FROM: SGHM 23 November 1971

SUBJECT: Research Proposal

TO: SGS

1. TITLE: Measurement thyroid hormone and Thyroid Stimulating Hormone in Pregnancy and Early Life.

2. PURPOSE: This study will have two major objectives:

a. The levels of thyroid hormone and TSH will be determined in normal pregnant women and their newborn.

b. The effectiveness of oral thyroid hormone to suppress maternal and neonatal thyroid stimulating hormone will be determined.

3. BACKGROUND:

Clinical: In pregnancy there are three specific clinical indications for thyroid hormone therapy. The first is the treatment of thyroid inadequacy in the mother.[1] The second is in the treatment of pregnant women with thyrotoxicosis.[2,3] The mother's thyroid overproduction may be controlled by antithyroid drugs which also block the child's thyroid gland.[4] To prevent hypothyroidism in the child, thyroid hormone has been given to the mother concurrently with the antithyroid drug.[3] However, it is not certain how much of this thyroid hormone passes through the placenta to reach the child.[5,6,7] Nor is it certain how this maternal thyroid hormone affects the child's peripheral tissues such as the TSH secretory mechanism.[8] There have been no reports of TSH levels of neonates from mothers who were given thyroid hormone therapy. The third clinical indication for thyroid hormone therapy in pregnancy is the development of a uni-nodular goiter where suppression of TSH is desired.[9] It has recently been found that the placenta can produce a substance very similar to pituitary TSH.[10, 11] Its pattern of secretion in pregnancy as measured by bioassay suggests that it is of greatest blood concentration in the first trimester.[11] Most likely this factor is not suppressable by thyroid hormone therapy. It is obviously important that pregnant women who have thyroid inadequacy receive thyroid hormone.[1] But it is not clear what efficacy thyroid hormone therapy has in preventing subtle developmental changes in the neonate whose mother is receiving an antithyroid drug.[6] And it is also not clear what effect this therapy has in reducing the size of a nontoxic uni-nodular goiter developing during pregnancy. To answer these questions, it seems important to study thyroid stimulating factors in pregnant women and their newborn; to determine whether these factors are similar to pituitary or placental substances and whether they suppress with a standard dose of thyroid hormone.[11]

4. Bibliography:

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5. Laboratory:

a. Thyrotropin human [illegible] thyroids by [illegible] extracts from placental tissue [illegible] in molecular size and biologic activity appears very similar to human pituitary [illegible].[11] However, [illegible] it is more closely related to bovine than human TSH.[11] [illegible] gives an incomplete non-identical dose response curve in the human radioimmunoassay system at 0-10% and complete identical response to bovine TSH in the b-TSH system.

Serum TSH levels during pregnancy are elevated[11, 15, 16] with the greatest values occurring during the first trimester. Most likely HCT levels are greatest during this period and if they are, the elevated levels of TSH as measured by the human TSH radioimmunoassay could represent cross reactivity of the placental material. These facts suggest that study of the nature of thyroid stimulating factors during pregnancy should include its identification and quantitation with a bovine TSH radioimmunoassay.

6. Technical Approach:

a. Clinical: Two groups of patients will be studied: The first group of 20 normal pregnant women, the second a group of 20 pregnant women who will receive a daily standard oral dose of thyroxine (0.1 mgm/day).[12] (This second group will be selected from those patients who by history appear to have normal thyroid function, and yet for whatever reasons were started on thyroid hormone prior to the onset of gestation.).

1) Upon entering the study each patient will receive a careful clinical evaluation by obstetrical and endocrine investigators.

2) Routine laboratory studies will be those of the normal prenatal care evaluation including CBC, urinalysis and blood urea.

3) Monthly blood specimens will be drawn for thyroxine by competitive protein binding technique, T_3 resin uptake and serum immunoreactive TSH.

4) Monthly clinical evaluation will be made by members of the Obstetrical Department unless the patient develops a goiter during pregnancy at which time she will be transferred into the obstetrical complications clinic and be evaluated monthly by both obstetrical and endocrine investigators.

5) At delivery maternal and cord blood will be drawn for serum total thyroxine, T_3 resin uptake, and TSH determinations. Thyroid hormone therapy will be discontinued at this time for patients in group two (2).

6) Six weeks postpartum all patients will have blood drawn for serum total thyroxine, T_3 resin uptake and TSH. Patients in group 2 will also have an I^{131} uptake and thyroid scan performed.

7. Laboratory:

a. The human TSH radioimmunoassay already developed for clinical research purposes will be used. Protocol #1-36 (71). A second radioimmunoassay to bovine TSH will be developed and patterned after the NIH Double Antibody Assay.[15] The steps in the development of this assay are as follows:[16]

1) Development of a suitable antiserum against bovine TSH by immunization of both guinea pigs and rabbits with partially purified b-TSH.

2) Radioiodination of purified bovine NIH TSH-B5 with $Na^{125}I$ or $Na^{131}I$ (17) (NIH TSH-B5 has already been received from the National Pituitary Agency.

3) Titering the antisera and establishment of a standard curve using the USP bovine thyrotropin.[18] (Available from the U.S. Pharmacopia Convention, Inc.).

The methods of calculation and the statistical concepts of Rodbard et. al., will be applied to the assays.[19,20]

8. Equipment:

a.	Sodium Iodide - 131 or 125 - 2 weeks. 20.00/2 mCi. Isoserve Division of Cambridge Nuclear Corporation Billeria, Mass.	
b.	Hamilton Repeating Dispenser Syringe 1-3 PB-600-10 5.0 cc volume Hamilton Company Whittier, California	$45.00 ea.
c.	Repipette - 1.0 ml (one) Curtin Scientific Company	$52.50
d.	Rabbits white, New Zealand (10)	$50.00
e.	Guinea Pigs (15)	$100.00
f.	Biopette Disposable Tips SChwartz Mann (1000)	$39.00
g.	Chromatography Column 30 x 1 cm Curtin Scientific Co. - 2	$25.00

h. Bio Gel P-10 - 200 gms $18.00
Bio-Rad Laboratories

i. DEAE Cellulose - 500 gms $29.00
Bio-Rad Laboratories

j. Carboxy Methyl Cellulose - 500 gm $23.50
Bio-Rad Laboratories

k. **Cost of TDY and Publication** **$700.00**

9. Investigation Schedule

Upon approval of the Wilford Hall USAF Medical Center Research Committee

a. Entering of patients into the study will begin. It is expected that all patients will have completed gestation by September 1972.

b. Development of antisera for the bovine TSH radio-immunoassay will begin.

10. Personnel Data:

a. Medical Facility Commander: Paul W. Myers, Colonel, USAF, MC

Principal Investigators: Ernest L. Mazzaferri, Lt Colonel, USAF, MC
Staff, Endocrinology and Metabolism

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

Alvin L. Brekken, Lt. Colonel, USAF, MC
Chief, Obstetric Service

Associate Investigators: Robert L. Young, Lt Colonel, USAF, MC
Chief, Endocrinology and Metabolism.

11. Manpower

Three (3) Staff Endocrinologists --2.4 hours/week
One (1) Sgt Laboratory Technician 12 hours/week.

Ernest L. Mazzaferri

ERNEST L. MAZZAFERRI, Lt Colonel, USAF, MC
Staff, Endocrinology-Metabolism

James C. Reynolds

JAMES C. REYNOLDS, Major, USAF, MC
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