2. Schmidt, N. J., Lennette, E. H., Proc. Soc. Exp. Biol. & Med., 1966, v121, 243.
3. Veronelli, J. A., Eckert, E. A., ibid., 1966, v121, 1223.
4. Stern, H., Nature, 1965, v208, 200.
5. Schell, K., Wong, K. T., ibid., 1966, v212, 621.
6. Vaheri, A., Sedwick, W. D., Plotkin, S. A., Proc. Soc. Exp. Biol. & Med., 1967, v125, 1086.
7. Sever, J. L., Ley, A. C., Wolman, F., Caplan, B. M., Crockett, P. W., Turner, H. C., Am. J. Clin. Path., 1964, v41, 167.
8. Norrby, E., Proc. Soc. Exp. Biol. & Med., 1962, v111, 814.
9. Stewart, G. L., Parkman, P. D., Hopps, H. E., Douglas, D. D., Hamilton, J. P., Meyer, H. M., Jr., New Engl. J. Med., v276, 554.
10. Schmidt, N. J., Lennette, E. H., Gee, P. S., Proc. Soc. Exp. Biol. & Med., 1966, v123, 758.

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Inhibition of Ovarian Ascorbic Acid Depletion with Ovine Luteinizing Hormone Antiserum.* (32286)

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Moudgal and Li(1) reported that rabbit antiserum prepared against ovine luteinizing hormone (LH) neutralized endogenous and exogeneous LH in the rat. This antiserum also neutralized LH preparations of pig, whale, and human pituitaries or pregnant mare's serum (PMS), but had no influence on chicken LH or human chorionic gonadotropin (HCG) when tested by ventral prostate weight in hypophysectomized rats. Bourdal (2) presented evidence that rabbit antiserum to ovine LH suppressed increases in ovarian and uterine weights of immature intact female rats, and subsequently, Bourdal and Li(3) blocked vaginal cycles in adult rats. Rennels(4) has reported that antibodies against LH prevented ovarian ascorbic acid depletion in immature pseudopregnant rats when injected ip 30 minutes prior to iv LH or when the antiserum and LH were incubated together *in vitro* prior to injection. He observed that sc injections of antiserum were ineffective, and hypothesized that the ineffectiveness was due to the rate of absorption of the antibody.

The present study was conducted to determine the influence of an iv injection of rabbit ovine-LH-antiserum on ovarian ascorbic acid depletion (OAAD) by LH in immature pseudopregnant rats. It was of interest, also, to determine if the ability of the antiserum to inactivate standard amounts of LH *in vivo* would correspond with estimates of the antibody predicted by a precipitin test.

Materials and methods. Adult male rabbits were immunized against ovine LH-S8 by 4 weekly 1.5 mg sc injections of LH dissolved in 1.5 cc of saline homogenized in an equal volume of Freund's complete adjuvant (Difco, Detroit, Mich). Two 2 mg booster injections of LH were given at 2-week intervals subsequent to the fourth 1.5 mg injection. After an additional time lapse of 12 weeks, a third 2 mg booster injection was administered followed by a final 2 mg injection 2 weeks later. Four days following the third and fourth booster injections 25-50 cc of blood was withdrawn (ear vein) from each rabbit. Adjuvant serum to Freund's complete adjuvant was prepared in the same manner in 2 male rabbits. Control serum was obtained from normal male rabbits. The sera within each treatment were pooled, and the globulins were concentrated to one-third of the original

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TABLE I. Effect of Antiserum Against Ovine-LH on LH Evoked Ovarian Ascorbic Acid Depletion in Pseudopregnant Rats.

Dose of LH* (μg) \ Type of serum	Saline	Control serum	Adjuvant serum	Low conc. antibody (LA)	High conc. antibody (HA)
	Ovarian ascorbic acid levels (μg/100 mg ovary) $\pm$ SE				
0	71.6[a] $\pm$ 3.2 (12)	85.6[a] $\pm$ 10.8 (4)	74.0[a] $\pm$ 3.2 (8)	77.8[a] $\pm$ 3.5 (12)	75.3[a] $\pm$ 3.2 (12)
0.4	73.1[a] $\pm$ 2.5 (12)	—	—	71.6[a] $\pm$ 3.4 (12)	68.6[a,d] $\pm$ 4.5 (4)
6.4	49.6[b] $\pm$ 1.3 (12)	51.2[b] $\pm$ 1.9 (8)	51.6[b] $\pm$ 2.1 (8)	66.6[a,d] $\pm$ 3.8 (10)	66.1[a,d] $\pm$ 3.5 (10)
25.0	38.1[c] $\pm$ 1.8 (12)	36.8[c] $\pm$ 1.8 (12)	38.3[c] $\pm$ 2.0 (4)	60.0[d] $\pm$ 2.3 (12)	68.0[a,d] $\pm$ 3.8 (12)

[a,b,c,d]: Means with the same superscript are not significantly different ($P > 0.05$). Least significant difference between means range from 10.0 to 23.6 μg depending on the number of rats per mean which are indicated by the figures in parentheses.

* NIH-LH-S_8 contains 0.73 $\times$ the LH potency of NIH-LH-S_1.

serum volume by repeated ammonium sulfate precipitation as previously described(5). An antibody titer of 1:32,000 was obtained by interfacial testing which indicated that 1 cc of pooled antisera should neutralize 1000 μg of LH-S8(6). On the basis of this estimate low concentration antiserum (LA) and high concentration antiserum (HA) titers were prepared so that 0.5 cc would neutralize, 6.4 and 25.0 μg of LH-S8, respectively.

Twenty-one-day-old female Sprague-Dawley rats (Hormone Assay Laboratory, Chicago, Ill.) were used in a modification of the 4-hour, 2-ovary OAAD assay described by Parlow(7). Luteinization was induced by injecting 75 IU PMS, sc on day 21 of age followed by a second injection of 50 IU 12 hours later. The technique was changed to include a second injection of PMS after initial trials using a single injection of 75 IU PMS had failed to produce ovaries weighing 100 mg each as recommended in the assay procedure(7). At 60 hours after the initial injection of PMS, 50 IU HCG was administered sc. The assay was conducted 6 days after HCG. Test substances were injected iv and both ovaries were removed 4 hours later for ascorbic acid determinations. Standard doses of LH-S_8 in 0.5 cc were injected iv immediately following injection of 0.5 cc of either antiserum, control or adjuvant serum.

A total of 176 rats were used in the 3 assays. Although 4 animals were used per subtreatment in each assay, each type of serum with each LH level was not run in all 3 assays which accounted for the disproportionate subclass numbers shown in Table I. Ovarian ascorbic acid content per 100 mg of ovarian tissue was calculated for each rat. Heterogeniety of variance among the 3 assays was not indicated and the data were pooled for analysis of variance. Differences in mean ovarian ascorbic acid levels following treatment with the 3 types of serum and 4 levels of LH were tested by least significant difference(10).

Results. Mean ovarian ascorbic acid (OAA) values for the various groups are shown in Table I. Standard doses of 6.4 and 25.0 μg of LH-S_8 significantly depleted OAA compared with the mean level of OAA in the saline control group ($P<0.05$). Although preliminary trials had indicated 0.4 μg LH provoked 10-15% OAAD, this level of LH had no significant effect on mean OAA in any of the groups tested. Neither control nor adjuvant serum administered with 6.4 and 25.0 μg of LH produced any significant variance in mean OAA values as compared with those obtained when LH was administered in saline.

Both LA and HA significantly depressed the OAAD evoked by 6.4 or 25.0 μg of LH in saline, control and adjuvant serum rat groups ($P<0.05$). When LA was administered with 25.0 μg of LH the mean μg OAA per 100 mg of ovarian tissue was 60; whereas when HA was administered with the same dose of LH the value obtained was 68. This difference in response approached significance ($P<0.10$). Twenty-five μg of LH with LA caused significantly greater OAAD than was evoked by saline, antiserum control or adjuvant serum alone, but the difference in the OAA LA column between the 6.4 and 25.0 μg LH treatment was not significant (Table I).

Discussion. Ovarian weights were elevated when two doses of PMS were administered at a 12-hour interval, but doses of LH below 0.4 μg rarely caused significant OAAD. The dose response curves obtained in Sprague-Dawley rats were similar to those reported by Yokota *et al*(8) and agree with those of Sakiz and Guillemin(9) who noted that two injections of 50 IU PMS would increase ovarian weights and OAA levels in Wistar rats without enhancing sensitivity to LH. Neither the modified priming procedure nor the fact that rats in our study were 4 days younger when initial priming began would account for the low responsiveness to LH since a subsequent assay designed to test these two variables indicated no significant effect of either initial age at priming or method of priming. However, in the latter trial both single and double injections of PMS produced rats with ovaries weighing 100 mg, and they responded to LH doses of one-half the magnitude of those needed in the earlier antiserum-trial. Levels of PMS-HCG required to stimulate ovarian weight and levels of LH needed to evoke OAAD appear related, but the mechanism responsible for the low sensitivity cannot be explained from these data.

Neutralization of endogenous rat LH and exogenous ovine LH in rats by chronic injections of antiserum prepared against ovine LH has been reported by several investigators (1,2,3). The data in the present experiment clearly indicate the antiserum effectively inhibited OAAD when injected iv immediately preceding iv injections of standard levels of LH. These data agree with and strengthen the results obtained by Rennels(4) in which anti-ovine-LH was administered ip 30 minutes prior to LH. Nonlinearity of OAA levels was not observed following control serum, adjuvant serum or saline with the different dosages of LH standard: a possibility which was not adequately tested in the study of Rennels.

A linear inhibition of the OAAD response to LH *in vivo* was not obtained using *in vitro* estimated dilutions of the antiserum. There was, however, a significantly lower mean OAA level following LA with 25.0 μg LH than following LA alone.

One cc of the antiserum diluted 1:40 effectively neutralized 25.0 μg of LH when OAAD was used as the end point. This amount of LH was also neutralized by 1 ml antiserum diluted 1:30 when inhibition of ovulation in the estrous rabbit was used as the index of anti-LH potency(11). Thus the OAAD procedure would appear to be the more sensitive indicator of antiserum potency.

Although Ely and Chen(12) have recently reported a serologically pure antibody against LH, no attempt was made to purify the antiserum used in the current investigation. A gel diffusion test indicated some contamination with FSH, TSH and possibly STH antibodies, but no prolactin antibody could be demonstrated. Since neither the absence of the anterior pituitary hormones, with the exception of prolactin(13,14), nor the addition of physiological levels of these hormone preparations(7) reportedly affect the OAAD-LH assay; the observed contaminating antibodies would not appear to have played a significant role in the results obtained.

Summary. Male rabbits were immunized against ovine luteinizing hormone (LH). Antiserum globulins were concentrated to one-third the original volume of antiserum by ammonium sulfate precipitation and tested by gel diffusions for specific and cross-reacting antibodies with other ovine pituitary preparations. Biological inactivation of ovine LH by the antiserum was tested by the LH dependent ovarian ascorbic acid depletion response. Standard doses of NIH-LH-S_8, 6.4 and 25.0 μg, significantly depleted mean ovarian ascorbic acid below saline control values ($P<0.05$). Antiserum administered iv immediately prior to LH injection prevented ovarian ascorbic acid depletion ($P<0.05$).

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1. Moudgal, N. R., Li, C. H., Arch. Biochem. & Biophys., 1961, v95, 93.

2. Bourdal, G., Gen. & Comp. Endocrinol., 1961, v1, 375.

3. Bourdal, G., Li, C. H., Acta Endocrinol., (Kobenhavn), 1963, v42, 473.

4. Rennels, E. G., Endocrinology, 1964, v74, 290.

5. Campbell, D. H., Garvey, J. S., Cremer, N. E., Sussdorf, D. H., In Methods of Immunology, Benjamin, W. A., Inc., New York, 1964, p118.

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

7. Parlow, A. F., In Albert, A. A., (ed.), Human Pituitary Gonadotrophins, Charles C Thomas, Springfield, Ill., 1961, p300.

8. Yokota, N., Igarashi, M., Matsumoto, S., Endocrinol. Japon., 1965, v12(2), 83.

9. Sakiz, E., Guillemin, R., Endocrinology, 1963, v72, 804.

10. Snedecor, G. W., Statistical Methods, Iowa State Univ. Press, Ames, 1962, p237.

11. Quadri, S. K., Harbers, L. H., Spies, H. G., Proc. Soc. Exp. Biol. & Med., 1966, v123, 809.

12. Ely, C. A., Chen, B., Endocrinology, 1966, v79, 362.

13. Baird, J. M., Wolf, R. O., Rennels, E. G., Proc. Soc. Exp. Biol. & Med., 1961, v106, 362.

14. Guillemin, R., Sakiz, E., Endocrinology, 1963, v72, 813.

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Purine Ribonucleotide Reductase: End-Product Inhibition and Stimulation of a Mammalian Enzyme.* (32287)

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The general area of metabolic regulation has considerable importance for studies of the oncogenic process. Specifically, control mechanisms exerted on DNA† synthesis should be of primary importance. The ribonucleotide reductases(1,2,3) catalyze the synthesis of the deoxyribonucleotides which serve as proximal precursors for DNA synthesis. Control mechanisms operating on the reductases should ultimately influence DNA synthesis. Also, chemical agents that modify the catalytic function of the reductases should interfere with the synthesis of DNA. This paper reports studies on the end-product inhibition and stimulation of purine ribonucleotide reductases, from a mouse tumor, by the natural proximal precursors for DNA. Two carcinostatic agents, β-D-arabinosylcytosine (4) and the 5′-phosphate of β-D-deoxythioguanosine(5), were also found to act as modifiers of deoxyribonucleotide formation. β-D-arabinosylcytosine (ara-c) inhibited deoxyadenylate synthesis, and deoxythioguanosine-5′-phosphate (dTGMP) was found to stimulate the formation of deoxyguanylate.

Materials and methods. Female A×C57BL/6 mice (Jackson Memorial Laboratory, Bar Harbor, Maine) were each implanted with 2×10^6 Carcinoma 755 ascites cells of a line resistant to 6-mercaptopurine. The ascites cells were harvested on day 5 and washed free of red blood cells. The ascites cells were centrifuged at 1470 × *g* for 2 minutes, diluted 1:1 with distilled water and thus subjected to osmotic shock for 2 minutes. The swollen cells were homogenized by 20 passes in a glass homogenizer with a teflon plunger. The homogenate was centrifuged at 16,000 × *g* for 90 minutes at 4°C. The crude enzyme extract was decanted and stored at —20°C. The reaction mixtures used in the enzyme tests contained AMP-8-^{14}C (Schwarz BioResearch, Inc.), 1.6×10^6 cpm/μmole, or GDP-8-^{14}C (Schwarz BioResearch, Inc.) 1.0×10^7 cpm/μmole; ATP, 0.25 μmoles; Tris-HCl, pH 7.4, 5 μmoles; $MgCl_2$,

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† Abbreviations used: DNA, deoxyribonucleic acid; ATP, adenosine-5′-triphosphate; dADP, deoxyadenosine-5′-diphosphate; ara-c, β-D-arabinosylcytosine; dGTP, deoxyguanosine-5′-triphosphate; dGDP, deoxyguanosine-5′-diphosphate; dGMP, deoxyguanosine-5′-monophosphate; dTGMP, β-D-deoxythioguanosine-5′-monophosphate; Tris-HCl, tris(hydroxymethyl) aminomethane hydrochloride buffer; AMP, adenosine-5′-monophosphate.