Influence of the Frequency and Route of Injection of HCG and FSH Antisera on Their Gonadotropic and Progonadotropic Responses[1] (37029)

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Maxwell (1) showed that 6 injections daily for 4 days of ovine pituitary gonadotropic preparations produced ovaries in the immature rat twice as large as those obtained by giving the same total dose in a single daily injection for 4 days. This finding is in accord with the short half-life of ovine pituitary LH (15 min) in the rat (2). Parlow and Reichert (3) found that 2 injections of FSH daily for 3 days were more effective than if the same total dose was given in a single injection daily for 3 days in the Steelman–Pohley (4) assay for FSH; FSH pituitary extracts from 6 species were used in this study. Salhanick (5) studied the FSH activity of human urine extracts by the Steelman–Pohley method (4) and found that the response of the ovaries of immature mice was greater when 2 injections daily for 4 days were made as compared to one injection daily.

Woods and Simpson (6) showed that relatively crude FSH or LH given ip to immature intact or hypophysectomized female rats in conjunction with homogenates of rat pituitaries given sc would inhibit the gonadotropic response obtained with the homogenate alone; the most highly purified FSH preparations however, were ineffective in inhibiting the response and increasing purification of LH preparations reduced but did not obliterate their inhibitory power. Pituitary extracts containing high concentrations of other pituitary hormones were ineffective in producing gonadotropic inhibition.

We have shown (7–9) that serum of ewes or mares immunized against HCG may show gonadotropic activity when injected alone into intact immature female rats or hypophysectomized male rats. When given concurrently, the antiserum may also enhance the response of either crude or purified FSH pituitary preparations in intact or hypophysectomized female rats as well as the response of purified LH pituitary preparations in hypophysectomized male rats (the so-called progonadotropic response). The serum of mare 665b immunized against crude FSH showed gonadotropic activity as evidenced by an increase in the weight of the testes and of the ventral prostates in hypophysectomized male rats (8).

The objectives in this study were to determine if the gonadotropic and progonadotropic responses to the HCG or FSH antisera from mares are influenced by either the frequency or route of injection as is true of gonadotropic preparations from the pituitary. Further, we wished to determine if using the antiserum as the vehicle for administration of the pituitary gonadotropin used in the progonadotropic tests would be more effective than giving the antiserum and the gonadotropin at separate sites.

Materials and Methods. The sera of two immunized mares were used. Mare 665a (Forbes) was injected 3 times weekly with 5750–11500 IU of HCG, once in Freund's complete adjuvant and twice in saline during the following periods: 8/28/'60–9/11/'60, 12/14/'60–2/10/'61, 4/10/'61–4/18/'61, 6/28/'61–7/12/'61, 1/3/'62–2/12/'62, 11/28/'62–1/7/'63, 1/6/'64/–1/31/64. Mare 665b (Curley) received 5750 IU or more per in-

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TABLE I. Effect of Frequency and Route of Injection of Ovine LH (NIH-LH-S16) on the Ventral Prostate Response of Hypophysectomized Male SD Rats.

Dose of LH (μg)	Wt of ventral prostate (mg)[a]			
	Subcutaneous		Intraperitoneal	
	1 Injection	3 Injections	1 Injection	3 Injections
None	$7.9 \pm 0.5^{1,a}$	$7.9 \pm 0.5^{1,a}$	$7.9 \pm 0.5^{1,a}$	$7.9 \pm 0.5^{1,a}$
20	$11.5 \pm 1.5^{1,b}$	$15.9 \pm 1.1^{2,b}$	$12.8 \pm 0.8^{1,2,b}$	$20.0 \pm 2.3^{3,b}$
40	$16.4 \pm 2.0^{1,c}$	$22.6 \pm 1.5^{2,c}$	$16.0 \pm 1.7^{1,b}$	$25.5 \pm 1.3^{2,c}$

[a] Values in the horizontal columns with the same superscript numbers do not differ significantly ($p > 0.05$) from each other. Values in the vertical columns bearing the same superscript letters do not differ significantly from each other.

jection of HCG during the first half of the first 2 series and 200 mg of crude FSH during the second half; subsequently only 200 mg of FSH were administered. Three injections were given weekly, once in Freund's complete adjuvant, and twice in saline during the following periods: 7/5/'61–9/29/'61, 1/3/'62–2/26/'62, 11/30/'62–1/9/'63, 1/2/'64–1/31/'64, 6/17/'64–7/27/'64. The FSH preparation was estimated to contain 0.2–0.3 times the amount of FSH contained in NIH-FSH-S1 and about 1/100 the activity of NIH-LH-S1.

In one experiment, the gonadotropic responses obtained by giving a single injection versus 4 or 8 injections sc were compared (see Table II). In others, single versus multiple injections, both sc and ip were compared. In all experiments each value represents the average of 5 or 6 rats.

Hypophysectomized, immature, Sprague–Dawley (SD) or Long–Evans (LE) males were used in the ventral prostate test for LH in the immune sera and in the progonadotropic tests designed to determine the augmentation of the ovine LH response by the immune sera. The SD and LE males were hypophysectomized on the 21st day of age, injections were begun on the 24th day with autopsy on the 28th day.

Intact immature Long–Evans female rats were used in the progonadotropic tests designed to determine the ability of the immune sera to augment the response of crude FSH. The injections of the female rats were begun on the 23rd day of age with autopsy four days later. In most of the progonadotropic tests, the gonadotropin (LH for males and FSH for females) was administered in saline sc in the neck region in 3 daily injections; in others the gonadotropin was suspended in normal or in immune serum. When the antiserum was given sc in conjunction with a gonadotropin, it was injected in the abdominal area and the gonadotropin was injected in the neck region to obviate mixing with the gonadotropin and thus to preclude the possibility of nonspecific augmentation with serum proteins (7).

The significance of the difference between values has been determined by the standard t test.

Results. Effect of frequency or route of injection of gonadotropic antisera on gonadotropic and progonadotropic responses in hypophysectomized male rats. As a basis for comparison with immune serum, the effect of the frequency and route of injection of ovine pituitary LH in hypophysectomized males was studied; 3 injections in each instance produced a significantly greater response than a single injection of the same total dose of hormone (Table I). On the other hand, the route of injection of ovine LH had no influence on the response in the male rat [this merely confirms the finding of Lostroh *et al.* (10)].

Unlike gonadotropic extracts of the pituitary, the frequency of injection of immune sera with LH activity had no significant effect upon the ventral prostate response in hypophysectomized male rats (Table II).

The increased response obtained when the immune serum, as compared to equivalent amounts of control serum, is given in conjunction with pituitary LH is referred

TABLE II. Effect of the Number of sc Injections of Antiserum with LH Activity on the Ventral Prostate Weight Response in Hypophysectomized Male LE Rats.[a]

Date serum drawn	No. of injections compared	Total dosage employed (ml)	Mean ventral prostate weights, respectively (mg)
3-12-65	1 and 8	2	9.3 ± 1.0, 10.9 ± 1.2
		8	18.7 ± 1.9, 15.2 ± 0.7
3-22-65	1 and 4	2	18.3 ± 1.4, 15.8 ± 0.9
		8	17.5 ± 1.5, 19.7 ± 1.7
4-2-65	1 and 8	2	18.3 ± 1.4, 15.8 ± 0.9
		8	17.5 ± 1.5, 19.7 ± 1.7

[a] The sera were obtained from mare 665b in which secretion of LH had been stimulated by injecting FSH as antigen. Multiple injections were given over a 4-day period.

to as the progonadotropic response. Thus, when ovine LH (in 3 daily sc injections) was given with 0.125 ml of immune serum, the responses obtained were significantly greater than when the ovine LH was given with 0.125 ml of control serum (Table III). Increased responses were likewise obtained in 3 of 4 instances with 0.0234 ml of the immune serum as compared to the same dose of control serum. This immune serum in the dosage employed had no effect when given alone to hypophysectomized male rats. When the ovine LH and immune serum were given separately, the route or frequency of injection had no consistent effect upon the ventral prostate responses. The same lack of effect was obtained by modifying the frequency or route of injection when the ovine LH was suspended in the immune serum when 2.0 ml was administered; why multiple injections were more effective when a total dose of 0.5 ml was injected is not known.

Effect of frequency and route of injection of HCG antisera on the progonadotropic response in intact immature female rats. Unlike pituitary gonadotropic extracts there is no indication that either the frequency or route of injection of an antiserum against HCG given concurrently with ovine pituitary FSH (injected in saline in 3 sc injections) influenced the ovarian response (Table IV). The antiserum used in this study was by itself ineffective at the dosages employed; however, 5 ml when injected alone did significantly increase ovarian and uterine weights.

Discussion. Earlier studies showed that the injection of HCG as an antigen may result in either increased gonadotropic or progonadotropic activities or of both activities of the sera of treated animals (7, 8). Evidence has also been obtained which was interpreted as indicating that HCG antiserum may contain 2 types of antibodies, (1) neutralizing antibodies and, (2) non-neutralizing antibodies which form a soluble complex with heterologous gonadotropins (9). These latter antibodies may complex with the endogenous gonadotropin(s) in the antiserum or with the endogenous gonadotropins secreted by intact test rats, increase their half-lives and thus account for the gonadotropic activity of the serum. When the antiserum is injected in conjunction with pituitary LH or FSH preparations the formation of a soluble complex between the non-neutralizing HCG antibodies and the exogenous gonadotropin increases its half-life and augments the response to the gonadotropin—the so called progonadotropic response.

The present study provides further circumstantial evidence of non-neutralizing complexing; that is, the fact that a single injection of antiserum by itself will give as good a ventral prostate response in hypophysectomized male rats as 4 or 8 injections of the same total amount of antiserum suggests that the half-life of LH in the antiserum is greatly extended over that of the pituitary LH. In like manner, it is suggested that a single injection of HCG antiserum given in conjunction with pituitary FSH into intact immature females or in conjunction with LH into hypo-

TABLE III. Effect of Route and Frequency of Injection of Control and of Anti-HCG Serum (Forbes, Bled 3-11-64) on the Progonadotropic Response when Given in Conjunction with Ovine LH (NIH-LH-S15).

LH injected (μg)	Serum injected		Wt of ventral prostate (mg)[a]			
	Control (ml)	Immune (ml)	Intraperitoneal		Subcutaneous	
			1 injection	3 injections	1 injection	3 injections
None	None	None	$10.1 \pm 0.9^{1,e}$	$10.1 \pm 0.9^{1,e}$	$10.1 \pm 0.9^{1,f}$	$10.1 \pm 0.9^{1,e}$
120[b]	0.0234	—	$23.0 \pm 1.5^{2,d}$	$19.6 \pm 1.1^{1,2,d}$	$18.5 \pm 1.2^{1,d,e}$	$20.5 \pm 1.5^{1,2,d}$
120[b]	0.125	—	$23.0 \pm 2.5^{1,d,f}$	$20.8 \pm 2.0^{1,c,d}$	$21.2 \pm 0.9^{1,c,d}$	$19.9 \pm 1.8^{1,d}$
120[c]	0.5	—	$15.0 \pm 2.8^{1,e,f}$	$20.2 \pm 2.2^{1,2,c,d}$	$16.2 \pm 1.8^{1,e}$	$27.4 \pm 3.2^{2,b,c,d}$
120[b]	—	0.0234	$25.0 \pm 1.2^{1,d}$	$25.6 \pm 1.6^{1,c}$	$24.7 \pm 1.5^{1,c}$	$26.2 \pm 1.6^{1,c}$
120[b]	—	0.125	$38.4 \pm 4.2^{1,2,a,b}$	$37.1 \pm 3.2^{1,a,b}$	$35.9 \pm 2.4^{1,b}$	$28.4 \pm 2.1^{2,b,c}$
120[b]	—	0.5	$37.1 \pm 3.0^{1,b,c}$	$34.7 \pm 1.8^{1,b}$	$35.4 \pm 4.3^{1,b}$	$30.5 \pm 1.0^{1,b}$
120[c]	—	0.5	$35.9 \pm 3.8^{1,2,b,c}$	$41.7 \pm 1.9^{2,a}$	$32.3 \pm 2.6^{1,b}$	$42.5 \pm 3.2^{2,a}$
120[c]	—	2.0	$48.4 \pm 3.9^{1,a}$	$50.6 \pm 5.3^{1,a}$	$49.4 \pm 4.4^{1,a}$	$41.7 \pm 2.4^{1,a}$

[a] Values in the horizontal columns bearing different superscript numbers differ significantly from each other ($p < 0.05$). Values in the vertical columns bearing different superscript letters differ significantly from each other.

[b] LH given in saline in 3 daily sc injections.

[c] LH suspended in control or immune serum for injection.

physectomized males results in the formation of soluble complexes with these exogenous gonadotropins with consequent extension of their half-lives and an enhancement of their biological responses.

Unlike crude pituitary LH (6), the ip injection of HCG antiserum with LH activity concurrently with FSH given sc does not inhibit the ovarian response to the FSH; rather one obtains an augmented response to FSH by either sc or ip injection of the antiserum. This finding is in accord with the conclusion of Woods and Simpson (6) that the "antagonist" effect of pituitary preparations given ip is probably not due to pituitary LH.

In 4 comparisons of progonadotropic tests, the gonadotropin was either suspended in the HCG antiserum or injected at a separate site. In both ip and sc injections of 0.5 ml of the antiserum, the greater response was obtained by suspending the gonadotropin in the antiserum when multiple injections were made but not when a single injection of antiserum was given. On the other hand no differences

TABLE IV. Effect of Route and Frequency of Injection of Equine Anti-HCG serum (Forbes Mare, Bled 3-4-'64) on the Progonadotropic Response when Given in Conjunction with 3 Daily SC Injections of Ovine FSH to Intact Immature Female Rats.[a]

Dose of FSH (mg)	Dose of control serum (ml)	Dose of anti-HCG serum (ml)	Wt of ovaries (mg)			
			Subcutaneous		Intraperitoneal	
			1 injection	3 injections	1 injection	3 injections
None	None	None	$20.9 \pm 1.9^{1,a}$	$20.9 \pm 1.9^{1,a}$	$20.9 \pm 1.9^{1,a}$	$20.9 \pm 1.9^{1,a}$
	—	0.05	$17.5 \pm 0.7^{1,a}$	$19.6 \pm 1.2^{1,a}$	$18.4 \pm 1.6^{1,a}$	$19.9 \pm 1.6^{1,a}$
	—	0.2	$18.9 \pm 1.3^{1,a}$	$19.5 \pm 2.0^{1,a}$	$16.7 \pm 0.7^{1,a}$	$16.9 \pm 0.8^{1,a}$
9	0.2	—	$42.1 \pm 1.3^{1,b}$	$49.4 \pm 5.6^{1,b}$	$41.1 \pm 2.8^{1,b}$	$49.8 \pm 4.5^{1,b}$
	—	0.05	$74.2 \pm 9.5^{1,2,c}$	$59.0 \pm 5.9^{1,b,c}$	$77.8 \pm 4.2^{2,c}$	$69.6 \pm 6.8^{1,2,c}$
	—	0.2	$97.5 \pm 5.0^{1,c}$	$78.4 \pm 7.5^{1,c}$	$94.2 \pm 9.0^{1,c}$	$94.4 \pm 4.6^{1,d}$

[a] Values in horizontal columns with different superscript numbers differ significantly from each other ($p < 0.05$). Values in vertical columns with different superscript letters differ significantly from each other ($p < 0.05$).

depending upon frequency of injection of antiserum were observed with a 2-ml dose of antiserum. Presumably, suspending the gonadotropin in the smaller amount of antiserum for 48 hr after the initial injection allowed for more complexing to occur. On the other hand when a large amount of antiserum was used, sufficient complexing occurred rapidly enough to obliterate the difference between single and multiple injections.

Summary. In 6 comparisons, a single injection of a gonadotropic antiserum alone was as effective in increasing the ventral prostate weights in hypophysectomized males as were 4 or 8 injections. In 13 of 14 comparisons, a single injection of antiserum in conjunction with either FSH in intact immature females or with LH in hypophysectomized males, the progonadotropic response was as great as that obtained with 3 injections of the same total dose of the antiserum; also no significant differences were found between sc and ip injections of the antisera. It is suggested that these data provide further evidence of soluble complexing of non-neutralizing gonadotropin antibodies with the heterologous gonadotropin to prolong the half-life of the latter.

Suspending of the gonadotropin in immune serum gave a greater response than was obtained by injecting the gonadotropin separately when 3 injections were given by certain dosages of the hormones, but not by others.

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