FIG. 1. Electrophoretic patterns are situated on the left and the corresponding scan tracings on the right. Arrow on tracings indicates starting-point of the run. Uppermost separation obtained with a standard mixture (see text). Below, serum patterns 1-3 in descending order.

and in some instances partly overlapping the slowest alcian blue staining component.

Evidently, the exact chemical nature of the demonstrated alcian blue staining components cannot be determined solely on the basis of electrophoretic mobility. This study shows, however, that the AMPS pattern of normal human serum is more complex than previous studies indicate, since components are demonstrated in addition to those described by other workers. Work is in progress for the closer characterization of the serum AMPS components observed.

As the AMPS fraction electrophoresed in this study is derived from approximately 3-4 ml of serum it is obvious that the variability of the individual serum AMPS pattern in health and disease is open for detailed investigation.

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Comparison of Antisera to Various Gonadotropins as They Effect The Mouse Vaginal Cycle.* (31202)

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Inhibition of endogenous pituitary gonadotropins by antiserum to heterologous gonadotropins has been reported since the earliest studies of antihormones began after 1930(1). Particularly convincing were studies by Meyer and his associates(2,3,4,5). However, since these studies involved the use of crude glandular extracts, they were open to the obvious criticism that antigonadotropic effects were due to non-specific factors or to antibodies to pituitary hormones other than those with gonadotropic activity. The fact that many workers failed to demonstrate cross-reactions between pituitary extracts from various animal sources, or to relate antihormone activity to precipitins *in vitro*, or to find evidence of endogenous hormone inactivity led to confusion, and there was some reluctance to accept the possibility that endogenous pituitary hormones of laboratory animals could be inhibited by antisera to pituitary hormones from other species. It was postulated that the use of more highly purified preparations would do away with the refractory state(6). Such doubts no longer appear to be justified.

With the exception of reports by Henry and van Dyke(7) and Gold *et al*(8), it has been demonstrated that antiserum to purified sheep luteinizing hormone cross-reacts with endogenous pituitary hormones of mice (9), rats(10-14) and rabbits(15), and antiserum to bovine thyrotropin was shown to inhibit the corresponding endogenous hormone in mice, rats, and guinea pigs(16). Conversely, antiserum to mouse thyrotropic hormone cross-reacts, though weakly, with beef thyrotropin(17). Among these studies reference to cross-reaction between endogenous gonadotropins and antiserum to follicle-stimulating hormone is missing. The present

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study is a comparison of the effect of antiserum to sheep follicle-stimulating hormone and sheep luteinizing hormone on the mouse vaginal cycle.

Materials and methods. Antiserum to luteinizing hormone (NIH-LH-S2) and follicle-stimulating hormone (NIH-FSH-S1) was developed in young adult male rabbits. Details of immunization procedures have been given elsewhere(9). In general, 1-10 mg of hormone were injected subcutaneously in Freund's complete adjuvant once or twice a week for a preliminary period of 4 to 6 weeks. After this, hormone injections were made at one- to two-week intervals with bleeding in alternate weeks. In addition, antiserum to crude sheep pituitary extract, sheep prolactin (NIH-LTH), human chorionic gonadotropin (HCG), pregnant mare's serum (PMS), and Freund's complete adjuvant were prepared for use in treatment of controls. Immunization procedures were essentially similar to those used for antiserum to LH and FSH with variations in the intervals between injections and in the relationship of the bleeding time to the last injection. The range of these variations in timing of immunization seldom exceeded one or two weeks. The hormone dose for a single injection of crude sheep pituitary extract was 5-10 mg; for LTH 5-10 mg; for HCG approximately 1500 IU; for PMS approximately 50 IU, and for Freund's complete adjuvant 0.5 ml.

Samples of 50 ml of blood were collected from the central artery of the ear after the initial injection period of 6 to 8 weeks and the serum stored in the frozen state in small lots until needed. Before experimental use the antisera were tested for antihormone activity by bioassay. Briefly, the antiserum effect is demonstrated when the hormone (antigen) and antigonadotropic serum (antibody) are injected simultaneously, but separately, into immature rats. A modification of the ventral prostate method of Greep (18) was used for assay of antiserum to LH and HCG, and a modification of the Steelman-Pohley method(19) for the assay of antiserum to FSH. Details of these two methods have been given elsewhere(9). The antihormone activity of antiserum to PMS and crude sheep pituitary extract was tested by a routine method for assaying antihormone activity described elsewhere(20). An amount of hormone was used sufficient to increase the immature ovary 200-600% in weight and an amount of antiserum necessary to inhibit this effect, usually 0.3-0.6 ml, was given simultaneously but separately. In any case only antisera showing strong inhibitory activity by these methods were used (Table I).

Vaginal smears were taken by lavage from young adult CF1 mice daily, 7 days a week. Animals were selected which showed 2 consecutive regular cycles of normal length previous to treatment, and subcutaneous injection of antiserum was begun at the onset of an estrous period. Daily injections were given continuously for 28-35 days. The animals were kept in groups of 4 or 5 in stainless steel cages measuring 5 × 6 × 12 inches and regularly supplied with water and a mixture of Rockland Mouse Diet and Purina Laboratory Chow pellets. Twelve separate experiments were carried out over a 3-year period in 10 different months. At the end of the injection period autopsies were performed. Ovarian, adrenal, uterine, thymic, and body weights were recorded. Tissues of interest were weighed and fixed in Bouin's fixative, embedded in paraffin, and subsequently stained with hematoxylin and eosin and by the periodic acid-Schiff technique with hematoxylin.

Results. Results of assays demonstrating antigonadotropic activity of antiserum to various gonadotropic hormones are given in Table I; results of the application of these antisera to young adult female mice are given in Table II.

Pretreatment controls initiated 31 cycles over a period of time corresponding to the length of the injection period of experimental animals when vaginal smears were taken. Vaginal cycling was virtually halted by anti-LH serum at dose levels of antiserum above 0.005 ml with less than one per cent of the expected cycles occurring. Neither antiserum to FSH nor antiserum to 4 other gonadotropic preparations had this effect at this dose level, but the number of cycles was reduced 43-71% below expectancy.

Absorption of anti-LH serum with sheep serum before injection did not change the

TABLE I. Demonstration of Antihormone Activity in Antisera to Gonadotropins.*

Treatment				Response		
Hormone	Dose	Anti-serum	Dose (ml)	Testis or ovary (mg)	Prostate or uterus (mg)	No. animals
A. Assay animal, normal immature female						
—	—	—	—	14.2	37.0	17
—	—	AFSH	1.2	12.3	38.6	3
FSH	250 μg	—	—	18.5	36.9	3
HCG	20 IU	—	—	37.3	102.0	12
FSH + HCG	250 μg + 20 IU	—	—	88.3	101.6	9
FSH + HCG	250 μg + 20 IU	AFSH‡	.3	35.5	97.3	6
HCG	20 IU	APMS	1.2	58.9	88.8	8
CSPE†	10 mg	—	—	55.3	78.8	15
CSPE	10 "	ACSPE	.3	28.0	57.8	11
FSH	1 "	—	—	52.6	102.7	3
FSH	1 "	AHCG	.6	39.7	140.9	3
PMS	50 IU	—	—	120.6	127.6	3
PMS	50 "	APMS	.3	12.7	26.2	9
B. Assay animal, hypophysectomized immature male						
—	—	—	—	116.2	6.5	12
—	—	AFSH	1.2	125.8	6.5	3
LH	45–54 μg	—	—	162.3	11.4	29
LH	*idem*	ALH§	.3	111.4	6.4**	8
LH	"	ALH‖	.3	158.9¶	6.8**	6
LH	"	AHCG	1.2	190.5	16.9	5
LH	"	APMS	.3	160.8	11.7	4
LH	"	APMS	1.2	186.0	10.4	2
C. Assay animal, normal immature male						
HCG	10 IU	—	—	448.5	64.2	3
HCG	10 "	AHCG	.3	396.9	31.1	12
—	—	—	—	371.8	33.3	6

* Portions of this table have been published elsewhere.
† CSPE = crude sheep pituitary extract, an aqueous extract of acetone dried pituitary powder.
‡ Hemagglutination titer = 1:100,000.
§ Hemagglutination titer = 1:20,000.
‖ Anti-LH serum absorbed with 2 mg/ml of FSH.
¶ Excess FSH probably responsible for higher testis weights.
** P value = <.001 when compared with hormone injected controls.

inhibitory activity of the anti-LH serum, but reduction of the dose to 0.005 ml greatly reduced it. A high dose (0.6 ml) of antiserum to either gonadotropic hormones or to control materials markedly decreased the frequency of vaginal cycling.

Ovarian and uterine weights were lowest after doses of 0.05-0.1 ml of anti-LH serum and high doses (0.6 ml) of antiserum to FSH and tended to be low after 0.6 ml of antiserum to crude sheep pituitary extract. Ovarian interstitial tissue in these groups showed a more intense development of the recognized signs of hormone deficiency. Areas where "wheel" cells were to be seen were more extensive and more nuclei showed the intensely stained chromatin characteristic of the ovary of the hypophysectomized animal.

Discussion. The finding that anti-LH serum brought a halt to vaginal cycling is most logically explained by assuming that there was a cross-reaction between the sheep antiserum and endogenous LH, and this finding is in agreement with the conclusion of others cited above. Conversely, the failure of antiserum to sheep FSH to influence vaginal cycling to any degree beyond that demonstrated in controls leads to the conclusion that there was not a cross-reaction between endogenous FSH and the demonstrably potent antiserum to sheep FSH.

There are few studies dealing with *in vivo* interaction of mouse and sheep FSH, except our brief notes(9) and that of Hayashida (21). Both studies showed inhibition of mouse pituitary homogenate activity by anti-

TABLE II. Comparison of the Effect of Antiserum to FSH and LH on the Mouse Vaginal Cycle.*

Treatment	BW (g)	Ovary (mg)	Uterus (mg)	Thymus (mg)	Cycles observed/expected (%)§	No. animals
			Controls			
Pretreatment	—	—	—	—	100	13
Saline	25.3	11.1	84.7	48.5	61	18
			Antiserum dose (0.005 ml)			
Anti-LH	29.1	15.4	112.4	62.9	38	6
			Antiserum dose (0.05-0.3 ml)			
Anti-LH	29.6	9.6	37.5	58.4	1	21
Anti-LH abs†	30.8	10.7	30.6	76.1	0	6
Anti-FSH	28.1	12.5	93.2	48.6	44	22
Anti-CSPE	31.5	12.9	82.7	53.2	43	4
Anti-LTH‡	27.1	12.7	92.4	48.3	71	4
Anti-HCG	27.3	14.9	115.9	55.5	68	9
Anti-PMS	27.4	10.4	73.1	49.8	52	4
Anti-ShS	27.3	17.7	89.2	54.9	113	3
Anti-BA + Adj	29.8	11.1	43.1	48.8	55	2
Anti-Sal + Adj	25.2	10.3	75.6	37.0	62	4
			Antiserum dose (0.6 ml)			
Anti-FSH	26.6	6.7	37.5	29.4	0	4
Anti-CSPE	31.9	10.6	46.5	70.0	9	4
Anti-HCG	29.7	15.7	66.8	47.1	21	15
Anti-PMS	30.6	14.2	83.8	49.5	30	15
Anti-ShS	29.5	13.8	85.6	37.4	21	11
Anti-Sal + Adj	28.6	12.8	58.7	49.5	33	5
NRS	29.3	12.7	69.2	41.2	20	14

* Abbreviations: BA + Adj = beef albumin in adjuvant; CSPE = crude sheep pituitary extract; NRS = normal rabbit serum; Sal + Adj = adjuvant in saline; ShS = sheep serum.

† Absorbed with sheep serum.

‡ Antiserum to LTH showed a single strong precipitin band by immunoelectrophoretic analysis and did not show cross-reaction with other hormones used in this study. Bioassay was not performed.

§ % of expected No. of cycles occurring.

FSH serum, but in neither case was the anti-FSH absorbed free of possible anti-LH activity which probably was responsible for the demonstrated inhibition.

The complete effectiveness of high doses of anti-FSH and the nearly complete effectiveness of antiserum to crude sheep pituitary extract in inhibiting vaginal cycling is best explained by assigning the responsibility to contaminating anti-LH. It was demonstrated that antiserum to NIH-FSH did have anti-LH activity, but it was considerably weaker than the activity in antisera to LH(9), and the fact that the anti-LH activity is not likely due to the presence of anti-FSH in anti-LH serum is demonstrated in Table I where anti-LH serum was absorbed with FSH without impairment of its inhibitory activity.

The observation that antisera to HCG and PMS were not substantially different from controls in their effect on vaginal cycling suggests that there was no cross-reaction with mouse endogenous pituitary hormones. Data given in Table I showing that antisera to these 2 heterologous hormones fail to inhibit sheep gonadotropins anticipate such a finding and are in agreement with earlier observations(1) and more recent studies(22,23,24) made with similar preparations when tested against gonadotropins of pituitary origin.

The prospect that the apparent results of a cross-reaction between antiserum to LH and endogenous mouse pituitary hormones are due to antibodies raised against pituitary protein hormones contaminating NIH-LH has been fully discussed(9). In brief, good evidence exists that antiserum to heterologous TSH cross-reacts with mouse pituitary gland extracts(9,10), and the antiserum to LH and FSH used in this study has been shown to have antibodies to TSH(3). But it should

be noted that no histological indication of impaired thyroid function was found after use of this same antiserum in another study (9), and it would seem likely that, if inhibition of vaginal cycling had been due to anti-TSH activity, anti-FSH serum would also have been inhibitory. Evidence of cross-reaction of endogenous hormones with growth hormone(25) and adrenocorticotropin(26) is not yet entirely convincing and the degree of contamination of NIH gonadotropins with these two hormones is known to be slight. Thus, at this time, evidence for the intervention of other pituitary hormones is not impressive.

Control data clearly indicate that, if sufficiently large amounts of any antiserum or normal serum are given, vaginal cycling can be impaired. Among controls 0.6 ml of rabbit antiserum to sheep serum, Freund's adjuvant, and normal rabbit serum itself considerably reduced the number of vaginal cycles. It seems likely that the effect of various antisera, including those to PMS and HCG, at this dose level was due to non-specific factors. But neither thymus weights nor body weights were markedly different in the various groups. At most, these findings point to the importance of the use of serum-injected controls when antigonadotropic effects are being sought.

Summary. Antisera to 6 different gonadotropins were given to young adult female mice and daily vaginal changes followed. Antisera to sheep luteinizing hormone virtually halted vaginal cycling. At comparable dose levels antisera to sheep FSH, HCG, and crude sheep pituitary extract failed to have this effect. It was concluded that there had been a cross-reaction between antibodies to the sheep luteinizing hormone and endogenous mouse pituitary hormones.

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