Evidence for a Specific Seminiferous Tubular Factor Affecting Follicle-Stimulating Hormone Secretion in Man

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ABSTRACT The interaction of the testis and gonadotropin secretion was studied in 15 men surviving chemotherapy for lymphoma. Azoospermia and complete destruction of all testicular germinal elements were present in 10 of the 15 men; however, Sertoli cells and Leydig cells were present. In these 10 men plasma follicle-stimulating hormone (FSH) levels were fourfold higher than in normal men of similar age whereas luteinizing hormone (LH) levels were normal. In contrast, both FSH and LH were normal in the remaining five men. Three had a full complement of spermatogenic tissue on biopsy and normal sperm concentrations. The other two men were azoospermic; one demonstrated full spermatogenesis in 30% of his tubules; the other had only a few spermatogonia in all tubules. In those patients with lower levels of gonadotropins pituitary insufficiency was excluded by the demonstration of appropriate responsiveness of FSH and LH to clomiphene administration. Similarly, Leydig cell function was normal since plasma testosterone was within the normal range in 13 of the 15 men and only slightly decreased in two. Thus, following chemotherapy, testicular damage was restricted to the germinal tissue, and this in turn was associated with a selective increase in FSH. The source of the FSH inhibitor is either the Sertoli cell or early germinal elements. However, since FSH levels are only half as high as those reported for castrate men, other testicular factors may modify FSH secretion.

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INTRODUCTION

The testis appears to have two functionally independent compartments, the Leydig cell, responsive to luteinizing hormone (LH),[1] and the seminiferous tubule, responsive to follicle-stimulating hormone (FSH) (1). The precise mechanisms of control and the interrelationships among the various components are poorly understood. Earlier work suggested that damage to the testis primarily affecting the germinal epithelium was associated with increased excretion of FSH (2–6). Since antitumor drugs, particularly the alkylating agents, are known to induce destruction of germinal tissue (7–11), we have evaluated a population of men who had received intensive chemotherapy for lymphoma (12) in order to study the roles of the components of the testis in the regulation of gonadotropin secretion.

METHODS

Patients. Gonadal-pituitary interaction was evaluated in 15 men in good health for 2 months to 7 yr after intensive multiagent chemotherapy for lymphoma. The mean age was 38 yr with a range of 21–52 yr. In all men, pubertal development and sexual potency were normal; 11 men had fathered children before diagnosis of lymphoma. One was single and three men were married for less than 1 yr. One of the men (W. W.) had had mumps orchitis as a child with resulting unilateral testicular atrophy; however, he had fathered three children. The clinical features are summarized in Tables I and II.

Karyotype. Chromosome analysis of leukocyte cultures from heparinized blood showed a normal male karyotype in all men.[2]

[1] *Abbreviations used in this paper:* FSH, follicle-stimulating hormone; LH, luteinizing hormone.

[2] Karyotype analyses were performed by Dr. Elizabeth Chu, Cytogenetics Laboratory, National Cancer Institute, National Institutes of Health, Bethesda, Md.

TABLE I

Clinical and Laboratory Features of Men in Remission following Chemotherapy

Patient	Age	Disease and stage	Total chemotherapy*	Length of remission	Children before treatment	Sperm concentration
	yr			*yr*		*millions/cc*
W. G.	35	Hodgkin 4B	N 58 V 12.6 M 4250 P 1400	3	1	0 n = 10
D. L.	26	Burkitt's lymphoma	C 17,500	6/12	0	0 n = 15
R. B.	26	Hodgkin 3B	N 72 V 16.8 M 6000 P 1120	8/12	0	0 n = 3
L. I.	30	Hodgkin 3B	MX 450 V 7.2 P 5185 C 3750	2	5	0 n = 2
W. P.	40	Lymphosarcoma 3B	V 21 P 7000 C 28000	2	2	0 n = 8
F. R.	51	Lymphosarcoma 4B	V 8.4 C 12,000 P 3000	6/12	3	0 n = 8
J. R.	48	Hodgkin 4B	N 96 V 22.4 M 4900 P 840	4	1	0 n = 6
W. W.	41	Reticulum cell sarcoma 3B	N 72 V 17 M 6000 P 3360	2	3	0 n = 10 (Rare spermatid)
C. P.	54	Hodgkin 4A	N 72 V 17 M 6000 P 3360	2	2	0.2 n = 10 (0–0.4)
F. G.	45	Hodgkin 3B	N 144 V 13 M 4000 P 5040	2/12	2	0.4 n = 7 (0–1)

* N, nitrogen mustard; V, vincristine; M, methylhydrazine; P, prednisone; and C, cytoxan in milligrams per square meter of body surface area; MX, methotrexate in total milligrams.
‡ Mean values; range in parenthesis.

Experimental design. To assess testicular-pituitary interactions, we evaluated sperm counts, testicular histology, plasma testosterone, and gonadotropin concentrations in all men. The patients collected serial seminal fluid specimens by masturbation. A 2 day period of sexual abstinence was required before each collection. A testicular biopsy was obtained under local anesthesia on each patient. Plasma testosterone and gonadotropins were measured before and

Who Demonstrate Absence of Germinal Epithelium on Testicular Biopsy

Testicular histology	Plasma FSH Control	Plasma FSH Clomiphene	Plasma LH Control	Plasma LH Clomiphene	Plasma Testosterone Control	Plasma Testosterone Clomiphene
	mIU/ml		*mIU/ml*		*μg/100 ml*	
Sertoli cells	54 (50–68) n = 6	90	12.9 (10.1–15.6) n = 6	23	0.72	0.74
Sertoli cells	42.5 (38–45.7) n = 9	78	17.6 (10.2–26.6) n = 9	32	0.61	0.85
Sertoli cells	39.6 (32.5–44.5) n = 5	53	21.2 (17.5–23.6) n = 5	32	0.31	0.70
Sertoli cells	26.6 (23–33) n = 4	48	9.5 (7.6–13.8) n = 4	23	0.40	1.00
Sertoli cells	41.1 (38–44) n = 4	67	21.2 (14.2–24.1) n = 4	24	0.25	0.48
Sertoli cells	50.3 (29.5–78.5) n = 4	59	19.3 (15.4–23.0) n = 4	27	0.44	0.64
Sertoli cells	51 (45–54) n = 7	59	23.9 (22–26.5) n = 7	32	0.64	0.68
Sertoli cells	43.5 (41–47) n = 4	64	17.0 (16.2–18.6) n = 4	29	0.22	0.52
Sertoli cells	38.6 (32–42) n = 5	54	15.2 (12.3–19.9) n = 5	26	0.46	0.54
Sertoli cells	44 (34–48) n = 5	60	20.5 (15.1–24.5) n = 5	35	0.82	0.85

after administration of clomiphene citrate orally, 200 mg daily for 5 days.

Gonadotropins. Plasma FSH and LH were measured by specific double antibody radioimmunoassay (13, 14) on multiple samples. Anti-FSH and anti-LH antisera were used as reagents. FSH and LH levels are expressed in terms of mIU of the second international reference preparation of human menopausal gonadotropin. The sensitivity of the FSH

TABLE II

Clinical and Laboratory Features of Men in Remission following Chemotherapy

Patient	Age	Disease and stage	Total chemotherapy*	Length of remission	Children before treatment	Sperm concentration
	yr			*yr*		*millions/cc*
D. R.	21	Hodgkin 3A	N 72 V 17 M 6000 P 3360	6/12	0	0 n = 9
J. Mc.	17	Hodgkin 4B	N 72 V 17 M 6000 P 3360	3	0	0 n = 8
J. M.	29	Hodgkin 4B	N 72 V 17 M 6000 P 3300	4	1	39 n = 9 (1–84)
N. S.	30	Hodgkin 3A	V 12 P 4500 C 6250 MX 720	7	2	21 n = 10 (61–141)
L. H.	43	Lymphosarcoma 4A	V 8.4 P 3000 C 12000	2	5	80 (42–100) n = 6

* N, nitrogen mustard; V, vincristine; M, methylhydrazine; P, prednisone; and C, cytoxan in milligrams per square meter of body surface area; MX, methotrexate in total milligrams.

‡ Mean values; range in parenthesis.

and LH methods is 2–4 and 4–6 mIU/ml, respectively. Interassay variation for both gonadotropins is < 20%. Normal values in men for FSH are 5–19 mIU/ml and for LH 10–30 mIU/ml.

Testosterone. Plasma testosterone was measured by New England Nuclear Corporation, Boston, Mass., using a double isotope derivative dilution technique (15). Normal values in men are 0.3–1.0 μg/100 ml (mean = 0.7 μg/100 ml).

Seminal fluid. Sperm concentration and morphology were examined in multiple semen specimens. Sperm counts were determined using a hemocytometer. Azoospermia was defined as no sperm seen on direct smear of the ejaculate. Immature and abnormal sperm were quantified from smears of the seminal fluid using MacLeod's criteria (16).

RESULTS

Seminiferous tubular function. 10 of the 15 men had only Sertoli cells within the seminiferous tubules on testicular biopsy (Table I and Fig. 1A). Numerous semen analyses showed that 8 of these 10 men were azoospermic while in 2, occasional ejaculates contained low concentrations of spermatozoa.

The remaining five men differed in that testicular biopsy demonstrated evidence of spermatogenesis (Tables II and III). The tubules of three of these patients showed complete spermatogenesis (Fig. 1D) and their ejaculates contained normal concentrations of sperm. The morphology of the sperm was normal except for a small increase in amorphous cells (Table IV). The other two men were azoospermic, yet on biopsy (D. R.) showed spermatogonia in all tubules and rare spermatocytes and spermatids in a few tubules (Fig. 1B). The other (J. Mc.) demonstrated a full complement of spermatogenic tissue in 30% of the tubules (Table III) but adjacent tubules had only Sertoli cells (Fig. 1C).

Gonadotropin concentrations. Plasma FSH and LH measurements are summarized in Tables I and II. In the 10 men with absent germinal epithelium on testicular biopsy the mean FSH level was 44.0 ±10.0 (SD) mIU/ml with a range of 23.0 to 79.0 mIU/ml; a 4-fold increase above the mean level in normal men (Fig. 2). In contrast, FSH levels were normal in the five men

Who Demonstrate Presence of Germinal Epithelium on Testicular Biopsy

Testicular histology	Plasma FSH Control	Plasma FSH Clomiphene	Plasma LH Control	Plasma LH Clomiphene	Testosterone Control	Testosterone Clomiphene
	mIU/ml		*mIU/ml*		*μg./100 ml*	
Scattered spermatogonia, spermatocytes and spermatids	11.9‡ (7.5–17.4) n = 6	36	13.4‡ (11.1–15.2) n = 6	36	1.10	1.80
30% of tubules with complete spermatogenesis	15.0 (13.2–17.3) n = 5	45	18 (14.6–23.5) n = 5	32	0.30 0.30	0.83 0.83
Complete spermatogenesis	7.6 (6.0–8.5) n = 5	16.0	10.4 (9.7–11.0) n = 5	17	0.91	1.11
Complete spermatogenesis	7.6 (6.0–9.3) n = 5	21	5.4 (4.2–7.7) n = 5	12	0.64	1.20
Complete spermatogenesis	14.3 (10.8–16.8) n = 4	20	6.3 (6.0–6.5) n = 4	14	0.61	0.79

TABLE III

Quantitation of Cells in the Ejaculate and in the Seminiferous Tubules of Men Demonstrating Spermatogenesis after Chemotherapy

Patient	Seminal fluid: No. of samples	Seminal fluid: Sperm* concn.	Seminal fluid: Total* sperm	Testicular biopsy: Sertoli cells	Testicular biopsy: Spermatogonia	Testicular biopsy: Spermatocytes	Testicular biopsy: Spermatids	Testicular biopsy: Sperm
		millions/cc	*millions*	%	%	%	%	%
D. R.	9	0	0	97.4	1.7	0.1	0.8	
J. Mc.	8	0	0	100‡	—	—	—	—
			Rare spermatid	7.6§	9.0	32.6	39.0	12.0
J. M.	9	39	94	13.1	16.2	24.5	30.0	16.2
N. S.	10	89	171	12.4	15.5	24.0	37.3	10.9
L. H.	6	80	198	16.3	14.4	21.6	16.1	31.9

* Mean values.

‡ Two-thirds of tubules show only Sertoli cells.

§ Cellular profile of remaining one-third of tubules which show spermatogenesis.

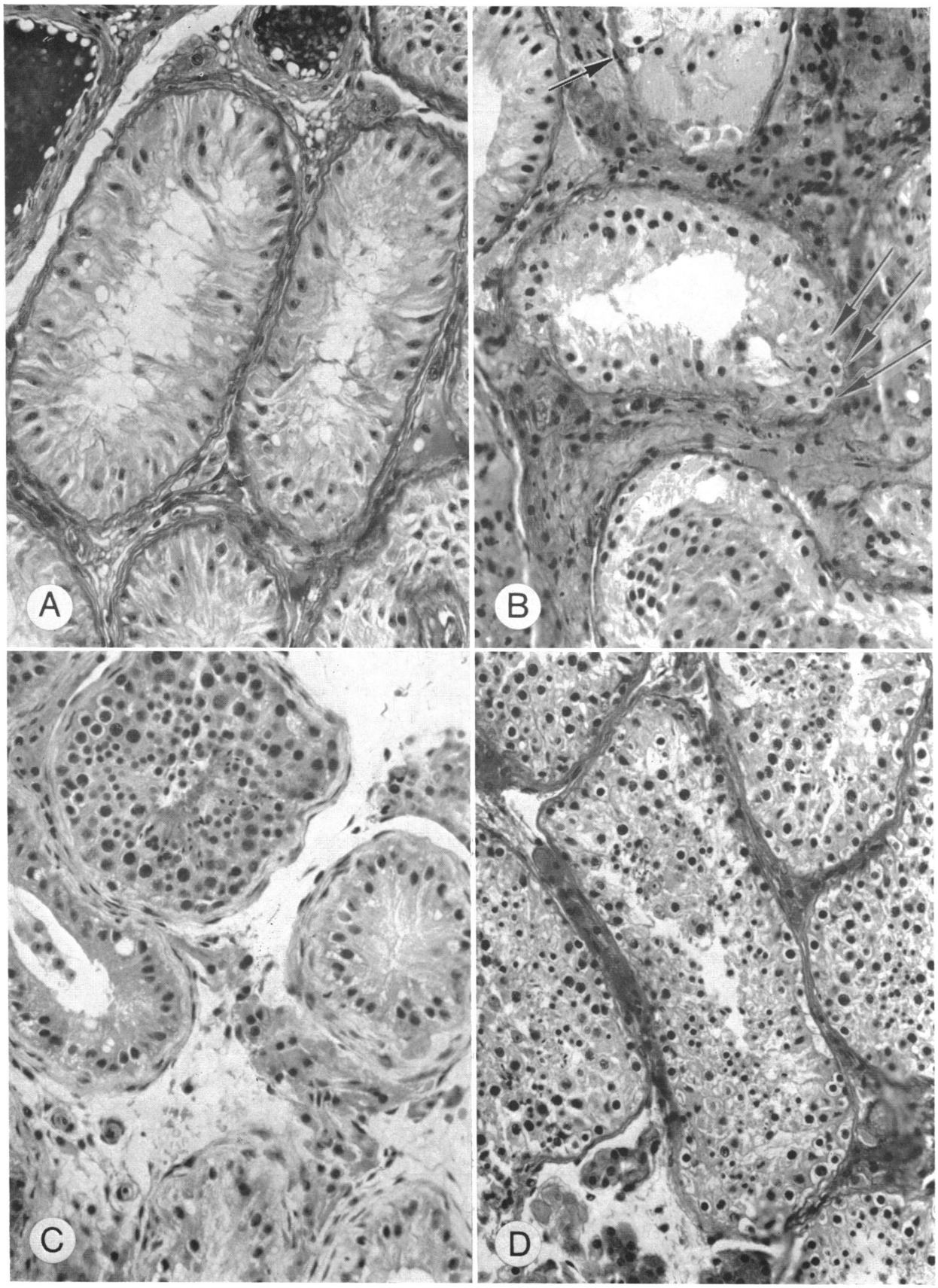
A
B
C
D

TABLE IV

Cellular Characteristics of Seminal Fluid of Men Demonstrating Persistent Mature Sperm in Ejaculates after Chemotherapy

Patient	No. of samples	Sperm concn.	Total sperm	Morphology							
				Oval	Large	Small	Tapering	Amorphous	Double head	Double tail	Immature
		million/cc	*millions*								
L. H.	6	80* (42–100)	198 (127–300)	61.0	—	6.5	4.5	26.7	0.3	0.2	1.0
J. M.	9	89 (1–84)	94 (1–216)	73.1	0.6	0.2	—	25.4	0.2	0.1	0.3
N. S.	10	89 (61–141)	171 (100–288)	67.9	—	2.7	1.8	25.5	0.8	0.2	0.9
Normals (MacLeod)§ 1967	1500	95	218	73	2.7	8.6	6.1	8.6	1.0	—	0.4

* Mean value.

‡ Range of sperm concentration.

§ Personal communication from Dr. John MacLeod.[3]

with germinal epithelium on biopsy (mean = 11.1 ±4.0 mIU/ml, range − 6.0 to 18.0 mIU/ml).

No such differences were noted for LH determinations. LH levels in the 10 men with totally absent germinal tissue (mean = 17.8 ±5.2 mIU/ml; range −7.6 to 26.6 mIU/ml) were higher ($P < 0.01$) than those for the five men with evidence of spermatogenesis (mean = 10.7 ±5.0 mIU/ml; range − 4.2 to 23.5 mIU/ml). All values were within the range reported for normal men (Fig. 3).

Testosterone concentration. Plasma testosterone was measured in a single sample from each of the patients before clomiphene administration. The data are presented in Tables I and II. Testosterone values were within the range for normal men in all but two of the subjects (Fig. 4). The mean level in the 10 men without germinal tissue (0.48 ±0.20 μg/100 ml) was lower than that in the remaining 5 men (0.71 ±0.30 μg/100 ml) but these differences were not statistically significant.

Clomiphene administration. FSH, LH, and testosterone responses to clomiphene administration are shown in Figs. 5–7 and Tables I and II. At least a 25% rise in concentrations of both FSH and LH occurred in 14 of the 15 patients. The mean increases were 83 and 73% respectively. Similar increases of testosterone (mean = 74%) were observed in 12 patients. This change is consistent with responses in normal men (17).

DISCUSSION

The recent use of intensive multiagent chemotherapy in patients with lymphoma has permitted increased survival (12). Since destruction of germinal tissue has been known to occur following the administration of alkylating agents to men (7, 11) with Hodgkin's disease, the availability of a population of men surviving lymphoma provided a unique opportunity to study the various components of testicular function.

Because this study was retrospective in design, the adequacy of testicular function before lymphoma and treatment was not known. However, since libido and sexual potency had been normal and since 11 of the men had fathered children before disease, it was assumed that the testes of these men had been normal before illness.

The findings of azoospermia and of severe depletion of germinal elements on testicular biopsy in 70% of the men studied are in keeping with the known destructive effects of alkylating agents in rodents (8–10) and man (7, 11). This particular susceptibility of the germinal tissue to alkylating agents and antimetabolites probably is a consequence of the rapid cell turnover present in the spermatogenic cells of the seminiferous

[3] Personal communication. John MacLeod, Department of Anatomy, Cornell University Medical School, New York.

FIGURE 1 Photomicrographs of representative testicular biopsies (H and E ×250). A. Testis of patient W. G. with absence of germinal epithelium; B. Testis of patient D. R. showing scattered spermatogonia (arrows), note predominant Sertoli cells; C. Testis of patient J. Mc. showing tubules with normal spermatogenesis adjacent to tubules with only Sertoli cells; D. Testis of patient J. M. demonstrating normal spermatogenesis. Leydig cells in all biopsies appear normal.

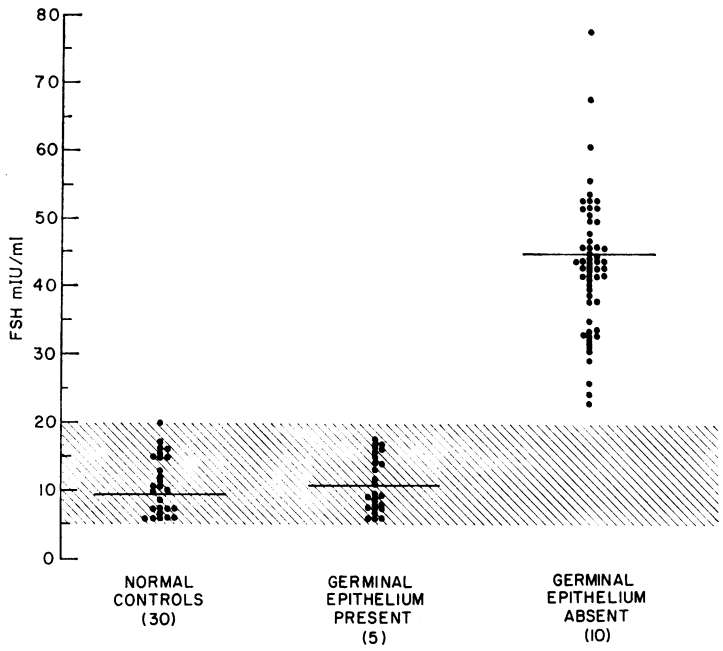

FIGURE 2 Plasma FSH levels in men treated for lymphoma are compared to levels in normal men of similar age. The range of values for normal men is given by the shaded area.

tubules. Previous studies indicated that the degree of germinal cell damage as well as the time for repopulation of the tubules appeared to be dose related (9). Whether this same relationship exists in men receiving intensive chemotherapy is not clear. The lengths of remission are shown in Tables I and II and refer to the duration of time since the last dose of chemotherapy. Although the men with biopsy evidence of germinal epithelium tend to be in remission longer, there are too few patients to state conclusively that return of germinal tissue is related to the interval of time since chemotherapy. The reversibility of germinal cell destruction may vary widely among men receiving chemotherapy. However, inherent resistence of the testis to alkylative agents in some men cannot be excluded.

The striking feature of this study was the selective increase in plasma FSH levels in those men with total absence of spermatogenesis. A state of increased FSH, germinal cell aplasia, and normal Leydig cell function thus provide direct evidence for a seminiferous tubular factor which specifically affects FSH secretion in man.

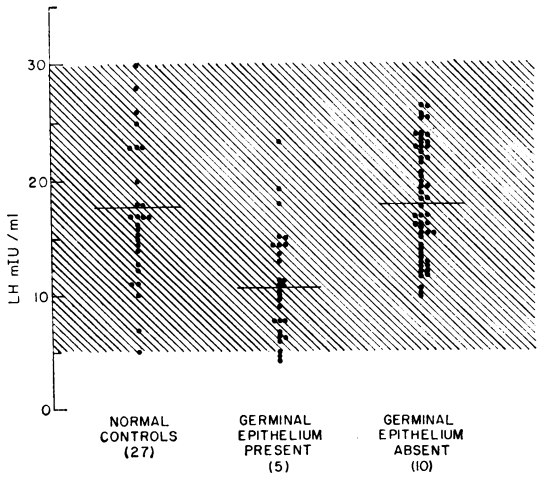

FIGURE 3 Plasma LH levels in man treated for lymphoma are compared to levels in normal men of similar age. The range of values for normal men is given by the shaded area.

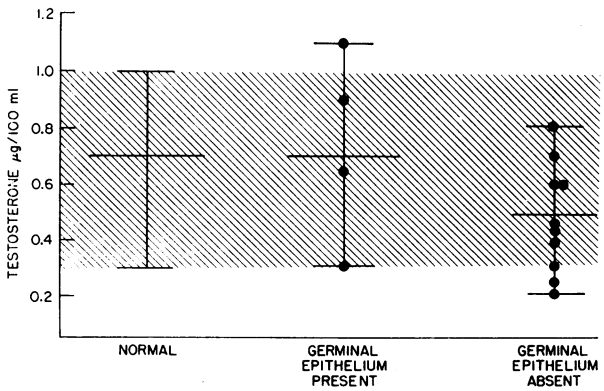

FIGURE 4 Plasma testosterone levels in men treated for lymphoma are compared to levels in normal men. The range of values for normal men is given by the shaded area.

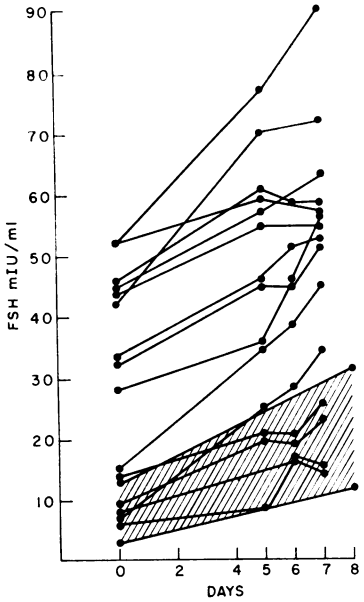

FIGURE 5 Plasma FSH levels before and after clomiphene administration to treated lymphoma patients. The range of response for normal men of similar age is shown in the shaded area.

The data suggest that the integrity of the precursor germ cells is important in the reinitiation of spermatogenesis, despite the elevated FSH levels. Sperm production may not proceed unless these precursor cells are viable. Recently, Paulsen has reported briefly that, when the testes of man are radiated, the germinal epithelium is temporarily damaged and Leydig cell function remains intact (6). During the period of tubular damage, he found urinary FSH titers increased, whereas urinary LH excretion remained unchanged. Our data provide specific evidence for an increase in plasma

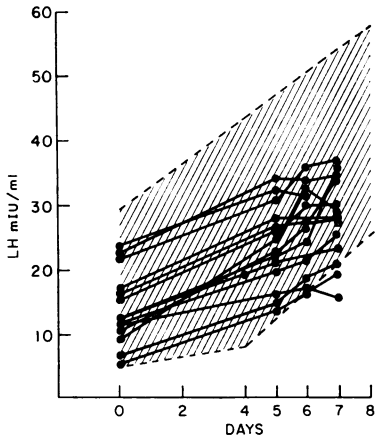

FIGURE 6 Plasma LH levels before and after clomiphene administration to treated lymphoma patients. The range of response for normal men is shown in the shaded area.

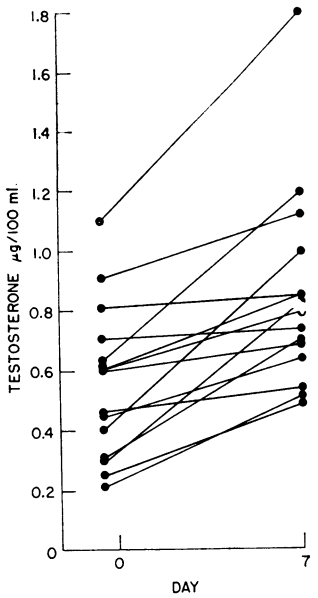

FIGURE 7 Plasma testosterone levels before and after clomiphene administration to treated lymphoma patients.

FSH following damage primarily to the seminiferous tubules.

Early investigators postulated that a water-soluble factor, "inhibin," of germinal epithelium origin was responsible for regulation of FSH secretion (2–5). However, this hypothesis has been accepted cautiously since the bioassays used to estimate the urinary FSH and LH excretion were relatively nonspecific. To date there has been no further characterization of "inhibin."

The anatomical site of origin for the FSH inhibitor similarly has been controversial. Earlier studies attempted to relate the casting off of spermatid cytoplasm during spermiogenesis with regulation of pituitary FSH secretion (18, 19). Recently, Johnsen has presented data for man which demonstrate a correlation between absence of late spermatids on testicular biopsy and elevation of total urinary gonadotropins (20). He postulated that some interaction between the Sertoli cells and the more mature spermatogenic cells is of major importance in the maintenance of high levels of the FSH inhibitor. In contrast, Leonard, Leach, and Paulsen failed to demonstrate any correlation between sperm count and serum FSH levels in normal men (21). Elevated FSH levels have been found in some patients with idiopathic oligospermia (21–23), however, Leonard et al. could show no differences in germinal cell numbers between men with normal and those with elevated FSH titers. Thus, they rejected the hypothesis of control of FSH secretion by the germ cells per se and postulated control by an "independent station" as yet undefined. We would support the hypothesis that the anatomical site for the FSH inhibitor is either the Ser-

toli cell or the early germinal elements since several of our patients (D. R. and J. Mc.) had normal FSH levels despite severe germinal cell destruction and azoospermia.

Despite the drug-induced changes within the seminiferous tubules, Leydig cell function appears to be normal. This interpretation assumes that the small difference noted in LH and testosterone for men with the greatest tubular damage are not physiologically important since all values, except for two testosterone measurements, are within the range reported for normal men. It is not possible, of course, to know whether testosterone levels decreased for any one man as a consequence of treatment. However, at least no striking increases in LH are evident as would be expected if testosterone secretion were subnormal. Furthermore, failure of LH release as a cause for lower testosterone levels is unlikely since LH concentrations were normal and they increased appropriately following clomiphene administration. Thus, if Leydig cell damage is present in even a few men, the dysfunction must be minimal.

Perhaps of equal importance to the selective increase of FSH with isolated seminiferous tubular damage is the question of whether other testicular factors influence FSH secretion. In this regard, although plasma FSH titers were increased fourfold in the men with germinal cell aplasia, FSH levels were still only half as high as those reported by Ross (24) in castrate adult men using the same assay method. Since Leydig cell function appeared to be intact, one could postulate that testosterone may partially suppress FSH release. This, of course, is inconsistent with data reported by several investigators that testosterone, when administered in high dose, does not suppress FSH levels (25–27). Thus, the maintenance of FSH at partially reduced levels in the men with germinal cell destruction could be related to release of the FSH inhibitor from the remaining Sertoli cells or small islands of germinal tissue not detected on the biopsy. On the other hand, other steroids secreted by Leydig cells could also affect FSH titers. The increase in FSH following clomiphene administration is in keeping with a steroidal effect on FSH release. From our data, we would propose that although FSH secretion may be modulated in part by testosterone or other secretory products of the Leydig cells, other factors related specifically to the germinal epithelium are involved in FSH feedback control. A similar conclusion was reached by Swerdloff, Walsh, Jacobs, and Odell from studies in the rat utilizing cryptorchidism as a means of inhibiting spermatogenesis (28).

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