ANTIHORMONE FORMATION TO SHEEP FOLLICLE STIMULATING HORMONE IN MEN:

I. PROPERTIES OF THE ANTIHORMONES

II. EFFECT OF THE ANTIHORMONES ON SPERM COUNTS AND URINARY GONADOTROPHIC HORMONE EXCRETION

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ANTIHORMONE FORMATION TO SHEEP FOLLICLE STIMULATING HORMONE IN MEN:

I. PROPERTIES OF THE ANTIHORMONES

Prolonged administration of gonadotrophic hormones derived from one species to an individual of a different species can elicit formation of substances in the plasma capable of preventing the action of the administered hormones. These substances have been named antihormones, or more specifically, antigonadotrophins. Such terminology infers a similarity between antihormone and antibody formation; however, it is not the purpose of this report to consider the immunological properties of antihormones. This subject has been amply reviewed by Zondek and Sulman (1).

Antigonadotrophins, besides being effective against the administered hormone, may also be effective against other gonadotrophic hormones. In experimental animals, it has been demonstrated that non-specific antigonadotrophins may even be effective against the normal, circulating gonadotrophins. For example, Rowlands (2) found that non-specific antihormones were formed in the serum of rabbits injected with an ox anterior pituitary extract. Administration of this serum to male or female rats was followed by atrophy of the gonads, similar to that occurring after hypophysectomy.

The problem of antihormone formation is therefore of considerable importance in clinical therapy with gonadotrophic hormones. Besides vitiating the effects of the administered hormone, the formation of non-specific antihormones could also nullify the actions of the patient's endogenous gonadotrophic hormones. This possibility has been recognized,

but as yet has not been demonstrated (3).

Antihormone formation in human subjects has followed administration of pregnant mare serum gonadotrophin (4,5,6), horse pituitary gonadotrophin (7), and a mixture of human chorionic gonadotrophin and sheep pituitary gonadotrophin (Synapoidin) (6). The antihormones forming against pregnant mare serum were specific (4,6), i.e., they were effective only against pregnant mare serum gonadotrophin. The antihormones formed against "Synapoidin" and against horse pituitary gonadotrophin were non-specific, as they were effective against pregnant mare serum as well as against the administered hormones (6,7). There was no evidence that the antihormones were effective against the patients' endogenous gonadotrophic hormones (6).

It is the purpose of this communication to report the results of studying antigonadotrophin formation in seven sterile men receiving a sheep anterior pituitary extract rich in follicle stimulating hormone* (hereafter referred to as sheep FSH). The following problems were investigated:

1. Rate, incidence and amount of antihormone formation.
2. Specificity of the antihormones.
3. Effects of the antihormones on the patients' endogenous gonadotrophins.
4. Mechanism of action of the antihormones.

Method of antihormone assay: Twenty-four day old female Sprague-Dawley rats were used as assay animals. A sample assay is presented

*Generously supplied by the Schering Corporation through the courtesy of Dr. Edward S. Henderson. (This preparation also contained small amounts of interstitial-cell stimulating hormone.)

TABLE 1. DETECTION OF ANTIGONADOTROPHINS

Units of sheep F.S.H. per rat*	Volume of plasma injected cc.	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
4	0	109	83	3
4	0.9**	146	15	2
4	1.8**	37	13	3
Uninjected controls***	0	36	13	63

*The F.S.H. and plasma were divided into 6 equal doses administered twice daily for 3 days

**Source of plasma: M.M. 82 days following initial F.S.H. injection.

***These values for uninjected controls were obtained from controls included in each assay and apply to all the data.

in Table 1. Each rat received a total of 4 units* of sheep FSH dissolved in 6.0 cc. of water, 1.0 cc. being injected subcutaneously twice daily for 3 days. One group of rats received only FSH, whereas others received in addition varying amounts of plasma suspected of containing antihormones (obtained from patient K. H. 82 days after initiating therapy). The plasma was divided into six equal doses and injected subcutaneously concurrently with the hormone, but at a separate site. The rats were killed 24 hours after the last injection, and the weights of ovaries and fluid-free uteri were taken as the assay end-points. (Controls receiving only FSH were included in each assay, because it was found that the amount of hormone varied from ampule to ampule. It should be stressed, however, that in each assay all rats received exactly the same amount of hormone from the same stock solution, freshly prepared prior to each assay.)

The data of Table 1 demonstrate that rats receiving only FSH showed marked uterine and ovarian stimulation. Rats receiving in addition 0.9 cc. plasma showed only uterine stimulation, whereas rats receiving 1.8 cc. of plasma showed neither ovarian nor uterine stimulation.

Certain details concerning the response of the immature female rat to gonadotrophic hormones should be emphasized. The first response of the ovary to gonadotrophic hormones is secretion of estrogen, which is detected by an increase in weight of the uterus. Only after the uterine weight increase has reached a maximum does the ovary increase

*"Units" refers to the manufacturers' stated potency. This also applies to the other hormones used.

in weight (8). The importance of this concept is illustrated by inspection of the data in Table 1. Compare first the data for rats receiving only FSH with those for rats receiving in addition 0.9 cc. plasma. If only uterine weight is considered, no difference between the two groups can be detected, because both 109 and 146 mg. can be considered as approximately maximal uterine weights (8). However, a clear-cut difference is found by comparing ovarian weights, 83 vs. 15 mg. Similarly, compare the figures for rats receiving 0.9 cc. plasma with those of rats receiving 1.8 cc. plasma. Ovarian weights show no detectable difference as both are at uninjected control levels. However, uterine weights now show a clear-cut difference, 146 vs. 37 mg.

The individual assay results for each patient are tabulated in Tables A to G in the appendix, and summarized in Table 2.

RATE, INCIDENCE AND AMOUNT OF ANTIHORMONE FORMATION

Antihormone titers before therapy: Antihormone titers were determined in 5 of the 7 patients before initiating therapy by assaying 0.9 cc. plasma against 2 units of sheep FSH. In 2 normal students 6.0 cc. of plasma was assayed against 4 units of FSH (Table H, appendix). In no instance were antihormones detected in untreated individuals.

Duration and amount of therapy: Each patient received 50 units of sheep FSH daily (self-administered, intramuscularly) except patients K. H. and G. M. Patient K. H. received 50 units twice daily for 12 days and then 50 units once daily for 54 days. Patient G. M. received 50 units twice daily for 45 days and then 50 units once daily for 11

TABLE 2. RATE AND AMOUNT OF ANTIGONADOTROPHIN FORMATION TO SHEEP F.S.H.

Patient	Detection of antihormones before therapy	Duration of therapy days (50 units sheep F.S.H. daily)	Antihormones first detected days	Maximal amount (units of antihormone* in circulation)	Last day present (after stopping therapy)	First day absent (after stopping therapy)
D.B.	None	65	52	2,000	37	86
H.B.	None	104	51	5,000	39	88
R.G.	None	84	60	7,000	60	125
L.D.	None	82	49	10,000	58	109
C.C.	----	60	56	7,000	283	—
K.H.	None	86	52	10,000	128	164
G.M.	----	56	45	3,000	21	121
Range	None (5 tested)	56-104	45-60	3,000-10,000	21-283	86-

*One "antihormone unit" is that amount just sufficient to prevent the action of 1 unit of sheep F.S.H.

days. Duration of therapy ranged from 56 to 104 days. Duration of therapy for each patient is tabulated in Table 2. Pretreatment intradermal sensitivity tests to the sheep FSH were negative in each patient.

Incidence of antihormone formation: Antihormone formation occurred in each of the 7 patients. By determining antihormone titers at approximately 2 to 4 week intervals it was found that all 7 patients developed antihormones within 45 to 60 days after initiating therapy (Table 2). At this time, antihormone titers were sufficiently great to nullify many times the administered daily dose of sheep FSH.

Amounts of antihormone present in the circulation: The amounts of antihormone present in the circulation can be roughly estimated as follows: from Table 1, it can be seen that 0.9 cc. of K. H.'s plasma almost, but not completely, prevented the action of 4 units of FSH. It can be estimated that approximately 3 units of FSH were inactivated. Defining an "antihormone unit" as that amount just sufficient to prevent the action of 1 unit of hormone, then 0.9 cc. of K. H.'s plasma contained about 3 units of antihormone. Assuming total plasma volume as 3,000 cc., then there are 3,000/0.9 x 3 or 10,000 units of antihormone in the total plasma. This is enough to neutralize 200 times the administered daily dose, or almost 3 times the total amount of hormone administered. Similar estimations for the other patients showed that at the time of maximal antihormone formation there were 3,000 to 10,000 units of antihormone present in the plasma (Table 2).

Time required for antihormones to disappear: Repeated antihormone assays performed after stopping therapy demonstrated that antihormones disappeared from the plasma within 3 to 5½ months after the last

injection of FSH in 6 of the 7 patients (Table 2). At the last date tested, 6.0 cc. of plasma of each of these patients did not alter the effects of 4 units of sheep FSH. Antihormones titers were still present in the other patient 283 days after cessation of therapy; at this time the titer was approximately one-fourth of the maximal value.

SPECIFICITY OF THE ANTIHORMONES

In order to test the specificity of the antihormones, plasma containing antihormones against sheep FSH was tested against the following gonadotrophic hormones:

1. Chorionic gonadotrophin derived from human pregnancy urine.
2. Pregnant mare serum gonadotrophin.
3. Anterior pituitary gonadotrophin derived from horse pituitaries (mainly FSH).
4. Urinary gonadotrophin derived from the urine of a castrated man (mainly FSH).

The assays were carried out as previously described.

Antigonadotrophic effect against human chorionic gonadotrophin: Two commercial preparations were tested: "Pranturon" (Schering) and "A.P.L." (Ayerst, McKenna and Harrison). In both instances, 1 unit of hormone elicited a definite gonadotrophic response which was completely prevented by the injection of 1.8 and 3.0 cc. of plasma of patients L. D. and C. G., respectively (Table 3).

Antigonadotrophic effect against pregnant mare serum gonadotrophin: Three cc. of patient C. G.'s plasma completely prevented the action

TABLE 3. ANTIGONADOTROPHIC EFFECT AGAINST HUMAN CHORIONIC GONADOTROPHIN

Units of chorionic gonadotrophin per rat	Volume of plasma injected cc.	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
Pranturon (Schering)				
1	0	91	22	3
1	1.8*	29	9	3
A.P.L. (Ayerst)				
1	0	125	19	3
1	3.0**	45	13	3

Source of plasma: *L.D. 105 days following initial F.S.H. injection
**C.G. 264 days following initial F.S.H. injection.

TABLE 4. ANTIGONADOTROPHIC EFFECT AGAINST PREGNANT MARE SERUM (PMS)

Units of P.M.S. (Antex, Ayerst) per rat	Volume of plasma injected cc.	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
5	0	135	15	3
5	3.0*	41	15	3

*Source of plasma: C.G. 264 days following initial F.S.H. injection

TABLE 5. ANTIGONADOTROPHIC EFFECT AGAINST HORSE ANTERIOR PITUITARY EXTRACT

Units of horse F.S.H. (Gonatrope, Forbes) per rat	Volume of plasma injected cc.	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
1	0	150	20	9
1	1.8*	120	18	3
1	3.0**	116	20	3
1	6.0**	138	24	3

Source of plasma: *D.B. 66 days following initial F.S.H. injection.
**C.G. 264 days following initial F.S.H. injection.

TABLE 6. ANTIGONADOTROPHIC EFFECT AGAINST HUMAN URINARY GONADOTROPHIN

Aliquot hours of human male castrate ultrafilter urine concentrate per rat	Volume of plasma injected cc.	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
2	0	114	55	3
2	0.9*	117	32	2
2	6.0*	21	5	3

*Source of plasma: K.H. 67 days following initial F.S.H. injection.

of 5 units of pregnant mare serum ("Antex", Ayerst, McKenna and Harrison) (Table 4).

Antigonadotrophic effect against horse FSH: The antihormones failed to alter the effect of 1 unit of horse pituitary gonadotrophin ("Gonatrope", Forbes). Although 1.8 cc. of patient D. B.'s plasma collected 66 days after initiating FSH therapy was capable of preventing the response of about 2 units of sheep FSH, the same amount of this plasma failed to affect the response of 1 unit of horse pituitary gonadotrophin. Similarly, 3.0 cc. of patient C. G.'s plasma collected 264 days after initiating therapy prevented the effect of approximately 2 units of sheep FSH, but 3.0 and 6.0 cc. of this plasma were without effect on 1 unit of the horse pituitary preparation (Table 5). Thus, plasma containing antihormones against sheep FSH was not antigonadotrophic against horse pituitary gonadotrophin.

Urinary gonadotrophin obtained from a castrated man: The urine was concentrated by the ultrafiltration technique (9). A two-hour aliquot gave a definite gonadotrophic response which was partially prevented by 0.9 cc. of plasma and completely prevented by 6.0 cc. of plasma (collected from patient K. H. 67 days after initiating therapy) (Table 6).

Antihormones that form in response to administration of sheep FSH are effective against hormones from sheep, horse, and human sources, and therefore are not species specific. The antihormones are effective against anterior pituitary, human chorionic, and pregnant mare serum hormones, and therefore are not hormone specific. However, this lack of specificity is not complete, because the antihormones are not

effective against horse pituitary gonadotrophin.

EFFECTS OF THE ANTIHORMONES ON THE PATIENTS' ENDOGENOUS GONADOTROPHINS

Since the antihormones were sufficiently non-specific to be active against human castrate male gonadotrophins, the question arose whether or not they were effective against the patients' own urinary gonadotrophins. Antihormones were tested against endogenous gonadotrophins in 4 of the patients. Urine collected after stopping therapy (in order to avoid recovering administered sheep FSH), was concentrated by ultrafiltration. Each rat in a given assay received an equal aliquot of the ultrafiltered urine concentrate. One group received only urine concentrate, whereas others received, in addition, plasma from the same patient. For example, patient L. D. collected urine from day 2 to day 7 after stopping therapy. The hormone concentrated from a twelve-hour urine specimen elicited definite uterine and ovarian stimulation; this was completely prevented by the addition of 3.0 cc. of his own plasma (Table 7). Similar results were obtained on the other 3 patients (Table 7).

The following control experiments were also performed. Six cc. of plasma from normal medical students R. B., L. C. and E. J. were tested against twelve-hour urine extracts from patients C. G., K. H. and L. D. respectively, without materially effecting the gonadotrophic response of the extracts (Table H, appendix). Also, the plasma of normal subject E. J. was not antigonadotrophic against his own urine extracts (Table H, appendix).

TABLE 7. EFFECT OF ANTIHORMONES AGAINST PATIENTS' ENDOGENOUS GONADOTROPHINS

Amount ultrafilter urine concentrate per rat	Total cc. plasma per rat	Days after therapy initiated plasma collected	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
12-hour urine per rat collected by R.C. days 54 to 63 after stopping therapy	0	---	86	12	3
	6.0	144	33	12	3
12-hour urine per rat collected by L.D. days 2 to 7 after stopping therapy	0	---	173	22	3
	3.0	91	28	13	3
12-hour urine per rat collected by K.H. days 6 to 9 after stopping therapy	0	---	113	12	2
	6.0	95	26	11	2
12-hour urine per rat collected by C.G. days 4 to 7 after stopping therapy	0	---	135	16	2
	0.9	77	28	11	2
4-hour urine per rat collected by C.G. days 126 to 131 after stopping therapy	0	---	82	14	4
	6.0	193	33	14	2

MECHANISM OF ACTION OF THE ANTIHORMONES

Although the antihormones are effective against endogenous gonadotrophic hormones, gonadotrophins are being excreted in the urine at a time when the antihormones in the blood are at their highest level (compare the time of urine collections (Table 7) with antihormone assays tabulated in the appendix). Also, urinary gonadotrophin titers were as high or higher than pretreatment levels (Table 1, section II). Therefore, antihormones do not destroy or irreversibly combine with gonadotrophins nor do they prevent production or release of gonadotrophic hormones from the hypophysis. ~~It is obvious that antihormones are not present in the urine extracts, for~~ As gonadotrophic potency is clearly demonstrable, when antihormones are present in the plasma, ~~Therefore,~~ separation of antihormone and hormone must occur. There are two possible sites where this could take place:

1. The kidney. The kidney could retain antihormone, but excrete hormone.
2. The ultrafilter concentration technique. It is possible that the kidney excretes both hormone and antihormone and that ultrafiltration recovers only hormone from the urine.

To differentiate between these two possibilities, plasma known to contain antihormones was diluted 1:100 with water and then concentrated by the usual ultrafiltration technique. The concentrate was then extracted with water to the original volume of plasma and its antihormone content compared with that of the original plasma by assaying against sheep FSH. The date of Table 8 show that the ultrafiltration technique is capable of retaining antihormones, and that

TABLE 8. RECOVERY OF ANTIHORMONES FROM PLASMA BY ULTRAFILTRATION

Units sheep F.S.H. per rat	Total cc. plasma per rat	Treatment of plasma	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
4	0	----	120	52	3
4	1.8*	untreated	97	15	3
4	3.0*	untreated	28	12	3
4	3.0*	ultrafiltration	80	17	3
4	0	----	101	77	3
4	0.9**	untreated	118	31	2
4	1.8**	ultrafiltration	118	29	3

Source of plasma: *C.G. 243 days following initial F.S.H. injection.
**K.H. 109 days following initial F.S.H. injection.

approximately 50% of the original potency is recovered. Thus, the second possibility is not in operation, and separation of antihormones and hormones must take place at the kidney.

DISCUSSION

Rate and incidence of antihormone formation: Clinical data concerning the formation of antigonadotrophins to pituitary extracts are meager. Meyer and Wolfe (10), referring to the unpublished data of Meyer and Sevringhaus, stated that antigonadotrophins formed in the blood of human females after the administration of anterior pituitary gonadotrophic preparations; no further information was given. Spence, Scowen and Rowlands (11) were unable to detect antihormones in the serum of 2 patients following treatment with 30 to 50 units of a pig pituitary extract twice weekly for 16 to 23 weeks. Leathem and Rakoff (7) reported that 6 of 13 patients treated with 300 to 400 units of horse pituitary gonadotrophin per month developed antihormones after 3 to 4 months of therapy.

To our knowledge there are no previous data concerning the formation of antigonadotrophins in the human following the administration of only sheep pituitary extracts. However, Rakoff and Leathem (12) reported the results of treating 25 patients with a mixture of sheep pituitary and human chorionic gonadotrophins (Synapoidin). Antihormones did not form in 22 patients treated for 2 to 5 months. Antihormones did form in 3 patients treated for more than 6 months with this preparation. The formation of antihormones in their experiments is most probably due to the sheep FSH in the extracts, since antihormone

formation has not been demonstrated following treatment with human chorionic gonadotrophin alone. (Segaloff and Parson [13] have reported antihormones effective against human chorionic gonadotrophin forming after the administration of this material. However, as antihormone assays were not performed prior to treatment with chorionic gonadotrophin, and as this patient had previously received protracted treatment with a pituitary extract, it is most likely that these antihormones formed in response to the pituitary extract--not the chorionic gonadotrophin.)

The low incidence of antihormone formation encountered by Rakoff and Leathem [12] compared to the 100% incidence found by us may be explained on the basis of the difference in dosages employed. Whereas we employed 50 units of sheep FSH daily, they used only 90 units of Synapoidin monthly ("15 rat synergy units...3 times weekly for the first 2 weeks of each cycle").

The lack of specificity of the antihormones formed in response to sheep FSH is in agreement with animal experiments [14,15]. The fact that the antihormones were not effective against horse pituitary gonadotrophin is puzzling, but not without precedent. Simmonet and Michel [16] found that antihormones formed in rabbits following administration of human chorionic, pregnant mare serum, and post-menopausal urine gonadotrophins were not effective against horse pituitary extracts. The only antihormone effective against horse pituitary gonadotrophin was that formed in response to horse pituitary extracts.

Our data demonstrate that antihormones do not destroy, irreversibly combine with or prevent pituitary production or release of gonadotrophins.

Two alternate mechanisms of action may be considered:

1. The antihormones have no effect on gonadotrophins, but act directly on the gonads, rendering them incapable of responding to gonadotrophins.
2. Antihormone and hormone combine, in which form the hormone is incapable of acting on the gonads. Such a combination could, by definition, be separated by the kidney.

The first mechanism of action seems highly improbable. It is difficult to visualize how an antihormone could render the gonads incapable of responding to one hormone but not another, unless the mechanism of gonadotrophic action varies as to the hormone employed. Okkels (17) has shown that thyroid tissue, unresponsive to the action of thyrotrophin, again becomes responsive to the same thyrotrophin when the thyroid gland is removed and placed in a perfusion apparatus. Selye, Collip and Thomson (18,19) have demonstrated that rats refractory to one gonadotrophic hormone are still capable of responding to other gonadotrophins.

The second mechanism of action adequately explains the data. Whether or not an antihormone was effective against a given hormone would depend upon the ability of the two to combine. Such a theory adequately explains the action of antihormones without assuming that they destroy hormones or act directly on the gonads.

Zondek and Sulman (20) demonstrated that antigonadotrophins do not destroy gonadotrophins in vitro. They treated a mixture of equal amounts of hormone and antihormone with N/10 to N/15 NaOH and found that

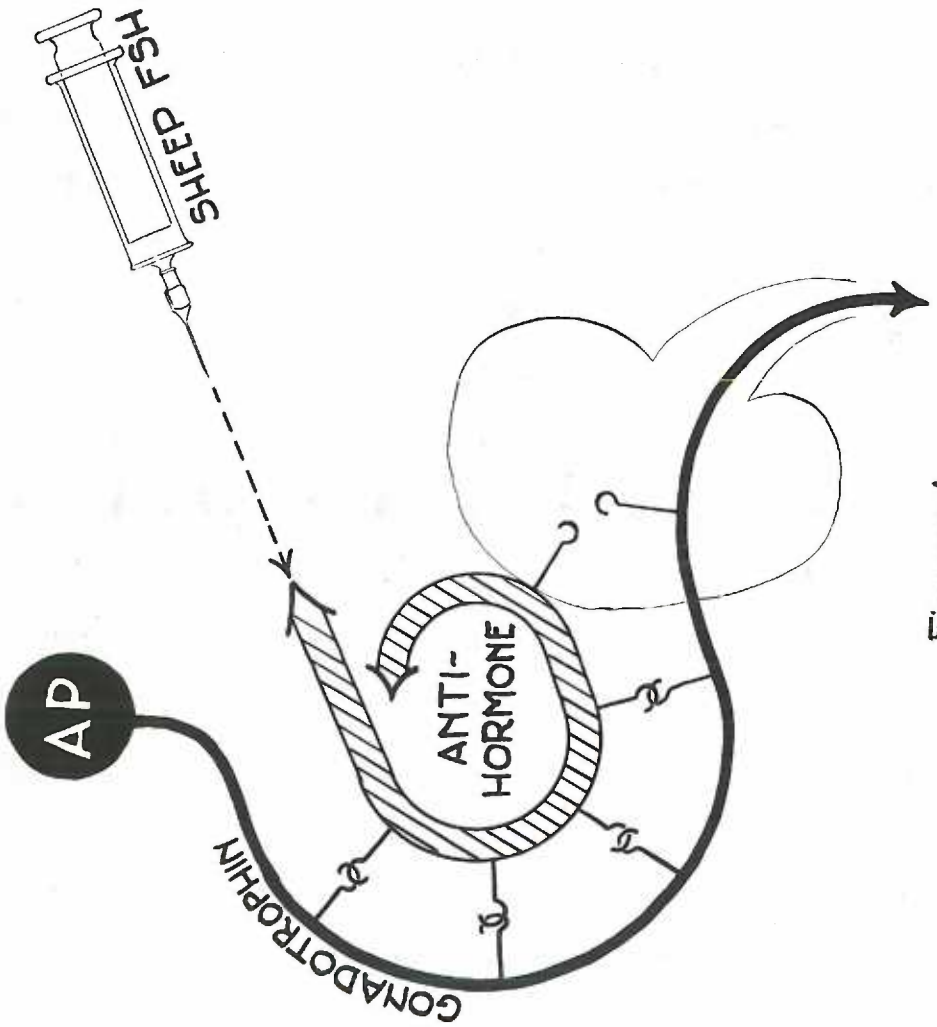


Figure 1

the antihormone was destroyed, leaving active gonadotrophin.

Kupperman, Meyer and Herts (21,22) have concluded from experiments in parabiotic rats that antihormones do not act on the pituitary gland, but that they "neutralize" gonadotrophins in the blood stream. We would agree with their conclusions and define "neutralization" as the formation of a combination of gonadotrophin and antihormone.

That antihormones are not excreted in the urine of men with high plasma antihormone titers agrees with work in experimental animals (23,24), where antihormones were not detected in the urine of animals having high titers of antihormones in the plasma.

The relationships between antihormones and endogenous gonadotrophins are summarized diagrammatically in Figure 1. Administration of sheep FSH elicits the formation of non-specific antihormones that are effective against the patients' endogenous gonadotrophins. Antihormones act by combining with gonadotrophins in the blood stream. The antihormone-hormone combination is separated by the kidney, antihormone being retained in the circulation and hormone being excreted in the urine.

SUMMARY AND CONCLUSIONS

The following problems were investigated in seven sterile men treated with daily injections of follicle stimulating hormone derived from the anterior pituitary glands of sheep (sheep FSH):

1. In what proportion of men given daily doses of sheep FSH does antihormone formation occur?

2. How soon after initiating therapy with sheep FSH is antihormone formation sufficiently great to neutralize the effect of the

FSH being administered?

3. How much antihormone is produced in response to the administered hormone?

4. How long does it take for the antihormones to disappear from the circulation after FSH therapy is stopped?

5. Are the antihormones species specific and/or hormone specific?

6. Do the antihormones formed against sheep FSH inactivate the patients' endogenous gonadotrophins?

7. What is the mechanism by which antihormones prevent the action of gonadotrophic hormones?

The following answers were obtained:

1. In each of the seven men given daily injections of sheep FSH, antihormone formation occurred.

2. Sufficient antihormones formed within 2 months to completely vitiate the effects of the injected FSH.

3. The maximal titer of antihormones, attained within 3 months after initiating therapy, was sufficient to neutralize the effect of from 50 to 200 times the daily injected dose of FSH.

4. Antihormones disappeared from the circulation 3 to 9 months after stopping therapy.

5. Inasmuch as the antihormones were effective against gonadotrophins derived from sheep, horse and human sources, they are not species specific.

Inasmuch as the antihormones were effective against anterior pituitary, chorionic and pregnant mare serum gonadotrophins, they are

not hormone specific.

6. The antihormones found in the patients' plasma were capable not only of inactivating gonadotrophins derived from a castrated man, but were able to prevent the action of their own urinary gonadotrophins. It is therefore concluded that antihormones are effective against endogenous gonadotrophins.

7. The mechanism whereby antihormones prevent the actions of gonadotrophic hormones is apparently a reversible combination between the molecules of hormone and the molecules of antihormone.

It seems unlikely that antigonadotrophins destroy gonadotrophins or irreversibly combine with them, because endogenous gonadotrophic hormones are being excreted in the urine when maximal amounts of antihormone are present in the plasma. It is concluded that the kidney separates the combination of hormone and antihormone by permitting the excretion of gonadotrophin and simultaneously retaining antigonadotrophins in the circulation.

APPENDIX

TABLE A. ANTIHORMONE TITERS OF PATIENT D.B. AGAINST SHEEP F.S.H.
Treatment: 50 Units Sheep F.S.H. Daily for 65 days

Units sheep F.S.H. per rat	Total cc. plasma per rat	Days after therapy initiated plasma collected	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
2	—	—	110	16	4
2	0.9	before therapy	100	29	4
2	—	—	51	11	3
2	0.9	24	53	9	3
4	—	—	109	83	3
4	1.8	39	102	70	3
4	—	—	109	64	3
4	1.8	52	124	32	3
4	—	—	101	77	3
4	1.8	66	107	41	3
4	—	—	93	54	3
4	3.0	81	98	33	3
4	—	—	120	52	3
4	3.0	102	111	21	3
4	6.0	102	115	19	3
4	—	—	90	67	3
4	6.0	151	108	56	3

TABLE B. ANTIHORMONE TITERS OF PATIENT H.B. AGAINST SHEEP F.S.H.
Treatment: 50 Units Sheep F.S.H. Daily for 104 Days

Units sheep F.S.H. per rat	Total cc. plasma per rat	Days after therapy initiated plasma collected	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
2	—	—	100	19	3
2	0.9	before therapy	111	31	3
2	—	—	113	37	3
2	0.9	24	103	41	3
2	—	—	138	66	3
2	0.9	51	130	18	3
4	—	—	109	83	3
4	1.8	80	116	36	3
4	—	—	109	64	3
4	1.8	93	129	15	3
4	—	—	101	77	3
4	1.8	105	127	20	3
4	—	—	120	52	3
4	1.8	143	121	27	3
4	3.0	143	122	17	3
4	—	—	90	67	3
4	6.0	192	111	67	3

TABLE C. ANTIHORMONE TITERS OF PATIENT R.C. AGAINST SHEEP F.S.H.
Treatment: 50 Units Sheep F.S.H. Daily for 84 Days

Units sheep F.S.H. per rat	Total cc. plasma per rat	Days after therapy initiated plasma collected	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
2	—	----	100	19	3
2	0.9	before therapy	103	17	3
2	—	----	51	11	3
2	0.9	45	72	12	3
4	—	----	109	83	3
4	1.8	60	146	54	3
4	—	----	109	64	3
4	1.8	73	151	20	3
4	—	----	101	77	3
4	1.8	87	41	12	3
4	—	----	120	62	3
4	1.8	137	124	59	3
4	1.8	137	133	48	3
4	—	----	101	50	3
4	3.0	144	154	39	3
4	—	----	111	51	3
4	6.0	209	149	54	3

TABLE D. ANTIHORMONE TITERS OF PATIENT L.D. AGAINST SHEEP F.S.H.
Treatment: 80 Units Sheep F.S.H. Daily for 82 Days

Units sheep F.S.H. per rat	Total cc. plasma per rat	Days after therapy initiated plasma collected	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
2	—	—	100	19	3
2	0.9	before therapy	104	28	3
2	—	—	113	37	3
2	0.9	22	107	38	3
2	—	—	134	32	3
2	0.9	36	138	26	3
2	—	—	138	66	3
2	0.9	49	143	12	3
4	—	—	109	83	3
4	1.8	78	143	13	3
4	—	—	109	64	3
4	0.9	91	133	17	2
4	1.8	91	29	12	2
4	—	—	101	77	3
4	0.9	105	130	29	3
4	—	—	120	52	3
4	0.9	141	106	45	3
4	1.8	141	113	26	3
4	—	—	90	67	3
4	1.8	190	97	70	3
4	3.0	190	102	66	3
4	—	—	111	51	3
4	6.0	227	106	53	3

TABLE E. ANTIHORMONE TITERS OF PATIENT C.G. AGAINST SHEEP F.S.H.
Treatment: 50 Units Sheep F.S.H. for 60 Days

Units sheep F.S.H. per rat	Total cc. plasma per rat	Days after therapy initiated plasma collected	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
2	---	----	139	39	4
2	0.9	56	33	12	4
2	---	----	106	39	3
2	0.9	77	41	13	4
2	---	----	98	27	5
2	0.9	95	29	12	5
2	---	----	113	37	3
2	0.9	125	54	17	3
2	---	----	134	32	3
2	0.9	137	27	15	3
4	---	----	109	83	3
4	0.9	180	151	40	2
4	1.8	180	44	16	3
4	---	----	109	64	3
4	1.8	193	36	19	3
4	---	----	93	54	3
4	1.8	222	107	23	3
4	---	----	120	52	3
4	1.8	243	97	15	3
4	3.0	243	28	12	3
4	---	----	101	50	3
4	1.8	264	136	44	3
4	3.0	264	93	20	3
4	---	----	102	64	3
4	3.0	343	110	30	3
4	6.0	343	59	19	3

TABLE F. ANTIHORMONE TITERS OF PATIENT K.H. AGAINST SHEEP F.S.H.
Treatment: 50 Units Sheep F.S.H. Twice Daily for 12 Days
Then 50 Units Once Daily for 54 Days

Units sheep F.S.H. per rat	Total cc. plasma per rat	Days after therapy initiated plasma collected	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
2	---	----	110	16	4
2	0.9	before therapy	113	25	4
2	---	----	113	37	3
2	0.9	27	100	25	3
2	---	----	138	66	3
2	0.9	53	41	11	3
2	---	----	51	11	3
2	0.9	67	23	8	3
4	---	----	109	83	3
4	0.9	82	146	15	2
4	1.8	82	37	13	3
4	---	----	109	64	3
4	0.9	95	118	21	3
4	---	----	101	77	3
4	0.9	109	118	31	2
4	---	----	120	62	4
4	1.8	159	114	30	3
4	---	----	90	67	3
4	1.8	194	99	87	2
4	4.5	194	99	47	3
4	---	----	111	51	3
4	6.0	230	127	47	3

TABLE G. ANTIHORMONE TITERS OF PATIENT G.M. AGAINST SHEEP F.S.H.
Treatment: 50 Units Sheep F.S.H. Twice Daily for 45 Days
Then 50 Units Once Daily for 11 Days

Units sheep F.S.H. per rat	Total cc. plasma per rat	Days after therapy initiated plasma collected	uterine weight mg.	ovarian weight mg.	Number of rats
4	---	---	109	83	3
4	1.8	22	95	74	3
4	---	---	101	77	3
4	1.8	45	115	55	3
4	---	---	120	52	3
4	3.0	77	77	18	3
4	---	---	102	64	3
4	6.0	177	85	65	3

TABLE H. CONTROL DATA, TESTING PLASMA FROM NORMAL MEDICAL STUDENTS R. B., L.C. AND E.J. AGAINST SHEEP F.S.H. AND URINARY GONADOTROPHINS

Hormone	Plasma: Amount- Source-	Assay rats uterine weight mg.	Assay rats ovarian weight mg.	Number of rats
4 units sheep F.S.H.	----	120	62	4
	6.0 cc. - L.C.	114	52	3
	6.0 cc. - E.J.	121	52	3
12-hour ultrafilter urine concentrate patient, C.G., 7 mo. after stopping therapy	----	89	77	3
	6.0 cc. - R.B.	107	86	3
12-hour ultrafilter urine concentrate, patient, K.D., 4 mo. after stopping therapy	----	125	23	3
	6.0 cc. - L.C.	132	14	3
12-hour ultrafilter urine concentrate patient, L.D. 3 mo. after stopping therapy	----	107	69	3
	6.0 cc. - E.J.	137	71	3
12-hour ultrafilter urine concentrate normal medical student, E.J.	----	105	44	3
	6.0 cc. - E.J.	112	35	3

ANTIHORMONE FORMATION TO SHEEP FOLLICLE STIMULATING HORMONE IN MEN: II. EFFECT OF THE ANTIHORMONES ON SPERM COUNTS AND URINARY GONADOTROPHIC HORMONE EXCRETION

The search for therapeutic agents effective in male infertility has been relatively fruitless to date. In recent years, hormonal therapy has been considered. Of the various hormones, the ones most likely to succeed would seem to be those that ordinarily stimulate spermatogenesis: the gonadotrophins. Of the gonadotrophins, (from anterior pituitary, pregnancy urine and pregnant mare serum sources,) one hormone that is known to specifically stimulate spermatogenesis is follicle stimulating hormone (FSH) of the anterior pituitary.(25)

It cannot be expected that FSH will prove effective in all types of male sterility. Several prerequisites are necessary before successful results can reasonably be expected. In addition to the obvious prerequisites, such as patent vas deferens, and the knowledge that the wife is potentially capable of conceiving, we have considered the following:

1. <u>Testes with potentially reversible defects</u>. In many cases of infertility, the testes are irreparably damaged and attempts at therapy of any kind are useless.

2. <u>Gonadotrophin production that is not already elevated</u>. If there were already an increase in endogenous gonadotrophic hormones, adding FSH from an exogenous source could not be expected to stimulate spermatogenesis.

Hypogonadotrophic eunuchoids are good examples of patients meeting

both these requirements, for they have infantile testes capable of responding to gonadotrophic stimulation, and lower than normal gonadotrophin titers. Administration of FSH to such patients, following treatment with chorionic gonadotrophin, will produce complete spermatogenesis (26).

3. A suitably concentrated source of FSH.

In studying over 100 infertile men with the view of determining whether prerequisites 1 and 2 were met, it was found that the majority were eliminated because of 1) irreparable testicular damage, and/or 2) elevation of gonadotrophins. In the current investigation, seven infertile, but otherwise normal, men were chosen who had either normal or low urinary gonadotrophin excretion and whose testicular biopsies revealed minor, but definite alterations in spermatogenesis. The third prerequisite was met when a potent and partially purified hypophyseal extract from sheep, containing predominantly FSH, was made available to us. Details concerning treatment are presented graphically in Figures 2 to 7, and are recorded in Table 2, Section I.

In the preceding report, it was demonstrated that the administration of sheep FSH elicited formation of antihormones that were capable of neutralizing the patients' endogenous gonadotrophic hormones. It is the purpose of this communication to correlate the effects of sheep FSH and subsequent antihormone formation on spermatogenesis and endogenous gonadotrophin excretion.

RESULTS

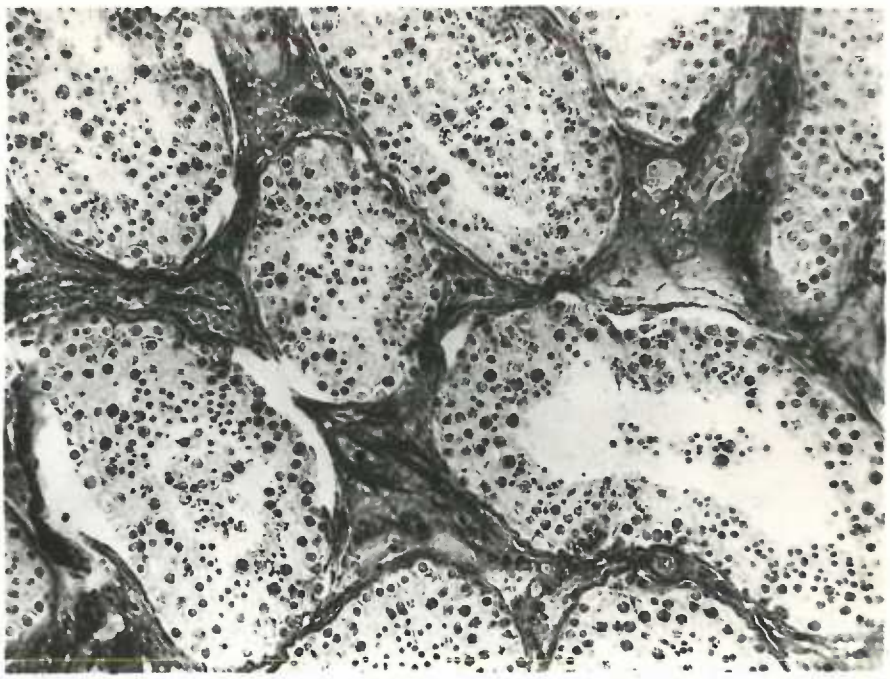
Testicular Biopsies: Testicular biopsies were obtained from six

Figure 1

a. Testicular biopsy from patient K.H. before therapy. Seminiferous tubules of approximately normal size, containing all stages of spermatogenesis, but a relative preponderance of the more immature steges. Sloughing of immature forms into the lumen of the tubules. There is a cluster of normal-appearing Leydig cells near the upper right hand corner. Note lack of any irreversible changes such as severe or complete hyalinization of the basement membranes. X175

b. Testicular biopsy from K.H. during antihormone formation. There is a striking decrease in the size of the seminiferous tubules as compared with the biopsy obtained before treatment. Mature germinal cells are scarce, leaving a preponderance of spermatogonia and primary spermatocytes. Tubules are completely filled by sloughing of immature cells into the lumens. Spermatozoa are present, however, these are deeply-staining and seemingly are old, retained sperm. Note the lack of any recognizable Leydig cells. The appearance is similar to that occurring in adult pituitary failure or in hypophysectomized animals. X175

a.



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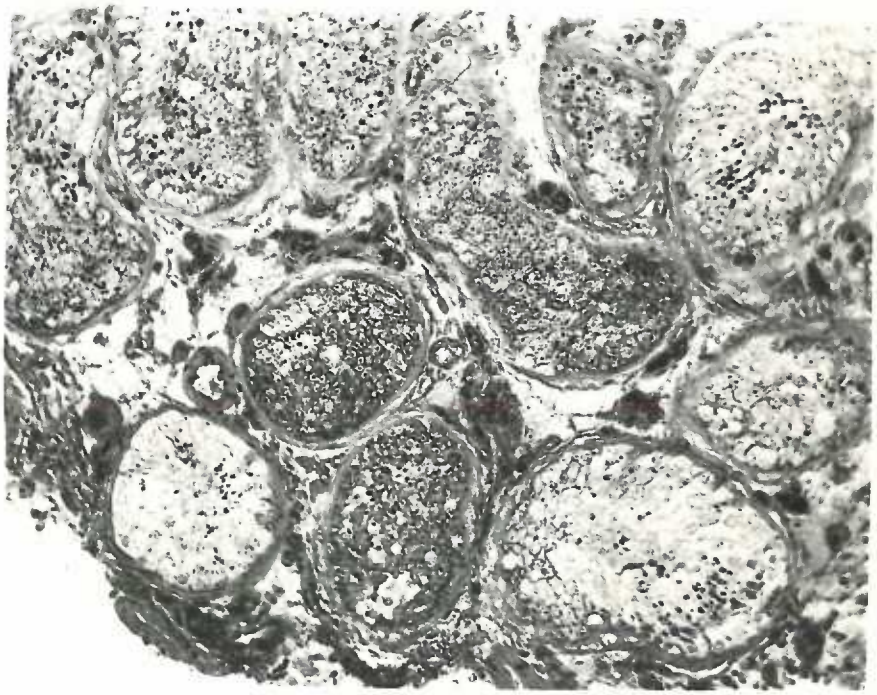

Figure 1

of the seven men before initiating therapy. (The seventh patient was included because he was found to have lower than normal titers of urinary gonadotrophins.) The principal defects encountered were disorganization of spermatogenesis, lack of maturation, and slougning of immature germinal cells into the lumen of the seminiferous tubules. There was a lack of irreversible changes such as severe or complete hyalinization of the basement membrane of the seminiferous tubules or lack of all germinal cells. The encouraging feature was the presence of germinal cells in various stages of maturation which seemingly could go on to sperm formation with the proper impetus. The Leydig cells appeared normal, which was in accord with lack of any clinical evidence of androgen deficiency. The biopsies showed a marked similarity; a representative biopsy (patient K. H.) is illustrated in Figure 1a.

Sperm Counts: Sperm counts were performed by diluting seminal fluid in a red or white cell pipette with dilute aqueous methylene blue, and then counting the sperm in a Neubauer counting chamber. Sperm counts are presented graphically in Figures 2 to 7. (As no more than an occasional sperm was ever encountered in R. C.'s specimens, data on his sperm counts are not presented.) Although abnormalities in sperm morphology and motility were encountered in some of the patients, no significant alterations occurred during or after therapy.

Great variations in the number of sperm were observed before, during and after instituting therapy. Averages before, during and after therapy, however, indicated an upward trend during FSH therapy in the 6 patients, followed by a decrease during the time of maximal

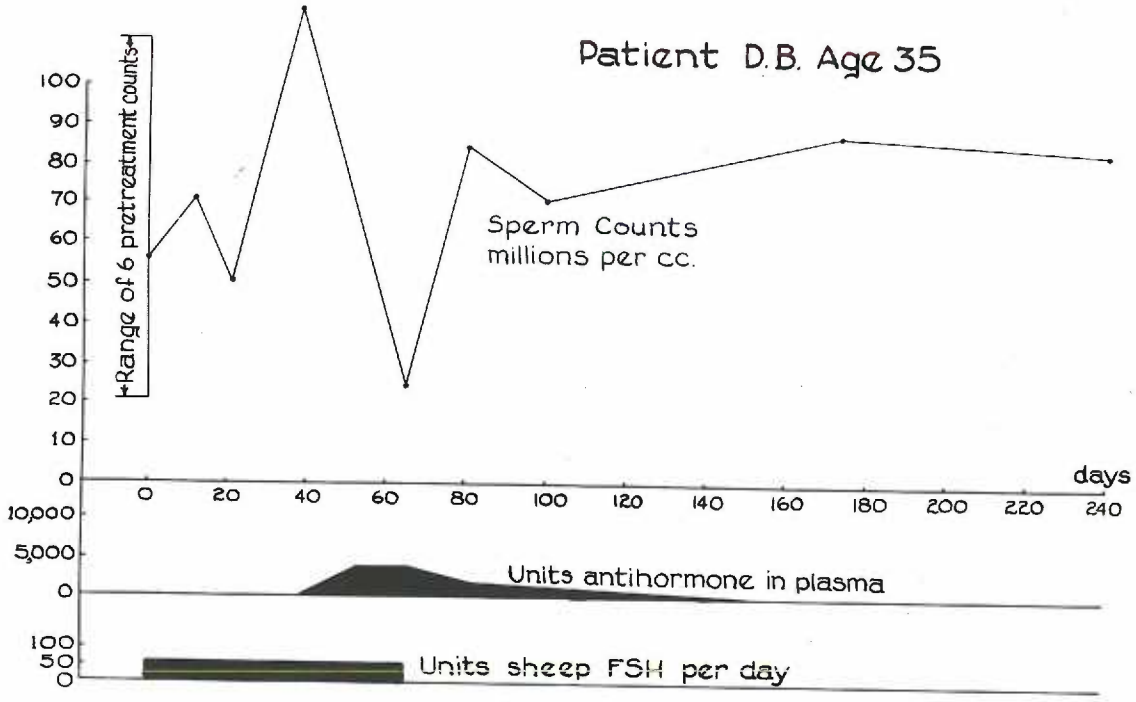

Figure 2

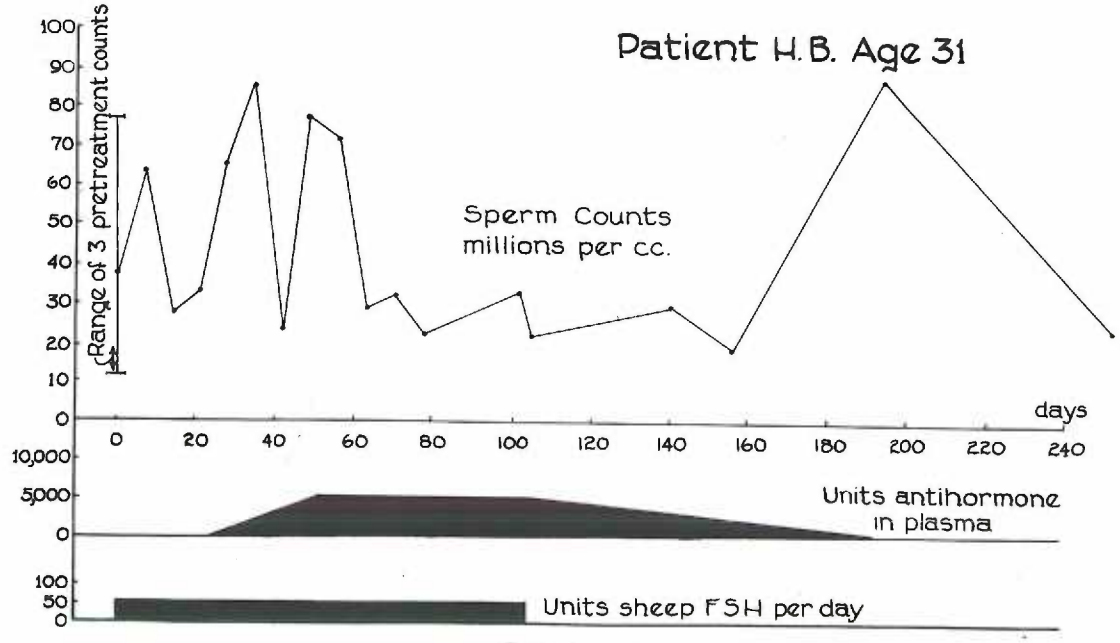

Figure 3

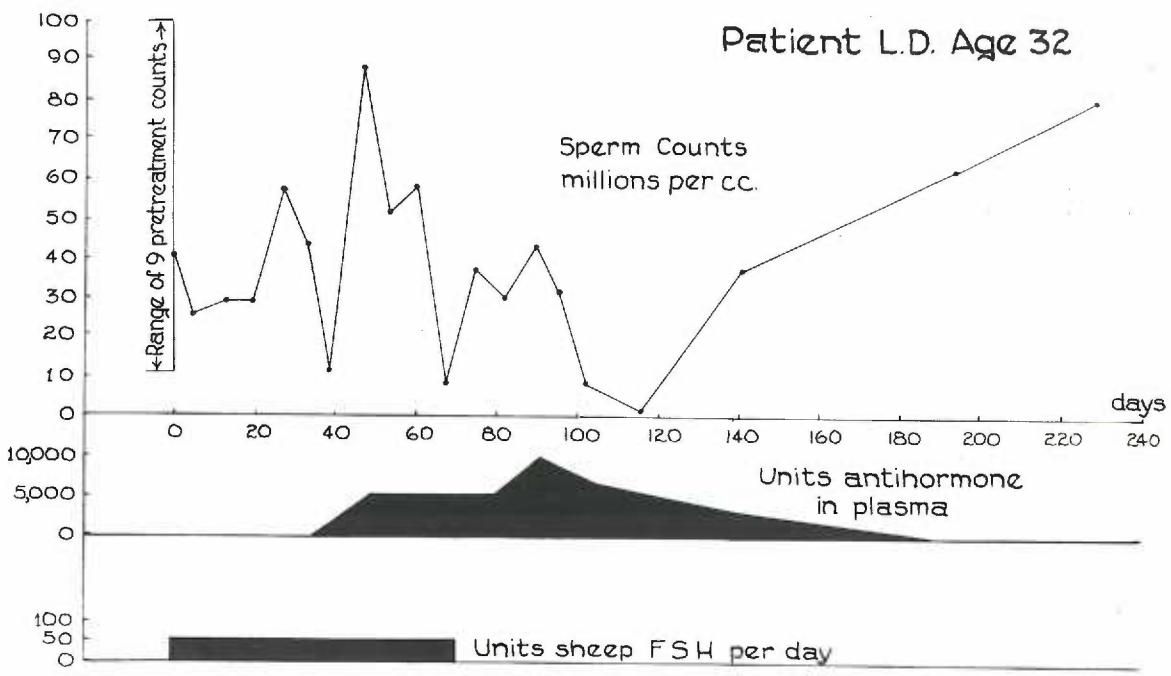

Figure 4

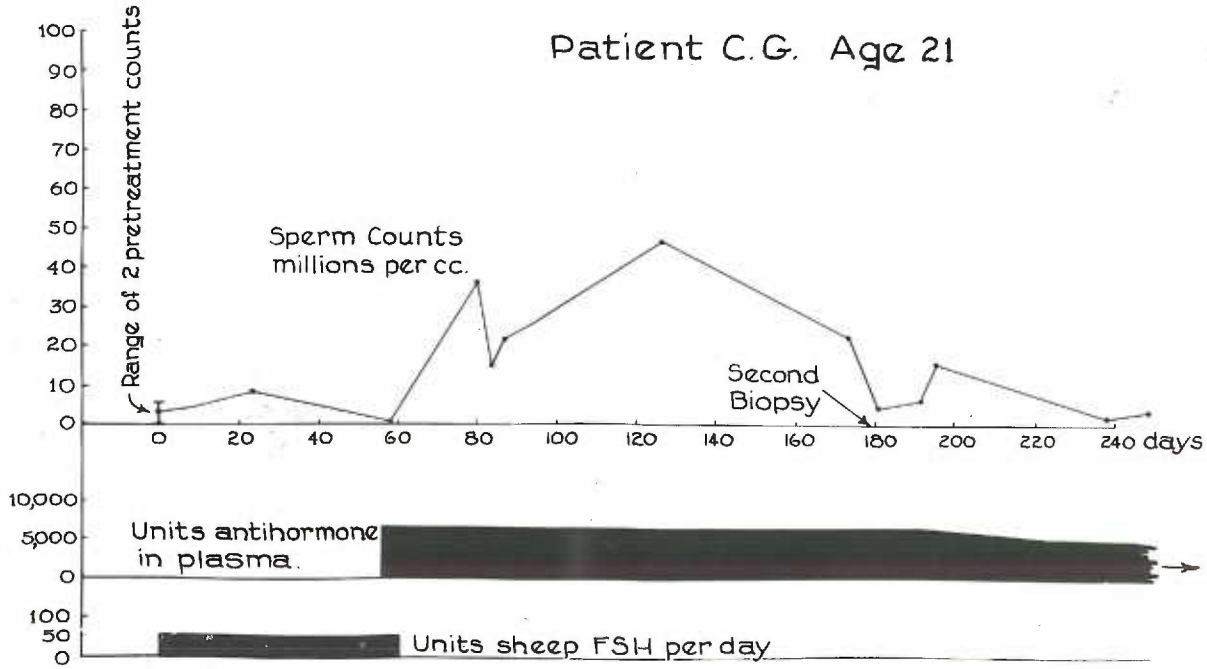

Figure 5

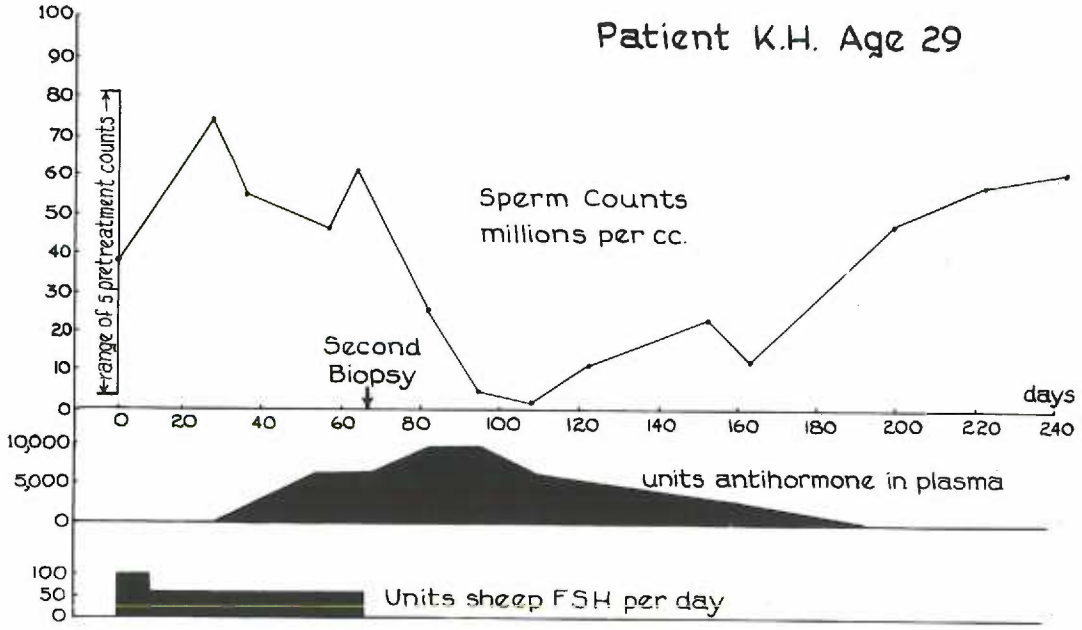

Figure 6

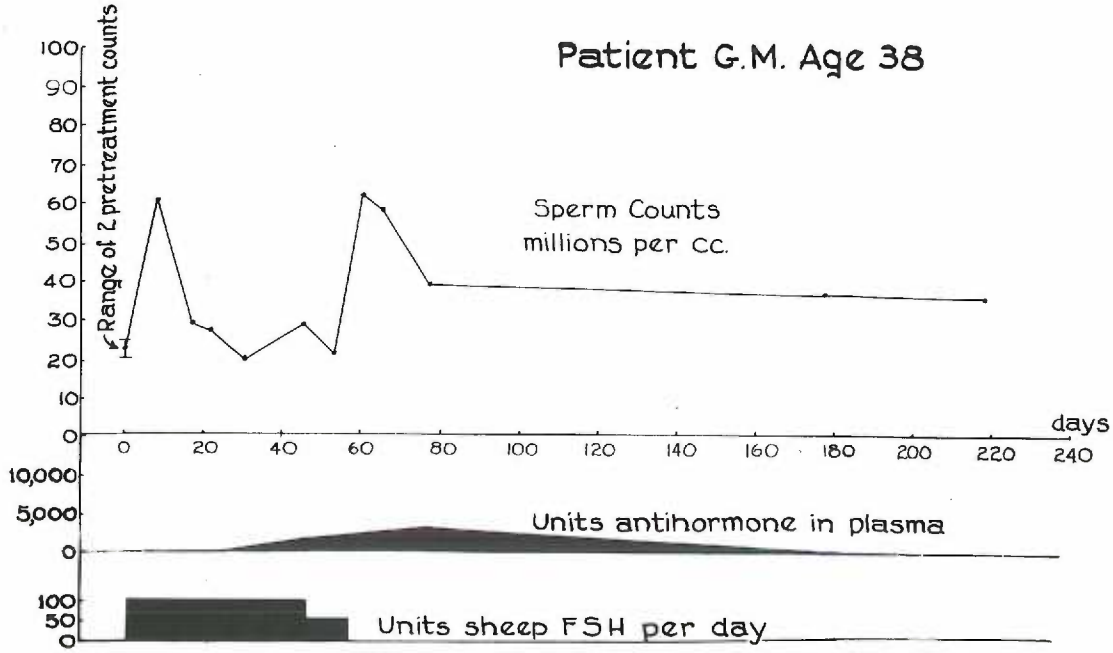

Figure 7

antihormone formation in three of the patients with high antihormone titers. The decrease in each instance was below the pretreatment average number of sperm. A fourth patient with similarly high antihormone titers also had a decrease in numbers of sperm during the time antihormones were elevated, but the onset of the decrease was delayed until two and one half months after initial antihormone detection. The two patients in whom numbers of sperm did not decrease experienced the least amount and shortest duration of antihormone formation.

Following the disappearance of detectable antihormones in the plasma, the average numbers of sperm rose to pretreatment levels or higher in the three cases in which a decrease had occurred, and remained at the pretreatment level or higher in the two in which no decrease was encountered. The patient with the delayed decrease continued to have a low sperm count, and continued to have antihormones in the plasma at the last date tested, 283 days after stopping therapy.

Urinary gonadotrophin excretion: Before treatment was instituted, gonadotrophin titers were normal in five patients, below normal in one and at the upper limits of normal in one (Table 1). Figures for normal individuals are presented in a previous publication (9).

During FSH administration (five patients tested) no increase in gonadotrophin excretion was noted. The titers fell below the pretreatment level in four patients and remained unchanged in one (Table 1).

After FSH administration had been stopped, and during the time of antihormone formation, gonadotrophin excretion was increased to the pretreatment level in six patients and was slightly above the pretreatment level in the remaining one (Table 1).

TABLE I. URINARY GONADOTROPHIN ASSAYS BEFORE, DURING AND AFTER F.S.H. THERAPY AND ANTIHORMONE FORMATION

Patient	Anti-hormone formation	FSH adminis-tration	Days after initiating therapy urine collected	Assay rats* uterine weight mg.	Assay rats* ovarian weight mg.	Number of rats
D.B.	0	Before	0	47	11	7
	+	After	65-68	56	10	4
	0	After	185-188	110	12	4
H.B.	0	Before	0	108	21	7
	+	During	66-69	58	9	4
	+	During	101-104	130	12	4
	+	After	141-146	115	22	3
	0	After	241-244	141	26	4
R.C.	0	Before	0	63	28	8
	0	During	45-49	90	13	4
	+	During	81-84	78	14	4
	+	After	87-92	59	13	3
	±	After	138-147	86	12	3
	0	After	200-203	84	44	3
L.D.	0	Before	0	123	23	8
	+	During	63-66	76	10	4
	+	After	83-88	173	22	3
	±	After	179-184	107	69	3
C.G.	0	Before	0	118	38	4
	+	After	64-67	135	16	2
	+	After	96-99	101	51	2
	+	After	167-172	126	45	4
	+	After	186-191	109	24	3
	+	After	249-253	100	64	3
	+	After	282-288	89	77	3
K.H.	0	Before	0	120	12	4
	+	During	64-66	36	8	3
	+	After	69-72	41	8	4
	+	After	73-76	113	12	2
	+	After	185-190	125	23	3
	±	After	218-221	120	18	4
G.M.	0	Before	0	72	18	7
	0	During	26-29	92	19	4
	+	During	54-56	133	23	4
	+	After	71-76	116	25	3
	±	After	162-168	125	17	4
Uninjected control rats				36	13	63

*Each rat received the ultrafilter concentrate of a 12-hour urine specimen.

After or at about the time of disappearance of antihormones from the circulation, gonadotrophin excretion was above the pretreatment level in three patients and equal to the pretreatment level in three others (Table 1).

DISCUSSION

Sperm Counts. Following the initiation of FSH therapy, there was an initial rise in the sperm output that soon reached a plateau or sharply declined to levels that were in some instances lower than before therapy was begun. With the exception of one patient, the rise in output of sperm was slight. There was no improvement in the impaired motility or the abnormal morphology of the sperm encountered in some of the patients. The lack of decided improvement, despite the fact that the seven subjects seemed suitable candidates for FSH therapy, may be due to 1) insufficient amounts of FSH administered, 2) the formation of antihormones which interceded so early that time for stimulating spermatogenesis may have been inadequate, or 3) the defects in spermatogenesis may not have been amenable to correction with gonadotrophins.

The slight rise in average numbers of sperm occurring in five patients and the sharp rise in one lends encouragement to the possibility that FSH therapy is potentially capable of stimulating spermatogenesis. The fact that an early plateau or early decline occurred could be correlated with the presence of antihormones in most instances. Thus, one limiting factor in adequate therapy appears to be the formation of antihormones. To circumvent early antihormone formation, perhaps other

sources of gonadotrophins or more highly purified forms of FSH, less apt to elicit antihormone formation (27), could be applied.

It would appear that antihormone formation did not cause permanent suppression of spermatogenesis, since output of sperm increased to pretreatment levels or above after the antihormones disappeared from the circulation.

The effects of antihormone formation on the microscopic appearance of the testis were studied in two instances in which biopsies were repeated after therapy when antihormones were present. In patient C. G., the biopsy was performed on day 179, and in patient K. H., on day 65 after initiating therapy. In both patients, sperm counts were decreasing at the time the biopsy was obtained.

Similar changes occurred in both patients and consisted of a reduction in size of the seminiferous tubules, reduction in the number of mature cells of the germinal series leaving a preponderance of spermatogonia and primary spermatocytes, sloughing of a large number of immature cells into the lumen of the tubules, an apparent retention of mature spermatozoa (which stained deeply and seemingly were old forms), and disappearance of recognizable interstitial cells of Leydig (Figure 1b, patient K. H.). The general appearance was similar to that encountered in hypogonadotrophic eunuchoidism (26) and in adult pituitary failure (28). The appearance thus was not unlike that seen in hypophysectomized animals (29).

The obvious explanation for the marked regression of the seminiferous tubules is that the antihormones prevented stimulation of the tubules by either the exogenous sheep FSH or the patients' endogenous

FSH. Thus, as concerns the seminiferous tubules, the patient had essentially been effectively hypophysectomized. Insert 47

The regression of the interstitial cells of Leydig suggests that either the sheep FSH contained enough interstitial-cell stimulating hormone (ICSH) to cause anti-ICSH formation, thus preventing stimulation of the Leydig cells by endogenous ICSH; or the antihormones to FSH were sufficiently non-hormone-specific to prevent endogenous ICSH stimulation. Apparently androgen production was not markedly interfered with for any great length of time, since none of the patients experienced androgen withdrawal symptoms.

Urinary gonadotrophins were tested during FSH therapy to determine whether appreciable amounts of hormone were being excreted. Since gonadotrophic titers were lower during treatment than before treatment, it seems reasonable to conclude that no appreciable amounts of the injected sheep FSH were excreted in the urine.

The decrease during FSH therapy cannot be accounted for by antihormone suppression. This was concluded from the fact that in one case the decrease occurred prior to antihormone formation, and from the fact that gonadotrophins increased to pretreatment levels during the presence of maximal antihormone titers soon after FSH therapy was stopped.

The rise in gonadotrophins encountered in four of the patients during the time of declining antihormone titers or soon after antihormones disappeared can be explained by the findings of Meyer, Kupperman and Finerty (30)(9). They noted that upon injecting antihormones in rats, a rise in the pituitary content of gonadotrophins occurred. Upon stopping

the injections of antihormones, evidence of increased secretion of gonadotrophin was obtained, following which gonadotrophin content of the pituitary decreased. They concluded that the increased pituitary secretion of gonadotrophins was due to the decrease in gonadal function caused by administering antihormones.

SUMMARY AND CONCLUSIONS

Criteria were set forth for the selection of candidates for treatment of male infertility with a purified preparation of sheep anterior pituitary glands containing mainly follicle stimulating hormone (sheep FSH). These included:

1. Testicular biopsies should reveal a suitable substrate for the action of the FSH, i.e., testicular defects that appear potentially reversible.
2. Gonadotrophin production should not be elevated. In such instances, addition of exogenous FSH would be superfluous.

Seven sterile men were judged to have fulfilled these prerequisites. They therefore were given daily injections of 50 units of sheep FSH for 2 to 3 months.

The average number of sperm increased somewhat initially, and then maintained a plateau or decreased. The lack of progressive rise seemed to coincide with the presence of circulating antihormones; as the antihormones disappeared, sperm production increased in those instances where it had formerly declined.

Testicular biopsies obtained from two patients at a time when

antihormones were present and when sperm counts were decreasing revealed a microscopic appearance not unlike that encountered following hypophysectomy in adult animals.

Urinary gonadotrophin excretion was determined before, during and after therapy. The injected sheep FSH was not excreted in the urine in appreciable quantities. Antihormones did not suppress endogenous gonadotrophin excretion.

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