716307

Reprinted from: THE HUMAN TESTIS
(Plenum Press, 1970)

THE ROLE OF FSH, ICSH, AND ENDOGENOUS TESTOSTERONE DURING TESTICULAR SUPPRESSION BY EXOGENOUS TESTOSTERONE IN NORMAL MEN

Carl G. Heller, Howard C. Morse, Mike Su, and Mavis J. Rowley

Division of Reproductive Physiology, Pacific Northwest Research Foundation, 1102 Columbia Street, Seattle, Washington 98104

Testosterone administered to normal men is know to depress spermatogenesis to azoospermia, as reflected in the ejaculate, and to depress Leydig cell morphology to the point that only mesenchymal cells and fibroblasts are identifiable. It is also know that urinary total gonadotropins are concomitantly depressed to the point that they are undetectable in 24 hour urine aliquots administered to single assay rats (1). It has been widely assumed, but not proved, that the depression of human testicular function by administering testosterone is secondary to pituitary suppression.

Three possible mechanisms of action of testosterone upon the testis were originally postulated in the following investigation: 1) that the depression is solely due to total pituitary gonadotropin suppression, 2) that the depression is solely due to a direct effect of testosterone upon the germinal epithelium and/or Leydig cells or 3) that the depression is due to a combination of direct testicular and indirect gonadotropin suppression.

To test which of these three postulates apply, the following protocol was planned. Testosterone enanthate (200 mg intramuscularly (I.M.) per week) was to be administered to normal men until the following three events occurred: azoospermia, Leydig cell disappearance and disappearance of gonadotropins in 24 hour urine aliquots. Then, while continuing the testosterone administration at the same level, human chorionic gonadotropin (HCG) was to be added for its interstitial cell-stimulating hormone (ICSH) effect (4000 International Units (I.U.) HCG I.M. per two days). If Leydig cell morphology could thus be restored to normal, as revealed by testicular biopsy examination, then a direct effect of testosterone upon

1 cy to Brooke, DBM - 11/16/70

3002164

Leydig cell depression could be eliminated from further consideration. The suppression of Leydig cells by testosterone would then be attributed to the suppression of pituitary ICSH (if no restoration occurred the direct effect of testosterone on Leydig cells must still be considered). Assuming restoration of Leydig cell morphology, the plan was to add follicle-stimulating hormone (FSH) in the form of Pergonal in order to restore spermatogenesis, while continuing to administer the testosterone enanthate and continuing the HCG at the same levels for each. If spermatogenesis were now restored we could conclude that the inhibitory effect of testosterone upon the germinal elements was mediated by suppression of FSH.

To this end three healthy men, ages 37, 33 and 29 were chosen to serve as subjects. They were determined to be reproductively normal by hormonal studies, sperm concentration and examination of control testicular biopsy specimens. Testicular biopsies were obtained and prepared using the methods of Rowley and Heller (2).

Testosterone enanthate (Delatestryl, Squibb) was administered at the rate of 200 mg I.M. once weekly for eight to 12 months. Human chorionic gonadotropin (APL, Ayerst, 4000 I.U. I.M. every second day) was added beginning four months after the initiation of testosterone enanthate administration. The duration of HCG administration was four and one-half months. Testosterone enanthate was continued alone on two subjects after HCG was discontinued.

Seminal fluid specimens were collected once weekly and examined for sperm concentration, motility and volume. Concentration was measured using the hemocytometer method. Each subject was used as his own control. Sperm morphology was examined by Dr. John MacLeod of Cornell University Medical College.

Twelve-hour (overnight) urine samples were collected and pooled in 48 hour collections. Total gonadotropins and ICSH determinations were performed on aliquots of the urine pools. Urines were kept below 5°C until the extraction procedures were carried out. Urinary gonadotropins were extracted using the kaolin-acetone method of Albert (3). Total gonadotropin levels were determined by measuring the ovarian weights of immature rats and expressed as mgU/24 hours of a standard human urinary postmenopausal preparation (Pergonal P-22C). Urinary ICSH was determined biologically using the method of Greep et al. (4), measuring the increase in ventral prostate weight in hypophysectomized immature male rats. The values were expressed as mgU/24 hours of the standard preparation (Pergonal P-26E). Testicular biopsies were examined quantitatively using the germ cell/Sertoli cell ratio method as developed by Heller et al. (5).

The administration of testosterone enanthate 200 mg I.M. weekly for four months resulted in barely detectable or absent total urinary gonadotropins, azoospermia as judged from ejaculated seminal

3002165

fluid samples, disappearance of Leydig cells as judged by testicular biopsy examination and marked reduction in numbers of Sd, Sc, Sb and Sa spermatids, marked reduction in pachytene, zygotene and leptotene spermatocytes, slight reduction in preleptotene spermatocytes and type B spermatogonia, questionable reduction of type A pale spermatocytes and no apparent reduction in type A dark spermatocytes (Figs. 1-2)

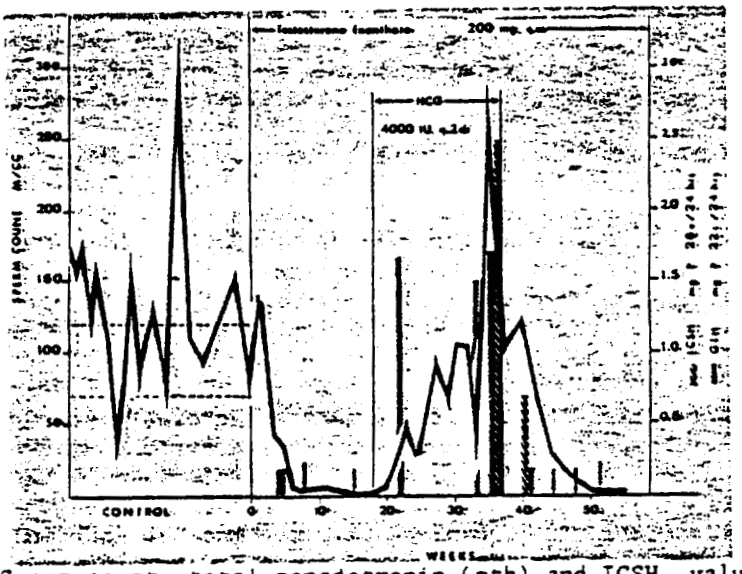

Fig. 1. Sperm count, total gonadotropin (gth) and ICSH values shown in a subject during control, testosterone and testosterone-HCG administration periods. Dashed lines across control period indicate range of normal gth and ICSH values. Bars with arrows pointing to bottom line indicate absent or barely detectable gth or ICSH.

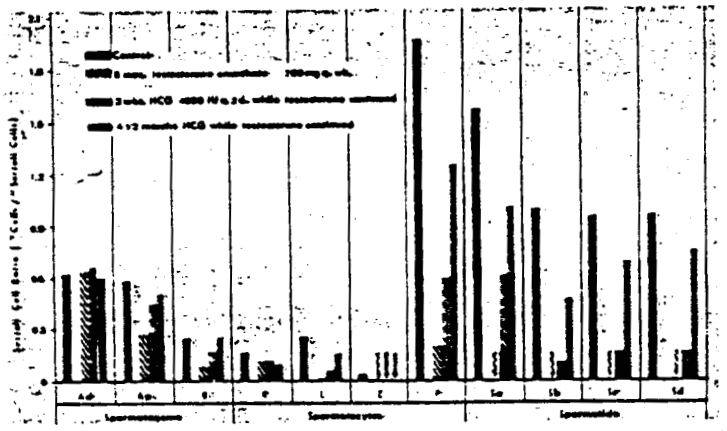

Fig. 2. Comparison of Sertoli cell ratios in biopsies taken during testosterone administration and twice during the additional administration of HCG. Control values for each cell type were determined from the average of the quantitation of 50 normal testicular biopsies. Arrows pointed to bottom line indicate zero values.

3002166

Adding 4000 I.U. of HCG administered I.M. every second day brought about complete restoration of Leydig cell morphology in all five biopsies taken during HCG administration. Note should be made that two of these biopsies were taken as early as three weeks after the initiation of HCG administration.

Therefore, since testosterone caused Leydig cell disappearance and adding HCG caused restoration of Leydig cells to normal, we conclude that testosterone-caused Leydig cell depression is mediated by suppressing anterior pituitary ICSH secretion or release.

In the meanwhile, much to our surprise, the weekly seminal fluid examinations revealed an increase in sperm concentration by one month in one subject and by the second or third months in the other two. Between the third and the fourth month of HCG administration each subject had reached pre-testosterone treatment control levels (Fig. 1). Two reached the average of the control levels and one rose above the minimal control levels but did not rise as high as his pre-treatment average.

Quantitation of the elements of the germinal series in the two testicular biopsies obtained three weeks after the initiation of HCG revealed a partial restoration of leptotene and pachytene spermatocytes and significant but small increases in each of the four spermatid types. The second biopsy, obtained at four and one-half months following initiation of HCG, revealed complete restoration of each of the depressed germinal cells to pre-treatment levels in the two subjects whose sperm concentration had returned to average pre-treatment levels (Fig. 2). The third subject, whose sperm concentration had not reached the average of his pre-treatment levels, revealed definite but incomplete restoration of each of the germinal cells.

These results suggest that 1) testosterone does not exert a direct depressive effect upon either the germinal cells or the Leydig cells since both elements can be restored to normal by administering HCG, 2) administering HCG during testosterone administration restores spermatogenesis either by providing sufficient FSH to obtain this effect or because testosterone does not depress FSH secretion or release in normal male human subjects, and further 3) HCG stimulates enough Leydig cell androgen production to aid in the restoration of spermatogenesis in the presence of FSH.

As a consequence of the unexpected germinal cell response to HCG administration without the addition of FSH there was no reason to follow the original protocol. Thus no Pergonal was administered and the HCG was discontinued. In order to confirm the effect of testosterone upon the two testicular systems the testosterone was continued for an additional four months in the two subjects who had complete restoration of spermatogenesis.

3002167

The two subjects responded as before in that the weekly seminal fluid values decreased to azoospermia, and the gonadotropins dropped to levels barely detectable or absent. The Leydig cells disappeared and the germinal elements (except type A dark spermatogonia) again became depressed in the subject whose biopsy was available for examination. Parenthetically, observations on sperm morphology revealed a drop in normal forms during the initial testosterone enanthate administration and a return to normal during the administration of HCG.

As a general proposition, spermatogenesis appears to reach completion in most mammals under the influence of two different hormones, testosterone and FSH. First testosterone must be present in sufficient amounts to bring about maturation of the seminiferous tubules. The tubules may be considered a part of the rest of the genital system which is dependent upon testosterone for its maintenance and development, e.e., epididymis, was deferens, prostate, seminal vesicle, etc. In man, when such maturation of the seminiferous tubules is induced by testosterone, whether exogenous or endogenous, there is an accompanying differentiation of the previously undifferentiated Sertoli cells, spermatogonia and the occasional spermatocyte, but no noticeable progression of germinal cell maturation. This can be judged from administering HCG to true hypogonadotropic eunuchoids (6). After testosterone has provided basic testicular support, the second hormone, FSH, is able to initiate progressive and complete spermatogenesis. Follicle-stimulating hormone however is unable to influence human spermatogenesis when administered alone as HMG (Pergonal) (7).

With the above in mind we wished to explore the proposition that testosterone might differentially depress ICSH completely without concomitantly completely depressing FSH. The preferred way of testing this proposition would be, of course, to determine plasma FSH and ICSH levels. These determinations were not performed on the three subjects reported and must await a repetition of the experiment. As a substitute that could be performed with relative rapidity normal subjects were chosen for administration of a high potency and more rapidly acting androgen. Thus, testosterone propionate (Oreton, Schering) was administered I.M. at 100 mg levels daily for five days. Five control observations were recorded for plasma FSH and ICSH using modifications of Odell's radioimmunoassay methods (8,9). Daily observations were made during androgen administration and every two to three days for two weeks following cessation of androgen. ICSH disappeared entirely within 24 hours of the first injection and remained undetectable for the five day injection period (any value below 2.0 mU/ml is considered as zero or undetectable in our laboratory). In distinct contrast the plasma FSH values, determined simultaneously, remained unaltered during and following testosterone propionate administration. Thus under the specific circumstances of this experiment it must be concluded that

3002168

testosterone can produce a dichotomous effect upon plasma FSH and ICSH levels (Table 1).

Table 1. Plasma Gonadotropin Before, During and After Testosterone Administration.

Date	SUBJECT PM 315 ICSH (mU/ml)*	SUBJECT PM 315 FSH (mU/ml)*	SUBJECT PM 214 ICSH (mU/ml)*	SUBJECT PM 214 FSH (mU/ml)*
Control				
3-9	5.66	8.07	4.43	7.03
4-11	3.13	7.47	3.98	7.30
4-12	6.00	7.03	4.23	7.30
4-14	5.25	7.20	3.58	6.75
10-11	4.40	7.75	3.45	6.50
Testosterone propionate 100 mg I.M. for five days				
10-12	1.90	7.65	1.43	6.40
10-13	1.70	7.17	2.08	6.75
10-14	1.63	7.25	2.05	6.50
10-15	1.73	6.63	2.03	6.18
10-16	1.75	6.50	1.68	6.60
Post-Testosterone				
10-20	8.25	7.20	5.23	6.45
10-23	7.38	8.25	6.90	6.68
10-27	5.03	8.03	3.70	6.73
10-30	5.15	7.53	3.93	6.85

* Standard:Human Pituitary Extract Reference Preparation LER 907

Swerdloff and Odell (10) administering 20 or 50 mg of fluoxymesterone daily for five days to five normal men and Franchimont (11) using a single injection of 50 mg testosterone propionate revealed a similar dichotomy in response, i.e., no change in FSH and undetectable ICSH (LH).

Neither Swerdloff and Odell's nor our experiments to date have been of sufficient duration to extrapolate the data to long-term effects. Bogdanove (12) has demonstrated a delayed effect of androgens on FSH release in rats.

The effect of androgen upon ICSH in the normal human male can be accepted as causing marked suppression. This is inferred from our observations of depression of total urinary gonadotropins, and suppression of urinary ICSH, in the one case, following testosterone enanthate administration. It is also inferred from the complete

3002169

early plasma ICSH suppression following administration of fluoxymesterone and testosterone propionate. Thus there appears to be a strong likelihood that a dichotomy exists between ICSH and FSH suppression.

Assuming that FSH is not suppressed or only incompletely suppressed by administering 200 mg testosterone enanthate I.M. per week, we speculate that the restoration of spermatogenesis following the addition of HCG is due wholly or in part to the presence of plasma FSH of anterior pituitary origin.

Continuing with this assumption, each result of the testosterone enanthate plus HCG experiment is readily accounted for except one: the suppression of spermatogenesis by administering testosterone enanthate (or other androgens). The enigma that requires explanation is based upon the assumption that the presence of only two hormones, testosterone and FSH, is required for completion of spermatogenesis in man. In this experiment each hormone is assumed to be present; testosterone being administered exogenously and FSH not having been suppressed. The only immediately apparent interference with regulation of testicular function is the suppression of ICSH. This in turn stops the endogenous production of androgens, which in turn provides the explanation for the cessation of complete spermatogenesis. It is likely that the concentration of androgen provided by the Leydig cells, which are in such close approximation to the seminiferous tubules, is far higher than that provided to the germinal cells by an injection of 200 mg of testosterone enanthate given I.M. once weekly. Thus the effect of testosterone enanthate given I.M. is to lower the androgen levels available to the germinal epithelium, due to the inhibition of ICSH.

In applying this explanation, reference must be made to observations on the maintenance of spermatogenesis in the hypophysectomized rat by exogenously administered androgens (13-16). Woods and Simpson (17) found that spermatogenesis in hypophysectomized rats could be poorly maintained by ICSH alone but well maintained by ICSH plus FSH or testosterone propionate plus FSH. In these and many confirmatory observations the dosage administered and the concentration of plasma testosterone attained are far in excess of those possible for so much larger a mammal as man.

That the location of the Leydig cell so close to the germinal epithelium is an important factor in providing concentrated amounts of androgen is seen in man in the case of the "fertile eunuch" (18). Maintenance of spermatogenesis has been produced in hypophysectomized rats by Dvoskin (19) and in hypophysectomized rhesus monkeys by Smith (20) by intratesticular administration of testosterone in pellet form. Smith found tubules nearest the deposited androgen undergoing complete spermatogenesis and those more remote remaining in the degenerate state. Dvoskin reported that if the same amount

3002170

of androgen was administered subcutaneously or intratesticularly the latter was far more effective in maintaining spermatogenesis.

We conclude that in normal men testosterone causes testicular Leydig cell and germinal cell depression without exerting a direct effect upon testicular activity, but does so indirectly by inhibiting production of ICSH. We postulate that FSH production is unaffected or only partially suppressed. Under these circumstances we assume that the inhibition of spermatogenesis is due to a decrease in the normally high androgen concentration at the germinal cell site due to the absence of ICSH effect upon Leydig cells.

ACKNOWLEDGEMENTS

We wish to thank Edward C. Reifenstein, Jr., M.D. of The Squibb Institute for supplying the Delatestryl, Jack Howard, M.D. of the Schering Corporation for the supply of Oreton and John Jewell, M.D. of Ayerst-McKenna for a supply of APL.

This investigation was supported by grants-in-aid from The Ford Foundation (680-0906), the Public Health Service (HD 00804) and the U.S. Atomic Energy Commission (AT (45-1)-1780).

REFERENCES

1. Heller, C.G. and Nelson, W.O., Recent Progr. Hormone Res., 3: 229, 1948.
2. Rowley, M.J. and Heller, C.G., Fertil. Steril., 17: 177, 1966.
3. Albert, A., Proc. Staff Meeting Mayo Clinic, 30: 552, 1955.
4. Greep, R.O., VanDyke, H.B. and Chow, B.F., Proc. Soc. Exp. Biol. Med., 46: 644, 1941.
5. Heller, C.G., Heller, G.V. and Rowley, M.J., In: Progress in Endocrinology, ed. Gual, C., Excerpta Medica Found., p. 1012, 1969.
6. Heller, C.G., Lalli, M.F. and Rowley, M.J., In: Pharmacology of Reproduction, Proc. 3rd Internat. Pharmacol. Meeting July 1966, Pergamon Press, Oxford and New York, 2: 61, 1968.
7. Paulsen, C.A., In: Gonadotropins, 1968, ed. Rosemberg, E., Geron-X Inc., Los Altos, Calif., p. 491, 1968.
8. Odell, W.D., Parlow, A.F., Cargille, C.M. and Ross, G.T., J. Clin. Invest., 47: 2551, 1968.
9. Odell, W.D., Ross, G.T., and Rayford, P.L., J. Clin. Invest., 46: 248, 1967.
10. Swerdloff, R.S. and Odell, W.D., In: Gonadotropins, 1968, ed. Rosemberg, E., Geron-X Inc., Los Altos, Calif., p 155, 1968.
11. Franchimont, P., Proc. Internat. Symp. on Protein and Polypeptide Hormones, ed. Margoulies, M., Excerpta Medica Found., Amsterdam, p. 99, 1968.
12. Bogdanove, E.M., Anat. Rec., 157: 117, 1967.
13. Walsh, E.L., Cuyler, W.K. and McCullagh, D.R., Amer. J. Physiol., 107: 508, 1934.

3002171

14. Nelson, W.O. and Gallagher, T.F., Science, 84: 230, 1936.
15. Cutuly, E., McCullagh, D.R., and Cutuly, E.C., Amer. J. Physiol. 119: 121, 1937.
16. Clermont, Y. and Harvey, S.C., In: Ciba Found Colloq. Endocr. 16: 173, 1967.
17. Woods, M.C. and Simpson, M.E., Endocrinology, 69: 91, 1961.
18. McCullagh, E.P., Beck, J.C. and Schaffenburg, C.A., J. Clin. Endocr., 13: 489, 1953.
19. Dvoskin, S., Anat. Rec., 99 : 329, 1947.
20. Smith, P.E., Yale J. Biol. Med., 17: 281, 1944.

DISCUSSION

ROSEMBERG: Dr. Heller, you indicated that testicular function was suppressed under testosterone administration; that is, the sperm count dropped. However, after subsequent addition of HCG, the sperm count increased. Hence, you postulated that HCG stimulated the Leydig cells to produce endogenous testosterone by virtue of the fact that testosterone may be near the germinal epithelium and then exerted a local effect. Do you have experimental proof for your statement? It seems to me that when exogenous testosterone is administered one is flooding the system.

HELLER: We do not have proof for our statement. The testosterone levels that we can conveniently produce in man by intramuscular injection are relatively low. In other words, the amount of testosterone theoretically reaching the germinal epithelium is much different in amount than what one can do, let us say in a hypophysectomized rat where one can maintain spermatogenesis by injecting testosterone. All the other evidence is rather indirect, as has been shown at this meeting. Dr. Steinberger showed a case with a testicular tumor in one testis in which the tubules that were near the testosterone-producing tumor continued to have normal spermatogenesis whereas on the other side no spermatogenesis was observed.

ROSEMBERG: Dr. Steinberger, what was the level of testosterone in this patient?

E. STEINBERGER: We are presently measuring the circulating hormone level, so I do not have the answer but I will be surprised if it is not extremely low.

ROSEMBERG: If this is so, it is difficult for me to visualize that the amount produced by the Leydig cells under HCG stimulation could overcome whatever is already there.

HELLER: The Leydig cells were morphologically altered by exogenous testosterone administration, suggesting lowered testosterone output.

3002172

But you ask me for proof; and I have no proof of this; it remains a logical assumption, but just an assumption.

SAVARD: There is a most important intra-testicular, as well as intra-ovarian, circulation of hormones produced within the gonad. I call your attention to the work of Lindner, some years ago in Dr. Mann's laboratories in Cambridge, who demonstrated the high testosterone content of the lymph of the testis. He found it many times greater than the peripheral testosterone concentrations, comparable and sometimes exceeding the concentrations of the spermatic venous blood. Lindner also suggested that this could perhaps explain the phenomenon of the syndrome of the fertile eunuch, who has sufficient local testosterone concentration to support spermatogenic function, yet not enough peripherally to masculinize him.

HUDSON: With the administration of fluoxymesterone, plasma levels of testosterone are suppressed. Continuing treatment with fluoxymesterone and administering HCG, peripheral plasma levels of testosterone are restored to normal. I really believe that Dr. Savard's comments are highly relevant: namely, what is the local concentration of testosterone around the tubules, and what is its significance on nurturing the germinal epithelium?

JOHNSEN: In Dr. Steinberger's case, there was only growth of the tubules and presence of a limited number of spermatocytes.

E. STEINBERGER: Dr. Johnsen, you are absolutely correct; as I clearly pointed out when discussing the photomicrographs, the spermatogenesis did not proceed beyond young spermatids. Dr. Heller's paper, together with data reported by Dr. Bergada on a case of Leydig cell hyperplasia showing localized areas of spermatogenesis and with our demonstration of spermatogenesis in a tumor-bearing testicle of a 6-year old boy, presents a good case for the critical role of testosterone in the process of initiation of spermatogenesis in the human testicle. Furthermore, we provided evidence that in the rat, FSH is necessary for the late stages of spermatid development. I think that demonstration of FSH by Dr. Heller in his experimental subject suggests that, in the human, FSH may also play an important role in maturation of the spermatids.

HELLER: Many years ago Carl Moore implanted crystals of testosterone. Wherever the crystals were, spermatogenesis was maintained; while peripheral to that, they were not.

PAULSEN: Dr. Heller, it occurs to me that something curious is going on which is not understandable. For example, when Maddock and Nelson administered HCG to normal adult men, oligospermia or azoospermia developed. Histologically, the seminiferous tubules became hyalinized and the Leydig cells became small and darkly stained.

3002173

They postulated that these changes were due to excessive steroid hormone production. Now suppose they had added testosterone to their HCG regimen. This would have further increased the intratesticular testosterone concentration. Thus the data of Maddock and Nelson appear to be in conflict with your experiment. Can you explain this?

HELLER: We have repeatedly confirmed the Maddock-Nelson work. And, for those of you who are not familiar with it, it consists simply of administering human chorionic gonadotropin long enough, in large enough amounts, resulting in azoospermia in normal men. During this time, not only are testosterone and the 17-KS steroids increased, but the hormone, in the urine at least, that increases the most is estrogen. I would postulate that human chorionic gonadotropin, under the circumstances of Maddock and Nelson's experiments and under the circumstances that we have given it, is causing failure of spermatogenesis because of the high concentration of estrogen.

PAULSEN: But you would still be getting the same equivalent amount of estrogen in the urine, Dr. Heller, with testosterone and HCG administration. Therefore, your explanation does not appear to be plausible. It still remains doubtful how you were able to restore spermatogenesis in your study. I would like to believe that it is the retention of normal endogenous FSH levels which was fundamental to the restoration of spermatogenesis in your study. But is it really fair, Dr. Heller, to compare your twenty-week administration of testosterone enanthate to the five-day administration of testosterone propionate?

HELLER: No it is not. Dr. Paulsen brings up the fact that we did not determine plasma ICSH and plasma FSH in the experiment with testosterone enanthate, which was a long-term experiment. This experiment is now under way.

DICZFALUSY: May I suggest another experiment. In the human female, those who tried to work on the mechanism of action of contraceptive steroids usually concluded that estrogen will inhibit FSH release and secretion, provided it is given for a long period of time. Dr. Heller, if you would give estrogen to your patients, you would see the following: 1) whether or not it was indeed the estrogen which, in combination with HCG, caused hyalinization, 2) whether or not you really need FSH from the pituitary and 3) whether you could replace FSH with HMG.

3002174

REPOSITORY: DOE-RICHLAND
COLLECTION: GSS HUMAN TEST SUBJECTS STUDIES
PRISONER STUDY

BOX: 046264

FOLDER: 1780 - HELLER PACIFIC NW RES. FOUNDA.

ASSIGNED NUMBER: RLHTS 94-0045

3002174 A