24 Aug 71

FROM: SGS/7226

SUBJECT: Research Protocol

TO: SGS

1. TITLE: The Radioimmunoassay of Human Thyrotrophin (TSH)

2. PURPOSE: The development of the radioimmunoassay of thyroid stimulation hormone is proposed to:

1) Support the study on the treatment of diffuse toxic goiter (Graves' disease).
2) Support future clinical studies on hypothyroidism (clinical detection, natural history and adequacy of treatment).

3. BACKGROUND: The TSH radioimmunoassay allows precise sensitive measurement of the blood TSH levels [1, 2, 3]. Clinically, there are two major uses for the assay: 1) the diagnosis of hypothyroidism, 2) the differentiation of primary from secondary hypothyroidism [4]. Primary hypothyroidism, caused by insufficiency of the thyroid gland itself, may be thought of as condition of inadequate circulating thyroid hormone to meet the peripheral tissue needs [5, 6, 7]. One of these "tissues" is the TSH secretory system which responds to low levels of circulating thyroid hormone with excessive TSH output [8]. The alteration in TSH secretion is reasonably rapid: fluctuations in the blood levels occur in a matter of hours [1, 2, 3] (it seems unlikely that the rate of peripheral metabolism of TSH changes so abruptly [9]). Since the distribution volume of TSH has been found to remain relatively constant in those conditions studied [3], measurement of blood TSH levels reflect the absolute pool quantity of TSH and in acute conditions TSH secretion. When the circulating thyroid hormone levels are inadequate, the TSH levels in the blood are elevated [1, 2, 3]. This has been shown to be true in patients with primary hypothyroidism of many different etiologies.

It is not known at the present time whether or not an elevated blood TSH level always means inadequate circulating thyroid hormone. There are cases of Hashimoto's thyroiditis [10], thyroid cancer [12], iodide deficiency [13] and subtotal thyroidectomy [14] who appear clinically eythyroid but have elevated TSH levels. Attempts have been made at correlating various measures of thyroid hormone with the TSH values but these have only been moderately successful [10, 11]. The free thyroxine value appears the closest related [11]. Although not settling the issue, Utiger has stated that if hypothyroidism is defined as a lack of sufficient thyroid hormone for peripheral tissue needs, then an increased blood level of TSH even with "normal" thyroid hormone levels means mild hypothyroidism [4]. Thus, it is concluded that the blood level of TSH is a most sensitive test for hypothyroidism and can be elevated prior to clinical changes in the patient.

The actual level of circulating TSH measured depends on certain variables. One variable is the population studied. The TSH level varies with the age of the patient [10] and the individual's iodide intake [13]. A second variable is the fact that the TSH blood level represents the product of both secretion and metabolism of the hormone. In the clinical conditions most often studied with this assay these two factors themselves vary [8]. The third variable lies in the nature of the radioimmunoassay technique which uses an immunologic detector (antibody and labelled hormone) that may be unable to distinguish the native hormone from intertering substances (Precursor hormones, metabolic products of the hormone, other similar hormones) [1, 15, 16, 17]. The National Institutes of Health make available a standard anti-human-TSH antibody and purified human-TSH for labelling in order that there can be correlation of results from one laboratory to another [18]. Looking at a variety of clinical conditions several authors have reported very comparable values when using these reagents [1, 10, 19]. Difficulty with interference by HCG and h-LH has varied from one laboratory to another [1, 10]. Others have used a bovine TSH system which gives higher values for circulating hormone [20, 21, 22]. It is difficult to relate the bovine and human radioimmunoassay systems in that by bioassay bovine and human TSH do not have parallel dose response curves [12, 23].

Thyrotoxicosis and blood TSH levels.

Except for one case report [24], the TSH levels measured during thyrotoxicosis have been less than normal or undetectable [1, 2, 3, 10, 12]. Patients with Graves' Disease, however, have a variable clinical course: some return to a euthyroid state [2, 5, 26], while others either from the natural course of the illness or from therapy may become hypothyroid [27, 28]. The development of hypothyroidism is heralded by elevation of the blood TSH levels. Although TSH levels have not been reported in thyrotoxic patients receiving propranolol, it is expected that these would be normal or low as Propranolol therapy has been found to not affect thyroid function [29]. Thus, an elevation of the TSH level would indicate a remission from the thyrotoxic state.

4. BIBLIOGRAPHY:

1) Odell, W.D., Wilber, J.F., Utiger, R.D.: Studies of Thyrotrophin Physiology by Means of Radioimmunoassay; Recent Progress in Hormone Research, 23:47-78, 1967.

2) Odell, W.D., Wilber, J.F., Paul, W.D.: Radioimmunoassay of Thyrotrophin in Human Serum; J. Clin. Endocrinol., 25:1179-1188, 1965.

3) Utiger, R.D.: Radioimmunoassay of Human Plasma Thyrotropin; J. Clin. Investigation, 44:1277-1286, 1965.

4) Utiger, R.D.: Thyrotrophin Radioimmunoassay: Another Test of Thyroid Function; Ann Internal Medicine, 74:627-629, 1971.

5) Sterling, K., Brenner, M.A.: Rat Diaphragm Uptake of Free Thyroxine (abstract), The ENdocrine Society Annual Meeting, New York, N.Y., June 17-19, 1965.

6) Sterling, K., Brenner, M.A.: Free Thyroxine in Human Serum: Simplified Measurement with the Aid of Magnesium Precipitation; J Clin Investigation, 45:153-163, 1966.

7) Ingbar, S.H., Freinkel, N.: Regulation of the Peripheral Metabolism of the Thyroid Hormones: Recent Progress in Hormone Research, 16:353-394, 1960.

8) Odell, W.D., Utiger, R.D., Wilber, J.F., Condliffe, P.G.: Estimation of the Secretion Rate of Thyrotropin in Man; J Clin Investigation, 46:953-959, 1967.

9) Sterling, K.S., Chodos, R.B.: Radiothyroxine Turnover Studies in Myxedema, Thyrotoxicosis and Hypermetabolism without Endocrine Disease; J Clinical Investigation, 35:806-813, 1956.

10) Mayberry, W.E., Sharib, H., Billstad, J.M., Sizemore, G.W.: Radioimmunoassay for Human Thyrotrophin; Ann Internal Medicine, 74:471-480, 1971.

11) Reichlin, S., Utiger, R.D.: Regulation of Pituitary Thyroid Axis in Man: Relationship of TSH Concentration to Concentration of Free and Total Thyroxine in Plasma; J Clin Endocrinol, 27:251-255, 1967.

12) Lemarchand-Beraud, Th.: Comparison Between Antibodies to Bovine and Human Thyrotrophin (TSH) for Radioimmunoassay in Plasma: Cross Reaction Studies with Clinical Results; acta Endocrinologica, 64:610-629, 1970.

13) Buttfield, I.H., Hetzel, B.S., Odell, W.D.: Effect of Iodized Oil on Serum TSH Determined by Immunoassay in Endemic Goiter Subjects; J Clin Endocrinol and Metab, 28:1664-1668, 1968.

14) Utiger, R.D.: Thyrotrophin in the Blood; Clinical Endocrinology, Ed:Astwood, E.B., Cassidy, C.E.; 1968, pp 25-39.

15) Berson, S.A., Yalow, R.S.: Principles of Immunoassay of Peptide Hormones in Plasma; Clinical Endocrinology, Ed:Astwood, E.B., Cassidy, C.E., 1968, pp 699-720.

16) Berson, S.A., Yalow, R.S.: Immunochemical Heterogeneity of Parathyroid Hormone in Plasma; J Clinical Endocrinology, 28:1037-1047, 1968.

17) Roth, J., Gorden, P., Pastan, I.: "Big Insulin", A New Component of Plasma Insulin Detected by Immunoassay; Proc Nat Acad Sciences, 61:138-145, 1968.

18) NPA Bulletin; National Institutes of Health, 1968.

19) Hershman, J.M., Pittman, J.A.: Utility of the Radioimmunoassay of Serum Thyrotrophin in Man; Ann Int Med, 74:481-490, 1971.

20) Lemarchand-Beraud, Th., Vannotti, A.: Radioimmunoassay of Human Plasma TSH: Current Topics Thyroid Research, Ed: Cassano, C., Andreoli, M., 1965, pp 527-541.

21) Lemarchand-Beraud, Th., Vannotti, A.: Relationships between Blood Thyrotrophin Level, Protein Bound Iodine and Free Thyroxine Concentration Under Normal Physiological Conditions; acta Endocrinologica, 60:315-326, 1969.

22) Lemarchand-Beraud, Th., Scazziga, B.R., Vannotti, A.: Plasma Thyrotropnin Levels in Thyroid Disease and Effect of Treatment; acta Endocrinol, 62:593-606, 1969.

23) Hershman, J.M.: Different Slopes of the Dose - Response Curves of Human and Bovine TSH in the McKenzie Bioassay; Endocrinol, 86:1004-1007, 1970.

24) Hamilton, C.R., Adams, L.C., Maloof, F.: Hyperthyroidism due to Thyrotropin Producing Pituitary Chromophobe Adenoma; New England J Med, 283:1077-1080, 1970.

25) Ingbar, S.H.: Physiologic Considerations in Treatment of Diffuse Toxic Goiter; Ann Internal Medicine, 107:198-217, 1961.

26) Hershman, J.M., Givens, J.R., Cassidy, C.E., Astwood, E.B.: Long Term Outcourse of Hyperthyroidism Treated with Antithyroid Drugs; J Clin Endocrinol, 26:803-807, 1966.

27) Wyse, E.P., McConahey, W.M., Woolner, L.B., Scholz, D.A., Kearns, T.P.: Ophthalmopathy without Hyperthyroidism in patients with Histologic Hashimotos' Thyroiditis; J Clin Endocrinol, 28:1623-1629, 1968.

28) Skillman, T.G., Mazzaferri, Gwinup, G.: Random Dosage of ^{131}I in the Treatment of Hyperthyroidism: Results of a Prospective Study; The Amer J of Medical Sciences, 257:382-387, 1969.

29) Stout, B.D., Wiever, L, Cox, J.W.; Combined Alpha and Beta Sympathetic Blockade in Hyperthyroidism; Ann Internal Medicine, 70:963-970, 1969.

30) Medical Research Council Bulletin, Division of Biologic Standards, World Health Organization, 1966.

31) Hunter, W.M., Greenwood, F.C.: Preparation of Iodine-131 Labelled Human Growth Hormone of High Specific Activity; Nature, 194:495-496, 1962.

32) Hunter, W.M.: The Preparation of Radioiodinated Proteins of High Activity. Their Reaction with Antibody in Vitro: The Radioimmunoassay; Handbook of Experimental Immunology, Ed: Weir, D.M., F. A. Davis Co. 1967, pp 608-654.

33) Berson, S.A., Yalow, R.S.: Immunoassay of Protein Hormones; The Hormones, Ed: Pincus, G., Thimann, K.V., Astwood, E.B., Vol IV, 1964, pp 557-630.

34) Morgan, C.R., Lazarow, A.: Immunoassay of Insulin: Two Antibody System; Diabetes, 12:115-126, 1963.

35) Rodbard, D., Bridson, W., Rayford, P.L.: Rapid Calculation of Radioimmunoassay Results: J Lab Clin Med, 74:770-781, 1969.

36) Rodbard, D., Rayford, P.L., Cooper, J.A., Ross, G.T.: Statistical Quality Control of Radioimmunoassays, J Clin Endocrinol, 28:1412-1418, 1968.

5. Technical Approach:

The TSH radioimmunoassay will be patterned after the methods of Odell, et al [1, 2]. This method has been summarized and is distributed to investigators by the National Pituitary Agency [18]. The National Pituitary Agency and Medical Research Council make available Human TSH, Rabbit anti-human-TSH antibody and the Human TSH Research Standard A [18, 30]. Radioiodination of the h-TSH will be done by the method of Hunter and Greenwood [31]. Chloramine-T is used to activate the radioiodide which is then able to substitute for hydrogen in the ring component of the tyrosine residues in TSH [32]. The iodinated hormone is then separated from free iodide and the damaged fragments of the hormone by gel filtration chromatography. The assay itself depends on the competition of labelled and unlabelled hormone for a specific antibody [33]. The antibody bound hormone is separated from the reaction mixture by a second antibody, an anti-rabbit gamma globulin antibody [34]. The methods of calculation and statistical concepts of Rodbard, et al will be applied to the assays [35, 36].

6. EQUIPMENT:

1)	Sodium Iodide - 131 or 125 (a2 weeks) Iso-Serve Division of Cambridge Nuclear Corporation Cambridge, Mass	$20.00/2mCi	
2)	Hamilton Repeating Dispensor Syringe PB-600-10 5.0 cc volume Hamilton Company Whittier, Calif	5 Syringes	$45.00 ea
3)	Repipette 1.0 ml (one) Curtin Scientific Co.		$52.50
4)	Lang-Levy micropipettes 300 ul #60 Scientific Products		$2.50 each

5)	Spanish Goats (4). (Development of Second Antibody)	$25.00 each	
6)	1.0 ml Graduated Pipettes Curtin Scientific Co.	60 -	$125.00
7)	0.2 ml Graduated Pipettes Curtin Scientific Co.	36 -	$ 75.00
8	Chromatography Column 30 x 1 Curtin Scientific Co.	2 -	$ 25.00
9)	Lambda Pipettes		
	5 ml	2	$ 5.00
	10 ml	2	$ 5.00
	20 ml	2	$ 5.00
	50 ml	2	$ 5.00
	100 ml	2	$ 5.00
10)	Disposable Lambda Pipettes 10 ml Scientific Products	500 -	$ 15.00
11)	Bovine Serum Albumin Pemtex Inc	2 gms	$ 10.00
12)	Chloramine T J T Baker Laboratory Chemicals	250 gms	$ 2.50
1.	Bio Gel P-10 Bio-Rad Laboratories	200 gms	$ 18.00

7. INVESTIGATION SCHEDULE: Development of the assay will begin following approval from the Wilford Hall Medical Center Research Committee.

8. PERSONNEL DATA:

Medical Facility Commander: Paul W. Myers, Col, USAF, MC

Principle Investigator: James C. Reynolds, Major, USAF, MC
Staff, Endocrinology and Metabolism

Associate Investigators: Edwin M. Bradley, Col, USAF, MC
Chief, Endocrinology and Metabolism

Ernest L. Mazzaferri, Lt Col, USAF, MC
Staff, Endocrinology and Metabolism

9. MANPOWER:

Three (3) Staff Endocrinologists	24 hours/week
One (1) SSgt Laboratory Technician	12 hours/week

James C. Reynolds.

JAMES C. REYNOLDS, Major, USAF, MC
Staff, Endocrinology and Metabolism