FROM: SGHGE 8 November 1972

SUBJECT: Research Protocol

TO: SGS

1. TITLE: The Radioimmunoassay of Serum Androgens: Testosterone, Androstenedione, and Dehydroepiandrosterone (DHA)

2. PURPOSE: The development of radioimmunoassay of serum androgens is proposed to: a. support existing clinical investigations in reproductive physiology and b. establish these procedures for studies in patients with Hirsutism.

3. BACKGROUND: Of the many androgenic steriods present in human sera, testosterone, androstenedione, and DHA seem to be the most important. All three are secreted in normal women by both the ovary and adrenal glands (10, 14, 16). In addition, peripheral conversion to testosterone from the weaker androgens, androstenedione and DHA, can take place in normal women (1, 8, 15). Even though androgens, notably androstenedione, are important precursors for estrogen production in the ovarian follicle, some evidence exists that the major fraction of secreted ovarian androgens may be synthesized in the stroma (22,23). Studies of ovarian vein blood either at the time of surgery or percutaneous ovarian vein catherterization, have indicated that the testosterone content in ovarian vein blood exceeds that of peripheral vein blood (10, 12). That the normal adrenal gland secretes testosterone has been well established by percutaneous catherterization of the adrenal vein. Testosterone gradients were present in 13 of 19 adrenal venous samples in normal women (10).

Certain pathological conditions of the ovaries and adrenals may lead to varying degrees of defeminization from mild hirsutism to complete virilization. In women with idiopathic hirsutism, peripheral testosterone levels vary from the normal range to elevated (9). Patients with polycystic ovaries also may have peripheral testosterone levels within the normal range but they to may have elevated levels (5, 9, 14). Venous effluents from polycystic ovaries, measured at the time of wedge resection, contained high levels of both testosterone and androstenedione (14). Certain ovarian tumors such as arrhenoblastoma, hilar cell tumors, and adrenal rest cell tumors of the ovary can secrete enough androgens to give male levels of testosterone in peripheral blood (7, 11, 18, 19). In many of these patients with ovarian virilizing tumors, urinary 17-ketosteroids may be within the normal range since testosterone is secreted in μg quantities and therefore contributes little to the mg measurement of urinary steriods (7).

Women with adrenal disorders such as congenital adrenal hyperplasia have peripheral testosterone levels in the range of normal men. Androstenedione levels in plasma from these patients are also increased and in general are higher than those of other virilized subjects (6). Adrenal neoplasms may secrete levels of testosterone and the weaker androgens at higher rates than normal men (9, 17).

In spite of all that is known about secretion of androgens by the ovaries and adrenals in normal women and pathological conditions, the clinical usefulness of peripheral blood values remains very ambiguous in many cases. Combined ovarian and adrenal vein catherization has shown that either the ovaries, adrenals, or both ovaries and adrenals may be the source of androgen overproduction in hirsute women, including subjects with polycystic ovaries (5, 10, 25). The relationship of serum gonadotropins to serum androgens and other serum steriods such as estradiol and progesterone has not been sufficiently studied in normal women and anovulatory women with or without hirsutism

Alternate use of ovarian suppression and stimulation with estrogens and human chorionic gonadotropin (HCG) combined with adrenal suppression and stimulation with glucocorticoids and ACTH has failed to identify the source of excessive androgens in most hirsute women (7, 9, 10, 17). The majority of these studies have had to depend on measurement of androgen metabolites in urine rather than the major steriods secreted into the blood by the ovaries and adrenals (4).

4. BIBLIOGRAPHY:

1. Bardin, C.W., and Lipsett, M.B., Jr.: Testosterone and androstenedione blood production rates in normal women and women with idiopathic hirsutism or polycystic ovaries. J Clin Invest 46:891, 1967.

2. Erlanger, B.F., Barek, F., Bieser, S.M., and Liberman, S.: Steroid-protein conjugates. I. Preparation and characteristics of conjugates of bovine serum albumin with testosterone and with cortisone. J Biol Chem 228:713, 1957.

3. Furuyama, S., Mayes, D., and Nugent, C.A.: Radioimmunoassay for plasma testosterone. Steroids 16:415, 1970.

4. Gambrell, R.D., Jr., Greenblatt, R.B., and Mahesh, V.B.: Serum gonadotropins and ancillary studies in women with Stein-Leventhal syndrome treated with clomiphene citrate. Obstet Gynec 38:850, 1971.

5. Gambrell, R.D., Jr: Is there inappropriate secretion of OH in the Stein-Leventhal syndrome? Presentation at Armed Forces District Meeting, American College of Obstetricans and Gynecologists, Seattle, October, 1972.

6. Gandy, H.M., and Peterson, R.E.: Measurement of testosterone and 17-ketosteroids in plasma by the double isotope dilution derivative technique. J Clin Endocr 28:949, 1968.

7. Greenblatt, R.B., Mahesh, V.B., and Gambrell, R.D., Jr.: Arrhenoblastoma: Three Case Reports. Obstet Gynec 39:567, 1972.

8. Horton, R., and Tait, J.F.: In vivo conversion of dehydroisoandrosterone to plasma androstenedione and testosterone in man. J Clin Endocr 27:79, 1967.

9. Kirschner, M.A., and Bardin, C.W.: Androgen production and metabolism in normal and virilized women. Metabolism 21:667, 1972.

10. Kirschner, M.A., and Jacobs, J.B.: Combined ovarian and adrenal vein catherterization to determine the site(s) of androgen overproduction in hirsute women. J Clin Endocr 33:199, 1971.

11. Lipsett, M.B., Kirschner, M.A., Wilson, H., and Bardin, C.W.: Malignant lipoid cell tumor of the ovary: clinical, biochemical, and etiological considerations. J Clin Endocr 30:336, 1970.

12. Lloyd, C.W., Lobotsky, J., Baird, D.T., Pupkin, M., Zanartu, J., and Puga, J.: Concentrations of unconjugated estrogens, androgens, and gestagens in ovarian and peripheral venous plasma of women: the normal menstrual cycle. J Clin Endocr 32:155, 1971.

13. Loriaux, D.L., Malinak, L.R., and Noall, M.W.: The contribution of plasma dehydroepiandrosterone sulfate to testosterone in a virilized patient with an arrhenoblastoma. J. Clin Endocr 31:702, 1970.

14. Mahesh, V.B., Greenblatt, R.B., Hydar, C.K., and Roy, S.: Secretions of androgens by the polycystic ovary and its significance. Fertil Steril 13:513, 1962.

15. Mahesh, V.B., and Greenblatt, R.B.: The in vivo conversion of DHA and androstenedione to testosterone in the human. Acta Endocr 41:400, 1962.

16. Mahesh, V.B., and Greenblatt, R.B.: Steroid secretion patterns of the normal and polycystic ovary. Recent Prog Hormone Res 20:341, 1964.

17. Mahesh, V.B., Greenblatt, R.B., and Coniff, R.F.: Urinary steroid secretions before and after dexamethasone administration and steroid content of adrenal tissue and venous blood invirilizing adrenal tumors. Amer J Obstet Gynec 100:1043, 1968.

18. Mahesh, V.B., McDonough, P.G., and Deleo, C.A.: Endocrine studies in the arrhenoblastoma. Amer J. Obstet Gynec 107:183, 1970.

19. Osborn, R.H., Bradbury, J.T., and Yannone, M.E.: Androgen studies in a patient with lipoid cell tumor of the ovary. Obstet Gynec 33:666, 1969.

20. Rodbard, D., Bridson, W., Rayford, P., Cooper, J.A., and Ross, G.T.: Statistical quality control of radioimmunoassays. J Clin Endocr 28:1412, 1968.

21. Rodbard, D., Bridson, W., and Rayford, P.L.: Rapid calculations of radioimmunoassay results. J Lab Clin Med 74:770, 1969.

22. Rice, B.F., and Savard, K.: Steroid hormone formation in the human ovary. IV. Ovarian stromal compartment; formation of radioactive steroids from acetate 1-^{14}C and actions of gonadotropins. J Clin Endocr 26:593, 1966.

23. Ryan, K.J., and Smith, O.W.: Biogenesis of steroid hormones in the human ovary. Recent Progr Hormone Res 21:326, 1965.

24. Sato, R., Shinada, T., and Matsumoto, S.: A clinical and metabolic study of masculinizing arrhenoblastoma. Amer J. Obstet Gynec 104:1124, 1969.

25. Stahl, N.L., Teeslink, C.R., Beauchamps, G., and Greenblatt, R.B.: Serum teststerone levels in hirsute women. A comparison of adrenal, ovarian, and peripheral vein values. Presentation at Armed Forces District Meeting, American College of Obstetricians and Gynecologists, Seattle, October, 1972.

26. Zourlas, P.A., and Jones, H.W., Jr.: Stein-Leventhal syndrome with masculinizing ovarian tumors: Report of three cases. Obstet Gynec 34:861, 1969.

5. TECHNICAL APPROACH:

It is necessary to obtain the following materials from Inter Science Institute for the development of a radioimmunoassay (RIA) for both testosterone and androstenedione: Testosterone Antisera and Androstenedione Antisera.

The steps for achieving the end goal in this project will be carried out as follows:

(1) Obtain the necessary materials from Inter Science Institute to set up the radioimmunoassays as soon as possible so that the serum samples already on hand can be analyzed.

(2) Prepare teststerone and androstenedione antisera in rabbits.

(3) Since antisera for dehydroepiandrosterone is not commericially available, attempts to prepare our own will be initiated as soon as the first two steps are accomplished.

The calculations of the data from the radioimmunoassays for serum androgens and statistical applications will be patterned after those of Rodbard, et al, (20, 21).

Serum has already been collected daily and frozen after assaying for FSH and LH from normal subjects during a menstrual cycle. Serum is being collected daily from anovulatory patients in the Infertility Clinic during cycles in which clomiphene citrate is given to induce ovulation. As the radioimmunoassays for serum testosterone, androstenedione, and DHA are established, these stored frozen sera will be extracted and assayed for serum androgens. Radioimmunoassays for estradiol, estrone, and progesterone are also being performed on the same sera. When all the assays are completed, correlation of serum gonadotropins and the major steroid hormones of the ovaries will be made in the normal menstrual cycle as well as in anovulatory patients treated with clomiphene citrate.

In addition, anovulatory patients with hirsutism will have additional studies with standard drugs prior to clomiphene citrate therapy. Following collection of baseline sera for gonadotropins, estrogens, progesterone, and androgens, the ovaries will be alternately stimulated with HCG and suppressed with estrogens while the adrenals are simultaneously suppressed with dexamethasone and stimulated with ACTH.

6. EQUIPMENT:

1.	Antisera:	
	Teststerone 500 tubes	$200.00
	Androstenedione - 500 tubes	225.00
2.	Tritiated steroids:	
	Testosterone - H^3 - 1 mCi	90.00
	Androstenedione - H^3 - 1 mCi	75.00
3.	Standard Chemicals: Methylene Chloride, Silica Gel, Liquifluor, and Benzene	300.00
4.	White rabbits - 12 each	
5.	Hamilton Repeating Dispensers and Syringes 4 each	on hand
6.	Repipetts - 2 each	on hand
7.	Chromatography columns	on hand
8.	Biopette Disposable Tips - 1000 each	39.50
	Total	$929.50

7. INVESTIGATION SCHEDULE:

a. Development of radioimmunoassay for serum testosterone and androstenedione will begin following approval of the Wilford Hall USAF Medical Center Research Committee and since commercial antisera are available the estimated completion time is three months.

b. Production of our own testosterone and androstenedione antibodies will be simultaneously undertaken by injecting the steroid - BSA complex into rabbits. Estimated completion time - four to six months.

c. Since no commerical antisera for radioimmunoassay of DHA is available production of this antibody will be undertaken by injecting the DHA - BSA complex into rabbits. Estimated completion time - six to twelve months.

8. SUBJECTS:

Serum has already been collected and frozen during the menstrual cycles of several normal ovulatory women and a few anovulatory patients treated with clomiphene citrate. These will be continued to be collected from patients in the Infertility Clinic as part of the on-going studies in reproductive physiology. In addition, women with hirsutism will be studied from the Gynecological Endocrinology Service.

9. DRUGS:

Standard drugs such as clomiphene citrate will be used to induce ovulation as currently used in clinical practice. Other standard drugs such as HCG, estrone sulfate, ethinyl estradiol, ACTH, and dexamethasone will be used to evaluate hirsutism as currently used in such clinical evaluations.

10. PERSONNEL DATA:

Medical Facility Commander: Paul W. Myers, Brig. General, USAF, MC

Principle Investigator: Richard D. Gambrell, JR., Colonel, USAF, MC

Associate Investigator: Larry C. Gilstrap,III, Captain, USAF, MC

11. MANPOWER:

Colonel, AFSC 9496A, 50 hours duty time, 50 hours off-duty time - 1
Sergeant, Laboratory Technician, AFSC 99127, 30 hours/week duty time
10 hours/week off-duty time - 1
Captain, AFSC 9491, 40 hours duty time, 40 hours off-duty time - 1

Richard D. Gambrell, Jr.

RICHARD D. GAMBRELL, JR., Colonel, USAF, MC
Chief, Gynecological Endocrinology Service
Department of Obstetrics and Gynecology