Pituitary Gonadotropin Content of Aspermatogenic Guinea Pigs* (32717)

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It is generally accepted, in laboratory animals at least, that interstitial cell stimulating hormone (or luteinizing hormone, LH) maintains the morphologic and endocrine status of interstitial tissue whereas follicle-stimulating hormone (FSH) acting synergistically with either LH or with androgens, induces tubular differentiation and maintains spermatogenesis. The effect of testicular components, particularly the spermatogenic elements, on pituitary gonadotropin function, however, is a more controversial subject.

For elucidation of this reciprocal relationship, specific deletion of the seminiferous epithelium is required. Experimentally induced aspermatogenesis seems to be ideally suited to this purpose because of the specific cell deletion resulting from immunization with antigen extracted from homologous testis or sperm (1). Interstitial tissue of the aspermatogenic animal continues to secrete androgens maintaining accessory organs of reproduction as in normal animals. It was decided, therefore, to employ the aspermatogenic guinea pig for studies involving the effect of aspermatogenesis on the pituitary content of FSH and LH.

Materials and Methods. Fifty male guinea pigs were immunized with 10 mg each of aspermatogenic antigen (ASA) extracted from homologous epididymal sperm emulsified in Freund's complete adjuvant and 50 male guinea pigs were similarly injected with saline emulsified in Freund's complete adjuvant. Procedures for extracting the antigen, emulsification in adjuvant, and injection have been provided in detail elsewhere (2). Two months postinjection animals were sacrificed by exsanguination, testes were rated for absence of spermatogenic tissue (2), anterior lobes of the pituitaries were removed and lyophilized. Pooled lyophilized adenohypophyses from normal and from aspermatogenic (ASP) male guinea pigs were each ground to a fine powder. Aliquots of each preparation were removed for FSH and LH assay. FSH content was determined in Spartan Sprague-Dawley immature female rats by the chorionic gonadotropin (HCG) augmentation method of Steelman and Pohley (3) modified by using a total dose of 50 IU of HCG and b.i.d. subcutaneous injections (4). Vehicle for all injections was Medium 199 (Microbiological Associates) adjusted to pH 7.2 with sodium bicarbonate. Relative potencies were determined by statistical analysis (parallel line assay) of log-dose response of ovarian weights.

LH activity was measured by the ovarian ascorbic acid depletion method described by Reichert and Parlow (5). Spartan Sprague-Dawley female rats, approximately 25-days old, were injected subcutaneously with 50 I.U. of pregnant mare serum (Gonadogen, Upjohn) and, 56 hours later, with 25 IU of HCG (Chorionic Gonadotropin, Upjohn). Test preparations, each in 0.5 ml of saline, were injected via the tail vein on day 6 after the HCG injection; four hours later the left ovary was removed for ascorbic acid analysis. Relative potencies were determined by statistical analysis (parallel-line assay) of log-dose response of ovarian–ascorbic acid concentrations.

Results and Discussion. Luteinizing hormone content did not differ significantly between adenohypophyses from normal and aspermatogenic guinea pigs (Table I). Relative to Armour's LH preparation, there is approximately 5 μg equivalents of LH per adenohypophysis. FSH content of adenohypophyses from normal guinea pigs was 0.38 times the FSH content of adenohypophyses from aspermatogenic guinea pigs (Table I). Relative to NIH-FSH-S3 there was 40 and 108 μg of

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TABLE I. FSH and LH Content of Adenohypophyses from Normal and from Immunologically Induced Aspermatogenic Guinea Pigs.

	FSH (Steelman-Pohley) assay			LH (ovarian ascorbic acid) assay		
Preparation	Dose	Number of rats	Ovarian wt. (mg)	Dose	Number of rats	Ovarian ascorbic acid (mg/100 gm of ovary)
Saline	0.5 ml	6	24.0	0.5 ml	11	69.9
HCG	50 μg	5	65.2			
NIH-FSH-S3	70 μg	5	113.9			
	140 μg	6	119.6			
	280 μg	6	221.1			
Armour LH lot no. R37729				2 μg	11	63.3
				6 μg	11	53.7
				18 μg	11	35.5
Adenohypophyses of aspermatogenic guinea pigs	0.5 gland equiv.	6	90.4	0.65 gland equiv.	11	59.9
	1.0 gland equiv.	6	132.7	1.95 gland equiv.	11	45.2
	2.0 gland equiv.	6	239.2			
Adenohypophyses of normal guinea pigs	1.25 gland equiv.	6	79.5	0.65 gland equiv.	11	56.8
	2.5 gland equiv.	6	156.6	1.95 gland equiv.	5	46.9
	5.0 gland equiv.	6	210.5			

Note.—Lamba (S/B) values: FSH, normal vs. ASP = .089;
FSH, adenohypophyses vs. standard = .132;
LH, normal vs. ASP = .254; and
LH, adenohypophyses vs. standard = .234.

FSH per gland, respectively.

A dualistic role for interstitial tissue and seminiferous tubule regulation of pituitary morphology and gonadotrophic hormone production was suggested (6,7). Witschi *et al.*, (8) concluded that a lack of spermatogenesis was responsible for the increase in hypophyseal secretion in the X-ray sterilized rat. Urinary excretion of total gonadotrophic hormone also increases in cases of azoospermia due to spontaneous hyalinization of testes tubules where normal Leydig-cell function is retained (9).

Cytological assessment of pituitaries from vitamin E-deficient (10) and from cadmium chloride-injected male rats (11) demonstrated characteristic castration features in peripheral pituitary gonadotrophs and essentially normal central pituitary gonadotrophs. In both of these animal preparations seminiferous tubules were degenerative whereas interstitial tissue function either remained at or had returned to an apparently normal state at time of evaluation. In an extended study of the effects of ovariectomy and cryptorchidism on FSH and on LH pituitary content in rats, Steinberger and Duckett (12) concluded the germinal epithelium inhibits FSH synthesis, but not release, from the pituitary.

The increased pituitary content of FSH in aspermatogenic guinea pigs is compatible with the thesis that FSH production is affected, either directly of indirectly (13,14) by a feedback machanism associated with tubular elements and corroborates the interpretations of the earlier studies. Whereas destruction of seminiferous tubules by rendering rats cryptorchid, a procedure which in guinea pigs severely affects testicular morphology but not accessory organ function or sexual behavior (15), leads to temporarily related increases of pituitary LH content accompanying increasing FSH content, immunologic suppression of spermatogenesis in guinea pigs does not significantly alter pituitary LH content.

Further studies immediately adaptable to analysis of the reciprocal relationship between seminiferous epithelium and anterior pituitary include assay of pituitaries removed at weekly intervals postimmunization to time of full development of aspermatogenesis (2 months) and then until repopulation of seminiferous epithelium occurs (6–8 months) (1).

Summary. Adenohypophyses from normal and aspermatogenic (ASP) guinea pigs were assayed for FSH (modified Steelman-Pohley assay) and for LH (OAAD assay) content. FSH content of adenohypophyses from normal guinea pigs was 0.38 times FSH content of adenohypophyses from ASP guinea pigs. Relative to NIH-FSH-S3 there was 40 and 108 μg of FSH per gland, respectively. There was no significant difference in luteinizing hormone content between adenohypophyses from normal and ASP guinea pigs. Relative to Armour's LH preparation, there is approximately 5 μg equivalents of LH per adenohypophysis. The increased pituitary content of FSH in the ASP guinea pig is compatible with the thesis that FSH production is affected, either directly or indirectly, by a feedback mechanism associated with tubular elements.

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Kinetics of the Effect of Vincristine Sulfate on the Reproductive Integrity of Proliferating Cultured Leukemia L1210 Cells* (32718)

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In order to cure leukemia, successful chemotherapy is dependent upon the eradication of every leukemia cell present in the host by drug dosages nonlethal to the host (1, 2). If host defense mechanisms are involved in leukemia cell eradication (3, 4), the total number of leukemia cells must at least be reduced to the number that can be eradicated by these processes. Recent investigations have indicated that certain antimetabolites of chemotherapeutic interest may be more effective in reducing *in vivo* leukemia cell populations when they are used on a multiple dose schedule than on a daily optimal dose schedule (5, 6). Pertinent to this multiple daily dose scheduling was the observation that when proliferating cultured L1210 leukemia cell populations were exposed to selected drugs which were effective only during a particular phase of the cell cycle, the kinetics of the reduction in the number of viable cells did not occur at a first-order rate (the fraction of cells killed

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