11. Hartman, C. G., Straus, W. L., Jr., eds., Anatomy of the Rhesus Monkey (*Macaca mulatta*), Williams & Wilkins Co., Baltimore, 1933, p244.

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Inhibition of Spermatogenesis and Ovulation in Rabbits with Antiovine LH Rabbit Serum.* (31610)

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Antiovine LH rabbit serum has been reported to neutralize endogenous gonadotropins in immature and mature rats of both sexes(1,2,3,4). Inhibition of the stimulatory effect of exogenous rat, pig and whale pituitary extracts on the ventral prostrate of immature hypophysectomized rats by concurrent administration of antiovine LH sera has likewise been observed(5). Ovulation has been blocked in rats by injecting antiserum between 1:00 and 4:00 PM on the day of proestrus(6). Although the rabbit was utilized for antibody production against tropic hormones, inhibition of endogenous and exogenous gonadotropins in rabbits following active and passive immunization has not been disclosed.

This study examined (1) the effect of active immunization of adult male rabbits to NIH-LH-S_8 and S_{10} on libido and testes function, (2) neutralization of endogenous LH and exogenous ovine LH in female rabbits given antiovine LH rabbit serum and (3) the duration of antibody persistence in female rabbits following passive immunization.

Materials and methods. Experiment I: Eight 3.5-4.4 kg New Zealand White male rabbits were randomly divided into 4 groups of 2 animals each. Each male was individually caged and trained with the aid of a dummy rabbit to serve an artificial vagina. Eight semen collections from each male at 4-day intervals provided estimates of variability in semen characteristics within and between individuals prior to experimental treatments.

Controls: Animals in the control group received no additional treatment. Semen collections were continued at 4-day intervals throughout the experimental period.

LH + adjuvant: Each animal received subcutaneously 1.5 mg NIH-LH-S_8 or S_{10} dissolved in 1.5 ml saline mixed with an equal volume of Freund's complete adjuvant (Difco, Detroit, Mich.) weekly for 4 weeks. Two 2.0 mg booster injections were given 2 weeks apart after the fourth 1.5 mg injection. After an additional lapse of 12 weeks, two additional 2.0 mg booster injections of LH at 2-week intervals were given.

Adjuvant: Animals in the adjuvant control group received injections of Freund's complete adjuvant only as described for the LH + adjuvant group.

LH + adjuvant + testosterone: Each animal in this group was injected with LH + adjuvant as described above. In addition beginning the sixth week after the initial LH + adjuvant injection, 1.0 mg testosterone propionate dissolved in 0.2 cc sesame oil was injected subcutaneously on alternate days for the remainder of the experimental period.

All animals were sacrificed 28 weeks from the beginning of immunization. The testes, epididymides, seminal vesicles, prostate, penis and pituitary glands were weighed and immediately frozen in liquid nitrogen or preserved in 10% formalin for histological evaluation. Samples of each tissue were sectioned at 10 mμ and stained with haematoxylin-acid fuchsin or haematoxylin-Mallory's triple stain. Experiment II. Blood from the LH + adjuvant treated males in Exp. I was collected at 3- to 5-day intervals following the 2 booster injections of 2.0 mg LH at 2-week intervals

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20 weeks following initial LH injection. Antisera, thus obtained, were concentrated to one-third saturation by repeated ammonium sulfate precipitation(7) and pooled.

Control blood serum was obtained from 2 untreated male rabbits and concentrated as described above.

Adult female rabbits were individually caged a minimum of 18 days prior to use. A single intravenous, subcutaneous or intraperitoneal injection of 3.0 cc to .005 cc antiserum was given to 21 females immediately prior to mating with a vasectomized male. Two rabbits received 3.0 cc each of control serum intravenously preceding mating. Each female was laparotomized 24 hours following mating, and the ovaries examined under a dissecting microscope for ovulation papilla or follicular luteinization. Anti-LH serum treated females that failed to ovulate on mating were placed with the male 10 minutes each day. Mating behavior and repeated subsequent ovarian examinations were observed to evaluate luteinization, possible progestin secretion and length of antibody persistence post injection. Also, to determine if the antibodies rendered the follicles nonovulatory and to estimate the level of exogenous ovine LH needed to produce ovulation in antiserum treated animals, nine anti-LH treated females were injected intravenously with varying levels from 15 to 200 μg of NIH-LH-S_{10}, sacrificed 24 hours later and their ovaries examined for ovulation papilla. Four animals also received control serum plus 15 or 25 μg of LH to establish that serum *per se* did not influence ovulation.

Results. Experiment I. The males in the control and adjuvant treated groups continued to serve the artificial vagina, and semen samples near termination of the 28-week experimental period successfully impregnated females. Semen volume and spermatozoa per mm^3 increased during the experimental period in both control and adjuvant treated groups, on the basis of pretreatment evaluation and training period data (Table I). In sharp contrast, the LH + adjuvant groups lacked libido, even when placed with estrous females, after the second week of immunization. Testosterone restored libido when injected from the sixth week following initial LH treatment, but the semen samples were void of spermatozoa, colorless and minute in volume compared with pretreatment semen samples from the same males (Table I).

Average testes weights, combined accessory organ weights and pituitary weights for the 4 groups at the end of the experimental period are shown in Table I.

Histological evaluation of the testes showed degeneration of spermatocytes and absence

TABLE I. Experiment I. Average Semen Volume, Spermatozoa Density and Weights of Testes, Accessory Glands and Pituitaries.

Treatment	Body wt (kg)	Semen vol (cc)		Spermatozoa No. (1000's/mm^3)		Avg weights		
		Prior treatment	Post treatment	Prior treatment	Post treatment	Testes (g)	Acc. glands (g)	Pituitary (mg)
Control	4.1 ± .1*	1.6 ± .7	2.1 ± .3	435 ± 90	490 ± 142	7.58 ±1.4	1.56 ± .2	31.0 ± 2.8
Adjuvant	3.5 ± .3	.8 ± .4	1.4 ± .4	361 ± 59	390 ± 180	5.61 ± .8	1.27 ± .4	33.5 ± 3.6
LH + adjuvant	4.4 ± .3	1.2 ± .6	0†	422 ± 95	0†	1.18 ± .2	.30 ± .2	41.9 ± 1.1
Idem + testosterone	3.7 ± .3	1.3 ± .2	0‡	331 ± 58	0‡	.85 ± .4	.24 ± .2	41.6 ± 1.1

* Numbers following ± indicate standard deviations.
† Libido lost the second week of injections.
‡ Libido lost after second injection of LH + adjuvant, but returned following testosterone treatment. Ejaculate volumes small, void of sperm, clear and watery.

of both spermatids and mature spermatozoa in the LH + adjuvant (Fig. 2) and LH + adjuvant + testosterone groups. Spermatocytes and spermatids were more systematically

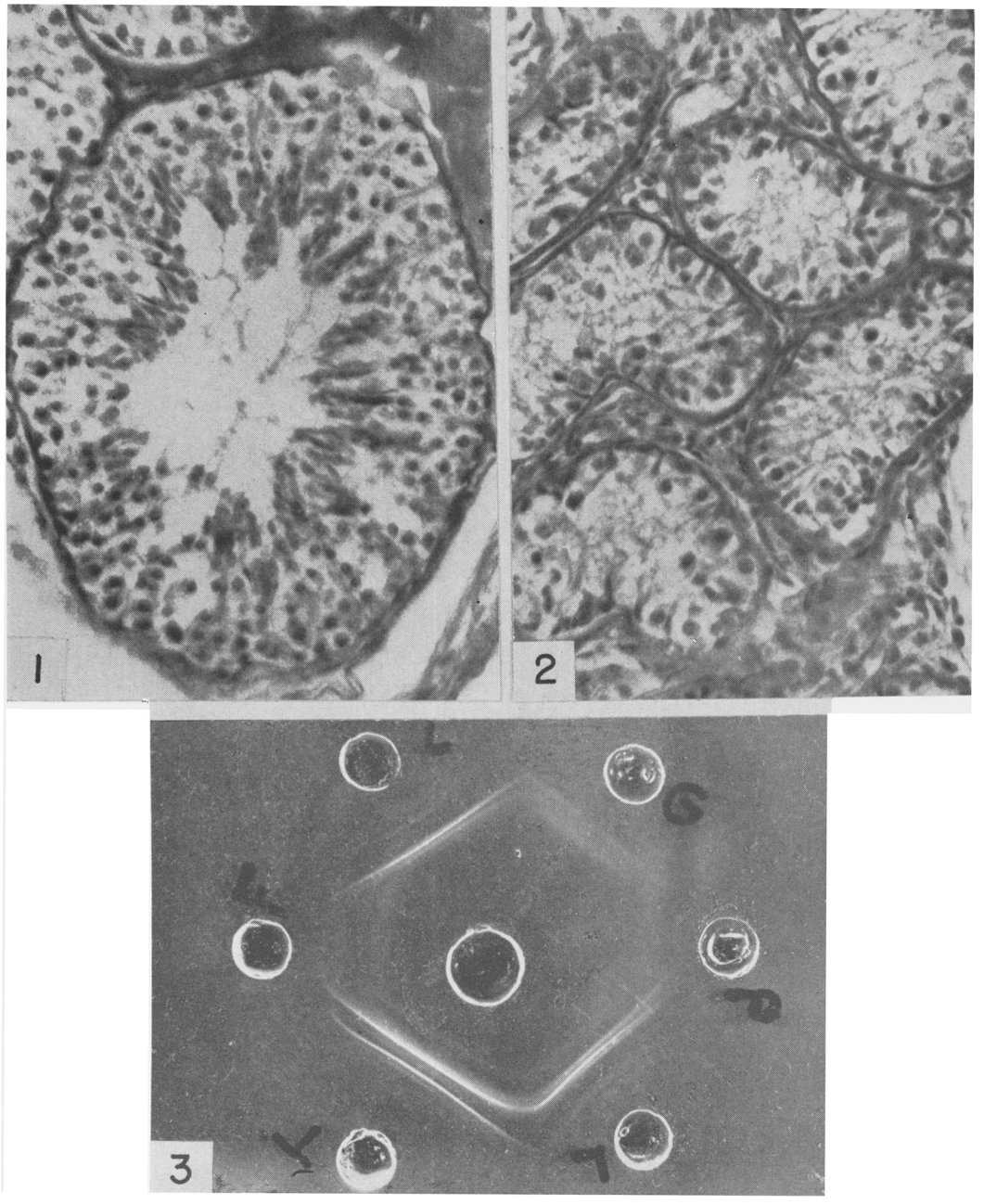

PLATE 1

FIG. 1. Control testes. Tubules show systemic arrangement of various cell types. 300 ×.

FIG. 2. LH + adjuvant testes. Tubules are degenerate and devoid of spermatozoa, spermatids and greatly reduced in spermatocytes. 300 ×.

FIG. 3. Ouchterlony plate with LH + adjuvant serum in the center well and NIH-FSH-S_1 (F), R-TSH-03 (T), NIH-LH-S_{10} (L), ovine STH (G) and ovine prolactin (P) in the outside wells. Precipitin bands suggested LH, TSH, FSH, and STH antibodies.

TABLE II. Ovulation Inhibition by Antiovine LH Rabbit Serum.

No. of rabbits	Treatment			Results	
	Dosage (cc)	Material*	Route	Avg No. ovulation papilla	Follicular luteinization
2	3.0	C.S.	I.V.	9.0	—
3	2.0 or 3.0	A.S.	I.V.	none	none
2	1.0 or 2.0	A.S.	I.P.	"	moderate
1	.25	A.S.	S.C.	"	slight
2	.12 or .25	A.S.	I.V.	"	"
5	.075	A.S.	I.V.	"	"
5	.06	A.S.	I.V.	7.3 in 3, none in 2	—
3	.005, .03, or .05	A.S.	I.V.	9.3	—

* C.S. = control serum; A.S. = antiserum.

arranged in testes of the control (Fig. 1) and adjuvant groups with many spermatozoa in the lumen of the tubules. Seminal vesicles and prostate glandular areas were regressed and devoid of epithelia in the LH + adjuvant group. Some glandular epithelia were in prostate tissues of the LH + adjuvant + testosterone group, but glands appeared greatly atrophied contrasted to similar histological samples of prostate from control and adjuvant treated males.

Antibody titer of antiserum from the LH + adjuvant group was 1:32,000. One cc of antiserum was estimated to neutralize 1 mg of NIH-LH-S_{10} *in vitro*(8). A gel diffusion test(9) of the antiserum revealed precipitin bands with ovine LH, FSH, TSH and possibly STH but not prolactin (Fig. 3). Antiserum from the LH + adjuvant + testosterone males formed a line of identity with the LH + adjuvant antiserum; control and adjuvant serum failed to react.

Experiment II. Ovulation was successfully suppressed by 3.0 to .075 cc of antiserum (Table II). Dosage levels of antiserum below .05 cc were ineffective (Table II). Of 5 animals that received .06 cc of antiserum, 3 ovulated; all 5 animals receiving .075 cc failed to ovulate. Control serum of 3.0 cc was ineffective in blocking ovulation.

Three animals injected with dosages of antiserum of 1 cc, 2 cc, or 3 cc failed to ovulate by day 7 post-treatment when mated daily to a vasectomized male; however, 2 females, repeatedly mated, ovulated on the sixth day following administration of .075 cc antiserum, the dosage that blocked initial ovulation in all animals tested.

An intravenous dosage of .075 cc of antiserum effectively neutralized the effect of a simultaneous intravenous injection of 25 μg of LH, while the same dosage of control serum did not neutralize 15 or 25 μg of LH and allowed ovulation to occur (Table III). Higher levels of antiserum effectively blocked ovulation following 25 to 75 μg of exogenous LH, but did not neutralize 200 μg of LH (Table III).

Discussion. The data suggest that antiovine LH rabbit serum neutralized endogenous gonadotropins and exogenous ovine LH in rabbits. Testes from immunized males showed marked degeneration of spermatocytes, presumed to be influenced by FSH(10,11), in addition to interstitial cell damage and absence of spermiogenesis as contrasted to adjuvant treated and control groups. Thus, FSH in addition to LH antibodies may have influenced results. Antibodies to TSH and STH, indicated by the gel diffusion test, probably did not play a major role. Neutralization of endogenous gonadotropins, pre-

TABLE III. Effect of Exogenous NIH-LH-S_{10} Following or Simultaneous with an Antiserum Injection on Ovulation.

No. of rabbits	Amt injected (i.v.)			Day LH injected following antiserum	Avg No. ovulation papilla
	cc	Serum*	μg LH		
2	1.0	A.S.	25 or 75	7	none
1	2.0	A.S.	15	4	"
1	3.0	A.S.	200	4	1.0
1	.075	A.S.	25	4	4.0
4	.075	A.S.	25	0	none
2	.075	C.S.	15	0	6.5
2	.075	C.S.	25	0	12.0

* A.S. = antiserum; C.S. = control serum.

sumably LH, in the LH + adjuvant group resulted in suppressing testosterone production followed by loss of libido and atrophy of testes and accessory glands. Administration of exogenous testosterone to LH + adjuvant + testosterone males restored libido, but failed to restore normal testes or accessory gland function possibly due to pronounced atrophy of tissues prior to testosterone injections. Others have reported that LH or testosterone alone failed to restore weights of testes or accessory organs following pronounced atrophy after hypophysectomy(12, 13,14). Additional evidence of neutralization of endogenous gonadotropins by antiserum was the increased pituitary weights and extensive vacuolation of cells resembling those of castrated animals.

Further evidence of neutralization of endogenous LH was suppression of ovulation in mated and exogenous LH treated females. The minimum dose of antiserum required to inhibit ovulation if administered immediately before mating by intravenous route was .075 cc. The same dosage of antiserum blocked ovulation when administered simultaneously with 25 μg LH while control serum plus 15 or 25 μg LH allowed ovulation. This antiserum appears to be more potent than antisera preparations tested in rats(2,4). Higher doses of antisera administered intraperitoneally or subcutaneously blocked ovulation, but greater preovulatory luteinization occurred compared with the same dosage levels of antisera given by an intravenous route at mating, possibly due to rate of absorption in relation to level of circulating LH(6).

A single injection of antiserum at various dosage levels did not permanently alter ovarian response as successful ovulations at different post injection periods were followed by normal pregnancies. A dose of .075 cc antiserum was unable to block ovulation on the fourth day after administration, but one and two cc antiserum permitted persistence of antibodies in effective amounts to inhibit ovulation on the seventh day following either mating or 25 or 75 μg of exogenous LH.

Summary. Active immunization of New Zealand White male rabbits with NIH-LH-S_8 and S_{10} resulted in loss of libido and suppression of semen production. Testes and accessory gland weights were reduced by 80% and pituitary weights were elevated 35%. Histological examination of the testes revealed absence of spermatozoa and spermatids and degeneration of spermatocytes. One mg testosterone on alternate days restored libido but semen production remained suppressed. No significant repair of atrophied testes or accessory organs occurred. The antiserum, concentrated by repeated precipitation with ammonium sulfate, gave an antibody titer of 1:32,000 and reacted with ovine LH, FSH, TSH and possibly STH in the Ouchterlony gel diffusion test. The antiserum used at dosage levels of 3.0 cc to .075 cc blocked ovulation in 13 females mated immediately with a vasectomized male and in four females injected simultaneously with 25 μg exogenous LH. The minimum antiserum dosage required to inhibit ovulation was .075 cc. Results obtained were attributed to neutralization of endogenous gonadotropins. Inhibition of endogenous testosterone was suggested among males.

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1. Bourdel, G., Gen Comp. Endocrin., 1961, v1, 375.

2. Bourdel, G., Li, C. H., Acta Endocrinol., 1963, v42, 473.

3. Young, W. P., Nasser, R., Hayashida, T., Nature, 1963, v197, 1117.

4. Hayashida, T., J. Endocrinol., 1963, v26, 75.

5. Moudgal, N. R., Li, C. H., Arch. Biochem. and Biophys., 1961, v95, 93.

6. Kelly, W. A., Robertson, H. A., Stansfield, D. A., J. Endocrinol., 1963, v27, 127.

7. Campbell, D. H., Garvey, J. S., Cremer, N. E., Sussdorf, D. H., in Methods in Immunology, W. A. Benjamin, Inc., 1964, p118.

8. Kabat, E. A., Mayer, M. M., in Experimental Immunochemistry, Ed. 2, Charles C Thomas, Springfield, Ill., 1961, p67.

9. Ouchterlony, O., Acta Path. et Microbiol. Scand., 1953, v32, 231.

10. Greep, R. O., Fevold, H. L., Endocrinology, 1937, v21, 611.

11. Simpson, M. E., Li, C. H., Evans, H. M., ibid., 1951, v48, 370.

12. ———, ibid., 1942, v30, 969.

13. Nelson, W. O., Am. J. Physiol., 1940, v129, 430.

14. Nelson, W. O., Merckel, C. H., Proc. Soc. Exp. Biol. and Med., 1937, v36, 825.

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Substitution of Dietary Starch for Dextrose in Hyperlipemic Subjects.* (31611)

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As many patients with lipemia continue to have elevated triglyceride levels on fat free diets(1), attention has been directed to the relation between carbohydrate ingestion and serum lipids. Two recent reviews have suggested that not only are triglyceride levels related to the proportion of total calories derived from fat, protein and carbohydrate, but that the type of carbohydrate itself influences blood lipid levels(2,3). For example, McDonald(4) found in a small group of normal men, that sucrose specifically increased serum triglycerides but maize starch did not. Kuo and Bassett(5) have reported 5 hyperglyceridemic subjects in whom substitution of sucrose for starch also elevated serum triglycerides. Despite these findings, there is still controversy about the precise influence of complex and simple sugars, since Lees(6) found that diets high in either sucrose or starch were associated with equivalent increases in plasma triglycerides in 7 normal subjects. Because caloric intake may be an important variable in the response of plasma triglycerides to dietary manipulation, we have investigated these conflicting results in studies of 2 lipemic subjects on a metabolic ward where precise control of dietary intake was possible.

Methods. Two men ages 58 and 47 were found to have lipemic serum after an overnight fast on an *ad lib* diet and were then hospitalized for study. For the first 4 days of study each was given a formula diet (containing corn oil, skim milk and dextrose) with total calories divided as 40% fat, 45% carbohydrate, 15% protein. During this period caloric intake was adjusted to maintain constant body weight. A standard 100 g oral glucose tolerance test and assay for post-heparin lipolytic activity(7) was performed. Both subjects had a normal lipolytic response to intravenous heparin and mild glucose intolerance(8). After weight had stabilized, the patients were then switched to an isocaloric formula diet containing only skim milk and dextrose, with 85% of total calories as carbohydrate (64% dextrose, 21% lactose) and 15% of total calories as protein (skim milk). This diet was maintained for 2 to 3 weeks at which time starch in the form of rice, potatoes, spaghetti, macaroni, oatmeal, matzos and corn starch was substituted for the carbohydrate in the diet. The starch diet was then maintained for another 2 to 3 weeks. The first patient was then discharged, but the second subject remained on the metabolic ward and the original 85% simple sugar and 15% protein formula was reinstituted. After an additional 3 weeks of this diet, total calories were slightly decreased by 5 calories per kilogram for 2 weeks and 10 calories per kilogram for a third week.

Weight was measured daily. Plasma triglycerides were determined by methods previously described(9), 3 times weekly.

Results and discussion. In both subjects, after one week of the high carbohydrate diet, plasma triglyceride levels reached stable values

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