for the elevated HTg levels in the circulation of patients with thyroid cancer. Likely more than one mechanism is operative in the release of thyroglobulin from a thyroid gland harboring a malignant tumor.

The decrease in the serum HTg levels after successful treatment of differentiated thyroid carcinoma and the persistent elevation in the presence of metastases or regrowth make the measurement of serum thyroglobulin a simple and inexpensive clinical test in the follow-up and health care delivery of patients with established differentiated thyroid carcinoma.

ACKNOWLEDGMENTS

Measurement of thyrocalcitonin was kindly performed by Dr. Frederick Singer of the University of Southern California Medical School and is gratefully acknowledged. We wish to express our gratitude to the following physicians who provided us with clinical information and serum samples of patients with thyroid cancer: Doctors D. Bernstein, L. Gershon, G. Kenyon, L. Lipman, N. Litman, J. Paris, S. Polin, R. Steele, and M. Weiss and all physicians of the Endocrine Division of the University of California at Los Angeles Medical Center. Our thanks also go to Dr. J. Brown for his critical review of the manuscript, and the skillfull technical help of K. Kellett and M. Greipfel is acknowledged. We like to thank Mrs. Charlotte Smith for

her excellent secretarial assistance.

This work was supported by U. S. Public Health Service Grant Ca 13447.

REFERENCES

1. Hjort, T. 1961. Determination of serum thyroglobulin by a haemagglutination inhibition test. *Lancet.* **1**: 1262–1264.
2. Assem, E. S. K. 1964. Thyroglobulin in the serum of parturient women and newborn infants. *Lancet.* **1**: 139–141.
3. Roitt, I. M., and G. Torrigiani. 1967. Identification and estimation of undegraded thyroglobulin in human serum. *Endocrinology.* **81**: 421–429.
4. Van Herle, A. J., R. P. Uller, N. L. Matthews, and Josiah Brown. 1973. Radioimmunoassay for measurement of thyroglobulin in human serum. *J. Clin. Invest.* **52**: 1320–1327.
5. Torrigiani, G., D. Doniach, and I. M. Roitt. 1969. Serum thyroglobulin levels in healthy subjects and in patients with thyroid disease. *J. Clin. Endocrinol. Metab.* **29**: 305–314.
6. Robbins, J., J. E. Rall, D. V. Becker, and R. W. Rawson. 1952. The nature of the serum iodine after large doses of ^{131}I. *J. Clin. Endocrinol. Metab.* **12**: 856–874.
7. Robbins, J. 1954. Thyroglobulin in serum after I^{131} therapy. I. Salting out. *J. Biol. Chem.* **208**: 377–386.
8. Owen, C. A., Jr., W. M. McConahey, D S. Childs, Jr., and B. F. McKenzie. 1960. Serum "thyroglobulin" in thyroidal carcinoma. *J. Clin. Endocrinol. Metab.* **20**: 187–204.
9. Valenta, L. 1971. Radioiodine-labeled proteins in the serum of patients treated with ^{131}I for thyroid disease. *Endocrinol. Exp.* **5**: 127–142.
10. Valenta, L., S. Lissitzky, M. Roques, and M. Rolland. 1969. A comparison of thyroglobulins from normal and carcinomatous thyroid tissue. *In* Thyroid Cancer. C. E. Hedinger, editor. Springer-Verlag New York Inc., New York. **12**: 234–237.
11. Glinoer, D., N. Puttemans, A. J. Van Herle, M. Camus, and A. M. Ermans. 1974. Sequential study of the impairment of thyroid function in the early stages of subacute thyroiditis. *Acta Endocrinol.* **77**: 26–34.
12. Greenspan, F. S., J. M. Lowenstein, M. N. West, and M. D. Okerlund. 1972. Immunoreactive material to bovine TSH in plasma from patients with thyroid cancer. *J. Clin. Endocrinol. Metab.* **35**: 795–798.
13. Greenspan, F. S., W. Lew, M. D. Okerlund, and J. M. Lowenstein. 1974. Falsely positive bovine TSH radioimmunoassay responses in sera from patients with thyroid cancer. *J. Clin. Endocrinol. Metab.* **38**: 1121–1122.
14. Shimaoka, K., J. E. Sokal, and J. W. Pickren. 1962. Metastatic neoplasma in the thyroid gland. *Cancer.* **15**: 557–565.