Effect of Ovarian Steroids on Lactic Dehydrogenase Activity in Endometrium and Myometrium of the Rat Uterus[1]

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ABSTRACT. Measurements of (a) lactic dehydrogenase activity (LDH) and (b) its isoenzyme distribution were made in both endometrium and myometrium of the rat uterus under 5 endocrine conditions: 1) during early pseudopregnancy; 2) following ovariectomy; 3) following treatment with estrogen; 4) after daily injection of progestogen; and 5) following daily treatment with combinations of these hormones. The response of endometrial tissue to steroid hormone deprivation or stimulation was much more dramatic than myometrium. Following the induction of pseudopregnancy, or after ovariectomy, LDH activity in endometrium declined. Treatment of ovariectomized rats with either estrone or, less effectively, progesterone stimulated both M-LDH and H-LDH. However, when administered simultaneously, progesterone blocked the action of estrogen. The pattern of LDH activity in endometrium during 6 days of treatment with 1 μg estrone and 2 mg progesterone was identical with that in intact pseudopregnant rats. Although the percentage of H-LDH in myometrium was higher than that in endometrium under all conditions, both tissues exhibited similar fluctuations in this parameter. Per cent H-LDH in both tissues paralleled changes in intrauterine oxygen tension, suggesting that *in vivo* oxygen levels may regulate the proportion of M- and H-LDH in both tissues. The inverse relation between specific activity of M-LDH and intrauterine pO_2 was not as consistent. The data suggest that ovarian steroids may modify LDH activity *in vivo* by affecting the oxygen tension to which the tissue is exposed. *Endocrinology* **89:** 358, 1971)

IT HAS BEEN DEMONSTRATED that the activity of LDH in the uterus is under regulation by ovarian hormones, and that differential synthesis of LDH subunits can be stimulated by these hormones (1). However, *in vivo* and *in vitro* studies have revealed that LDH subunit activity in the uterus also may be related to changes in oxygen tension (2,3). Because changes in LDH activity in the uterus of the rat occur predominantly in the endometrium, in areas exposed to fluctuating luminal oxygen levels (4–6), this enzyme may play a key role in the metabolism of the endometrium associated with implantation. Accordingly, it was of interest to examine in detail the effects of physiological dosages of estrogen and progestogen on LDH activity and isoenzyme distribution, and to attempt to relate these effects to previously measured levels of intrauterine oxygen under identical endocrine conditions.

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Materials and Methods

Animals. Adult female rats (200–300 g) of the Holtzman strain (Sprague-Dawley derived) were housed 1/cage in air-conditioned quarters (20-25 C) with a photoperiod of 14-hr fluorescent illumination and 10 hr-darkness/day. The midpoints of the light and dark periods were set to noon and midnight, respectively. All animals were fed Purina Lab Chow and water *ad lib.*, and vaginal smears of each rat were recorded daily for 2 or 3 cycles before use. Pseudopregnancy was induced by mechanical stimulation of the uterine cervix during proestrus and estrus. Day 0 of pseudopregnancy was designated as the last day of vaginal cornification (estrus) prior to the onset of pseudopregnancy.

Hormone treatment. Ovariectomy was performed under ether anesthesia on Day 0, and ovarian steroid hormones, dissolved in 0.1 ml sesame oil, were injected subcutaneously on Day 0 and daily thereafter. As shown by Clark and Yochim (1), LDH activity was highest during normal estrus or on Day 0 following cervical

stimulation. The present experiments were designed, therefore, to show the effect of replacement therapy following ovariectomy during a period of peak activity. This experimental design avoided the use of pharmacological doses, which often are necessary when the tissue undergoes complete regression as in long-term deprivation studies. Estrone (0.5, 1.0, or 5.0 μg), progesterone (2.0 or 4.0 mg) or a combination of these steroids was used in the present study. These dosages of hormones have been shown to simulate responses measured in intact rats during the estrous cycle and pseudopregnancy (7–9).

Preparation of homogenates. All animals were anesthetized with ether, killed by cervical dislocation, weighed, decapitated, and exsanguinated between 8:00 and 11:00 AM. Uteri were removed quickly and trimmed of adhering fat and mesentery on an ice cold glass plate. The cornua were weighed individually to the nearest 0.1 mg, split longitudinally, and the endometrium was scraped gently from each horn. Endometrial tissue scrapings from both horns were pooled and weighed on a plastic cover slip, and transferred to a chilled Kontes all-glass homogenizer containing 0.4–1.0 ml of 0.1M sodium pyrophosphate-EDTA buffer (pH 8.8). Following homogenization, the sample was centrifuged in a clinical centrifuge for 5 min, and the supernate was decanted and placed in an ice bath prior to enzyme analysis. Samples of myometrium (6–10 mg) were prepared in a similar manner.

Enzyme assay. LDH activity was measured as reported previously (4), using a method modified from Elevitch and Phillips (10) to record the fluorescence of NADH formed during the conversion of lactate to pyruvate. A sample of supernate containing 50–200 μg wet weight of tissue was added to a reaction mixture containing sodium lactate and NAD in sodium pyrophosphate-EDTA buffer at 37 C. The rate of formation of NADH was recorded for 1 min on an Aminco-Bowman spectrophotofluorometer using an activation wavelength of 340 mμ and an emission wavelength of 450 mμ. All samples were assayed at 2 concentrations, and the results were averaged after calculation of specific activity. Specific activity of LDH is expressed in the present report as nmoles NADH formed per min per mg wet weight of tissue.

Separation of LDH isoenzymes. Electrophoretic separation of the isoenzymes was accomplished using a Gelman apparatus and power source by methods reported previously (4). A volume of supernate was placed on Gelman Cellulose Polyacetate Sepraphore III strips which had been presoaked for at least 24 hr in barbital buffer. Electrophoresis was conducted for 1.4 hr at 23 C with the chamber troughs filled with barbital buffer; an emf of 320 V and a current of 2–3 mA per strip was used. The gel strips were removed, stained, and fixed as previously described (4), and then dehydrated and cleared prior to drying in an oven. The isoenzyme patterns were scanned at 400 mμ using an Aminco-Bowman scanner attached to the spectrophotofluorometer. From the recorded scan, the relative concentration of each isoenzyme band was determined (% of total activity), and from this the percentages of M- and H-sub-units were calculated by multiplying the relative concentration of each band by the fraction of M- or H-subunits which it contained, according to the accepted tetrameric structure of LDH (11–13). Estimates of M- and H-LDH activity were calculated by multiplying the % M- or % H-subunits by the total activity obtained by fluorometric analysis.

Results

Measurements of (a) LDH activity (nmoles NADH/min/mg tissue) and (b) its isoenzyme distribution were made in both endometrium and myometrium under five endocrine conditions: 1) during the first six days of pseudopregnancy (intact control); 2) following ovariectomy (ovariectomized control); 3) following daily injection of each of three dosages of estrogen; 4) following daily injection of each of two dosages of progestogen; and 5) following daily injection of each of three combinations of estrogen and progestogen. From the data collected, the proportion of M- and H-subunits and the activity of M- and H-LDH were calculated.

1) Intact control. Following the induction of pseudopregnancy, LDH activity decreased in the endometrium from 57 nmoles/mg tissue on Day 0 to 21 nmoles by Day 2 and remained at about this level through Day 6 ($p < .001$). The activity which could be attributed to M-subunits was 89% on Day 0, but only 80% by Day 6 ($p < .01$) (Table 1).

By contrast, enzyme activity in the my-

TABLE 1. Effect of estrone, progesterone or combinations of these hormones on LDH activity (M+H) and per cent M-subunits in endometrium

Treatment	*	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Intact	Act.	27.7±2.7	21.1±0.8	24.8±2.8	19.2±1.8	19.7±0.8	20.4±0.9
	% M	89.5 1.5	83.9 0.9	83.0 2.1	82.2 1.3	80.8 1.4	80.0 1.9
Ovx.	Act.	25.0 2.9	19.7 0.9	19.1 1.7	16.3 2.6	15.1 1.8	12.1 1.2
	% M	89.2 0.9	80.2 3.5	78.4 2.3	76.2 2.9	79.4 1.5	75.1 1.9
0.5 μg E	Act.	26.5 1.4	—	18.4 0.9	—	21.9 1.2	—
	% M	92.1 1.2	—	81.7 2.6	—	88.4 1.7	—
1.0 μg E	Act.	33.9 2.9	23.8 0.8	24.9 2.3 (3)	29.0 3.0 (5)	30.9 2.3	40.2 3.7
	% M	85.2 3.5	82.0 2.8	84.4 1.9	82.3 2.1 (5)	86.8 1.8 (5)	85.3 1.6
5.0 μg E	Act.	45.2 5.0	48.1 1.6 (3)	77.0 3.6 (6)	75.6 6.0	54.8 7.2	63.5 9.1
	% M	90.2 1.1	94.5 0.4 (3)	91.1 0.8 (6)	94.9 1.8	90.8 0.2	95.7 0.2
2.0 mg P	Act.	27.8 1.4	28.7 1.5	25.4 1.0	24.2 3.3	24.9 0.4	24.9 1.6
	% M	85.7 3.8	83.1 1.7	82.2 3.0	81.3 1.8	78.7 1.6	79.0 2.6
4.0 mg P	Act.	26.2 1.1	—	23.8 0.7	—	20.6 1.1	—
	% M	92.6 0.6	—	87.8 1.3	—	82.7 2.9	—
1 μg E/2 mg P	Act.	33.5 3.4	24.2 2.4	27.1 1.8	20.6 0.7	21.5 1.3	22.5 1.1
	% M	88.4 2.1	81.1 1.9	85.2 1.1	81.2 1.5	84.8 2.8	85.9 2.0
5 μg E/2 mg P	Act.	—	—	36.4 2.4	27.2 3.6	—	—
	% M	—	—	85.1 1.5	88.4 1.3	—	—
5 μg E/4 mg P	Act.	—	—	33.6 1.5	27.5 0.6	—	—
	% M	—	—	87.5 1.4	89.8 1.4	—	—

Day 0 (estrous) control values: Act. =56.5±2.7; % M =89.3±1.7; (7) and (5), respectively.
Number of animals in each group is 4 unless indicated in parentheses.
Intact =pseudopregnant; Ovx. =ovariectomy; E =estrone; P =progesterone.
* Values are means±standard error of mean expressed as nmoles NADH/min/mg (Act.) or as % M-subunits.

ometrium was significantly less than that in endometrium (Table 2). On Day 0 of pseudopregnancy LDH activity was 14 nmoles and declined to 10 nmoles by Day 4 ($p < .01$), followed by a rise to initial values by Day 6. M-LDH, which accounted for 73% of all subunit activity on Day 0, showed a slight but insignificant decline to 66% by Day 6.

2) *Ovariectomized control.* Following ovariectomy on Day 0, LDH activity in the endometrium declined from 57 to 12 nmoles by Day 6 ($p < .001$), with a corresponding decrease in M-subunits from 89 to 75% ($p < .001$, Table 1). By contrast, no significant change was observed in LDH activity in the myometrium, although the proportion of M-subunits declined sharply by Day 1 and remained significantly lower than Day 0 throughout the period measured ($p < .05$, Table 2). Thus, by Day 4 following ovariectomy, the enzyme activity in endometrium was not statistically different from that in the myometrium, while the percentage of M-subunits in the endometrium remained higher than that in the myometrium throughout the days measured.

3) *Estrogen replacement.* All dosages of estrogen used prevented the post-ovariectomy decline in total activity and in % M subunits in the endometrium (Table 1). The time and magnitude of response was dose dependent: with higher dosages of estrogen, peak responses were greater and occurred earlier. Fig. 1 depicts the activities of M- and H-LDH in endometrium following treatment with estrogen for five days. The hormone maintained the activities of both subunits proportionally (Fig. 1B). By contrast, no consistent effect of estrogen on

TABLE 2. Effect of estrone, progesterone or combinations of these hormones on LDH activity (M+H) and per cent M-subunits in myometrium

Treatment	*	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Intact	Act.	13.1±1.5	13.1±1.6	12.2±0.6	9.6±0.6	10.7±1.6	14.1±0.9
	% M	66.9 2.6	66.6 2.7	66.7 2.0	64.5 2.3	67.3 2.5	65.5 1.9
Ovx.	Act.	13.4 1.3	14.8 0.7	13.1 1.3	13.7 1.4	13.7 2.0	13.1 1.5
	% M	65.9 2.6	62.3 2.5	63.7 2.0	64.3 2.3	65.0 0.7	64.2 2.8
0.5 μg E	Act.	12.8 1.1	—	13.2 1.4	—	11.6 0.8	—
	% M	71.8 2.7	—	67.6 1.1	—	66.1 1.6	—
1.0 μg E	Act.	15.8 0.5	14.0 0.6	12.7 0.7	12.0 0.4 (5)	12.1 1.0 (5)	12.8 0.6
	% M	72.7 1.1	64.6 1.7	66.4 2.4	66.0 1.4 (5)	65.9 2.5 (5)	70.6 2.6
5.0 μg E	Act.	13.5 0.7	11.9 0.4 (3)	14.7 1.3 (6)	12.5 0.8	12.5 0.4	12.4 1.0
	% M	76.6 1.4	77.0 1.7 (3)	74.9 0.6	80.0 1.2	78.4 0.9	77.3 1.4
2.0 mg P	Act.	14.8 1.0	14.6 1.0	13.7 1.4	13.9 0.8	13.4 0.5	13.5 0.3
	% M	69.8 2.6	68.7 0.5	66.4 3.2	65.7 2.3	67.4 1.5	66.4 1.2
4.0 mg P	Act.	12.3 0.7	—	11.1 1.0	—	10.9 0.8	—
	% M	70.7 3.3	—	70.0 0.8	—	65.1 1.8	—
1 μg E/2 mg P	Act.	14.0 1.0	12.5 0.9	12.9 1.0	10.2 0.4	11.8 0.8	14.0 0.9
	% M	67.6 1.3	65.2 1.8	70.8 1.9	66.0 1.5	65.9 0.7	66.5 1.0
5 μg E/2 mg P	Act.	—	—	12.5 0.9	11.7 0.3 (3)	—	—
	% M	—	—	65.0 2.3	66.5 1.6 (3)	—	—
5 μg E/4 mg P	Act.	—	—	11.2 0.5	11.5 0.6	—	—
	% M	—	—	62.3 2.2	64.2 1.8	—	—

Day 0 (estrous) control values: Act. =14.1±0.6 (7); % M =73.0±2.8 (5).
Number of animals in each group is 4 unless indicated in parentheses.
Intact =pseudopregnant; Ovx. =ovariectomy; E =estrone; P =progesterone.
* Values are means±standard error of mean expressed as nmoles NADH/min/mg (Act.) or as % M-subunits.

LDH activity was noted in the myometrium other than a significant elevation of the proportion of M-subunits following treatment with 5.0 μg estrone (Table 2). Thus, estrogen was effective in preventing the post-castration decline in M- and H-LDH in endometrium, but had little effect in the myometrium.

4) Progesterone replacement. Daily treatment with 2.0 or 4.0 mg progesterone maintained LDH activity at a level similar to that in the intact pseudopregnant rat, and significantly higher than that measured in ovariectomized animals (Table 1). The proportion of M-subunits in endometrium following treatment with 4.0 mg progesterone was significantly higher than that in ovariectomized controls only through Day 3 ($p < .02$). A similar effect on the proportion

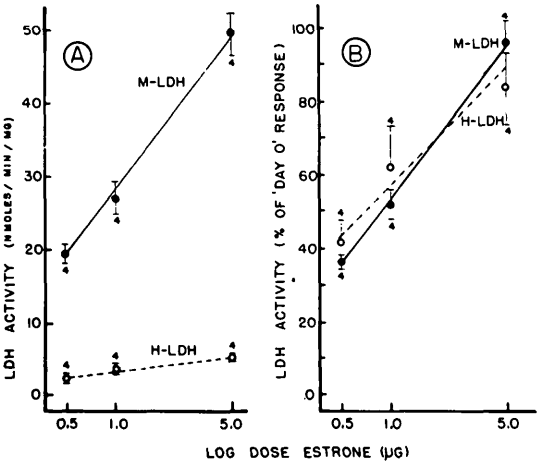

FIG. 1. Response of endometrial LDH to treatment with estrone for 5 days following ovariectomy on Day 0 (estrus). A: activity (nmoles NADH/min/mg tissue); B: % of control (Day 0) response. Number of rats is listed above or below each standard error of mean.

of M-subunits was observed in myometrium (Table 2). Thus, progesterone did not entirely prevent the post-castration decline, but maintained activity at a level comparable to that in intact pseudopregnant rats.

5) *Estrogen and progesterone interaction.* Following treatment with 1.0 μg estrone and 2.0 mg progesterone, LDH activity in endometrium was similar to that for progesterone-treated rats, while the proportion of M-subunits resembled that in estrogen-treated animals (Table 1). With higher dosages of estrogen, progesterone blocked both the activity and the proportion of M-subunits.

In the myometrium, treatment with 1.0 μg estrone and 2.0 mg progesterone caused responses similar to those measured following injection of either hormone alone, with the exception of values measured on Day 4. Combinations of either dose of progesterone with 5.0 μg estrone resulted in a proportion of M-subunits which was significantly less than that observed after estrogen treatment alone (Table 2), a response similar to that in the endometrium.

6) *Isoenzyme distribution.* LDH isoenzymes were numbered 1 to 5 in order of decreasing anodic migration. LDH-5, the major isoenzyme in the endometrium, was as high as 87% after six days' treatment with 5.0 μg estrone, and as low as 44% six days after ovariectomy. This isoenzyme consistently reflected changes in a direction parallel to that observed in % M subunits. The next isoenzyme, LDH-4, varied from a low of 9% after six days of treatment with 5.0 μg estrone, to a high of 34% after five days of treatment with 2.0 mg progesterone. Changes in this isoenzyme were generally in a direction inverse to shifts in % M subunits, *i.e.*, proportional to changes in H-subunits. LDH-1 varied directly with changes in % H subunits.

The theoretical (expected) isoenzyme distributions, assuming complete randomization of subunits into the tetrameric structure, were calculated from the individual % M and H values from endometrium. Comparison of the expected (E) with the observed (O) isoenzyme distribution revealed that the major 5^{th}-O band (M_4) was consistently but not significantly higher than the 5^{th}-E band. Also, the 4^{th}-O band (M_3H) was consistently and sometimes significantly lower than the 4^{th}-E band. These differences persisted regardless of hormone treatment and appeared to be inherent within the experimental procedure.

Discussion

a) *Effect of steroid hormones on LDH.* The present results show clearly that the major effect of physiological dosages of ovarian steroids occurs in the endometrium and not the myometrium. On Day 0, endometrial LDH was four times that in myometrium; four days after ovariectomy there was no significant difference in activity between these tissues. Six days after ovariectomy, endometrial LDH activity had declined 79% while only a 6% decrease in activity occurred in myometrium during the same period. Hormone therapy reversed these post-castration changes.

Consistently, the proportion of M-subunits in the endometrium was higher than that in the myometrium, regardless of treatment. There existed between these tissues about a 20% difference in % M on Day 0, but only a 10% difference six days after ovariectomy. These changes also were reversed by hormone therapy. Thus, it appeared that (a) the enzyme activity is basically the same in both tissues when these tissues are deprived of hormones, (b) the endometrium is more sensitive than the myometrium to the presence or absence of the hormones and (c) the endometrium exhibits a higher base-line composition of M-subunits.

Goodfriend and Kaplan (1) also have demonstrated greater M-LDH activity in the endometrium than in the myometrium of the rabbit uterus, and the phenomenon

TABLE 3. Effect of progesterone treatment for five days on M- and H-LDH activity

Treatment	N	Activity		% of Day 0	
		M-LDH	H-LDH	M-LDH	H-LDH
Day 0* control	5	51.8 ± 3.7	6.1 ± 0.8	100 ± 7	100 ± 13
Prog. 5 days, 0 mg	4	12.0 1.4	3.1 0.5	23 3	51 8
2 mg	4	19.6 0.3	5.3 0.4	38 1	87 7
4 mg	4	17.0 0.8	3.6 0.7	33 2	59 16

Values are means ± standard error of means expressed as nmoles NADH/min/mg or as per cent of Day 0 responses.
N = number of rats.
* Day 0 = estrus following cervical stimulation.

does not seem to be unique to LDH (9). Although LDH activity in the myometrium was stimulated by 5.0 μg estrone in the present study, the treatment resulted in changes greater than those observed in the intact rat (4, 5, 14), an indication that the tissue was exposed to dosages of hormone greater than those to which it is normally exposed *in vivo* (5, 16–18). Radioautography following injection of labeled estradiol has shown that localization of the label occurs primarily in endometrium, an indication, perhaps, of the basis for the increased sensitivity and responsiveness of this tissue to ovarian steroids (15). Thus, in the uterus of the rat, the changes in enzyme pattern and activity reflect a differential responsiveness of the cell types.

Goodfriend and Kaplan have demonstrated increased M-LDH activity following estrogen stimulation; progesterone treatment stimulated both subunits equally. The present results revealed that estrogen as well as progesterone prevented the postcastration decline in activity of both subunits (Table 3; Fig. 1B, 2). Although the effect of estrogen on M-LDH was more striking (Fig. 1A, 2), the maintenance of activity of both subunits following hormone therapy was equivalent when each was compared with its initial value recorded on Day 0 of the experiment (Fig. 1B). Thus, under the present experimental design, treatment with estrogen maintained activity of both M- and H-LDH on Day 5 at levels similar to those measured on Day 0.

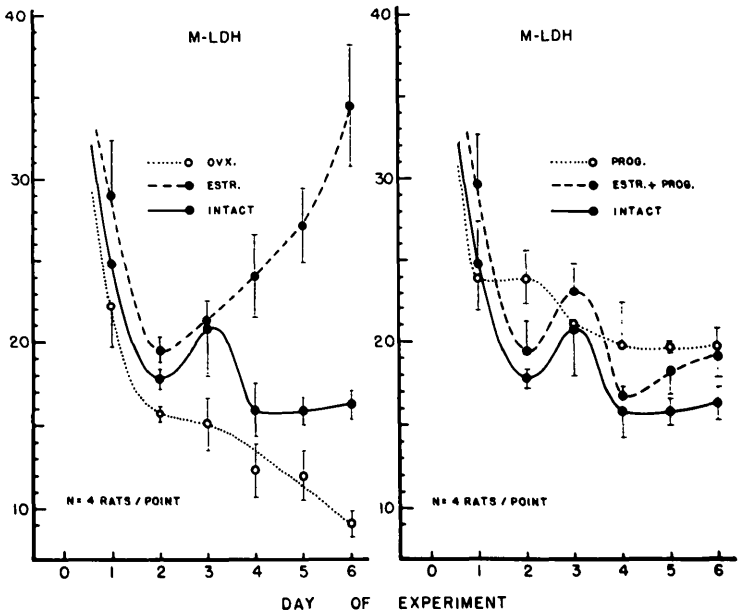

FIG. 2. Activity of M-LDH in endometrium following ovariectomy (OVX.), treatment with 1.0 μg estrone daily (ESTR.), 2.0 mg progesterone daily (PROG.) or the combination of steroids. Data are compared with intact, pseudopregnant rats (INTACT). Values are means ± standard errors of means; N = 4 rats/point. Ordinate: nmoles NADH/min/mg tissue.

TABLE 4. Block of estrone-induced M-LDH activity by progesterone

Treatment, 4 days		M-LDH activity		% Inhibition
Estrone	Prog.	nmoles	SE	
—	—	12.3	1.7	—
1 μg	—	24.0	2.6	—
1 μg	2 mg	16.7	0.5	30.4
5 μg	—	71.5	4.6	—
5 μg	2 mg	24.0	3.0	66.4
5 μg	4 mg	24.7	0.9	65.6

N = 4 rats/exp; values are means ± standard error of mean expressed as nmoles NADH/min/mg tissue.

By contrast, progesterone treatment at low dosages prevented the decline in H-LDH more effectively than M-LDH (Table 3). Thus, although low dosages of progesterone maintained both subunits over comparably timed castrates (Table 3; Fig. 2), its effect on H-LDH was proportionally greater, when compared with Day 0 controls.

During progestation *both* estrogen and progestogen are secreted and affect uterine metabolism. The interaction of these two steroids on metabolism has been demonstrated and is important for the process of implantation (8, 9, 19). The present experiments depict a similar interaction on M-LDH. Although either steroid stimulated M-LDH activity in the endometrium when given individually, when both were injected simultaneously the action of estrogen was suppressed (Table 4). The time course of this interaction in comparison with the intact pseudopregnant rat is presented in Fig. 2: under the conditions of the present experiments, progesterone suppressed the estrogen-induced enzyme activity to result in a response identical with that measured in the intact psuedopregnant animal, similar to that measured in the progesterone-treated rat, and significantly higher than that in the ovariectomized rat.

b) Intrauterine oxygen tension and LDH activity. Low oxygen tension has been shown to stimulate M-LDH in the rabbit uterus *in vitro* (2). A relationship between M-LDH activity, % H subunits and intrauterine oxygen tension also has been shown to exist *in vivo* in the uterus of the rat during the estrous cycle (4, 6). Since luminal oxygen is regulated by ovarian steroids (20), it was of interest to compare the present results with the changes in intrauterine pO_2 which occur following hormone therapy. A direct comparison can be made since the experimental design was identical in both studies and the levels of intrauterine oxygen which result from these doses of ovarian steroids are well within the range found effective in stimulating M-LDH *in vitro* (2). Fig. 3 depicts the relationship between % H subunits, M-LDH activity and intrauterine pO_2 following ovariectomy and replacement with 1.0 μg estrone and 2.0 mg progesterone as an example. It is evident that: (a) M-LDH activity in endometrium, but not myometrium, was inversely related to intrauterine oxygen; (b) the proportion of H-subunits fluctuated directly with pO_2 in both tissues, despite the fact that (c) the per cent of H-subunits in myometrium was higher than that in endometrium.

The relationship between the proportion of H-subunits and pO_2 was evident with all hormone dosages and combinations used: rapid changes in pO_2 were followed by a corresponding change in the per cent of H-subunits within 24 hours. The inverse relation between specific activity of M-LDH and intrauterine oxygen was not as consistent: it was obscured in animals receiving no estrogen, in rats treated with high dosages of estrogen, and in animals treated with high dosages of progesterone. That myometrium contains a greater proportion of H-subunits than endometrium may be an indication that the muscular portion of the uterus is oxygenated *in vivo* more efficiently than the glandular portion. The smaller proportion of glucose metabolized to lactate in myometrium is consistent with this notion (19, 21).

Thus, the present experiments demonstrate (a) a differential responsiveness of endometrium and myometrium following

hormone manipulation, (b) an antagonistic action of progesterone on estrogen-induced LDH subunit activity and (c) a relation between hormone-induced enzyme activity and intrauterine oxygen tension [measured under identical endocrine conditions (20)]. Because of the correlation between intrauterine pO_2 and LDH, it is possible that one mode of action of the ovarian steroids on subunit activity *in vivo* may be through regulation of the level of oxygen to which the tissue is exposed (3).

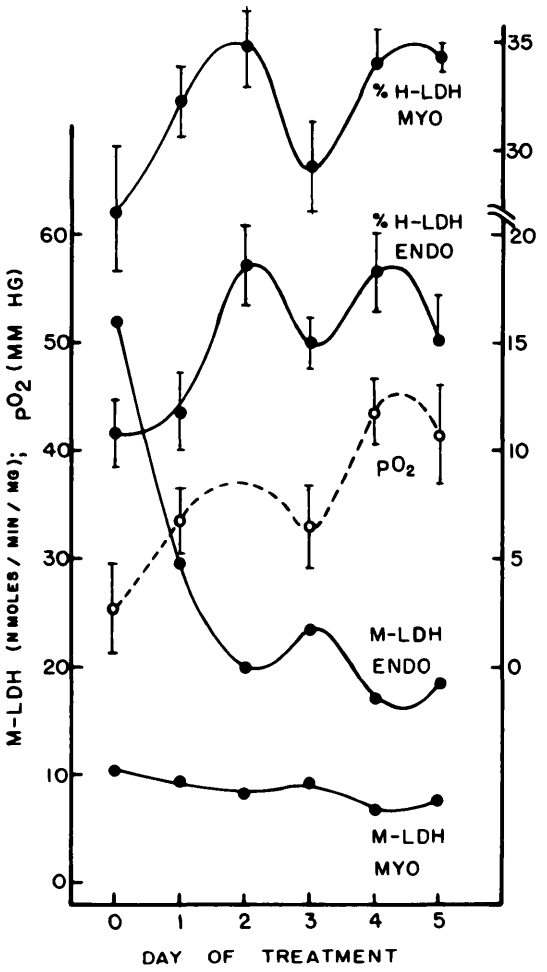

FIG. 3. Comparison of M-LDH activity and % H-subunits with intrauterine oxygen tension. Animals were ovariectomized and treated daily with 1.0 μg estrone and 2.0 mg progesterone. Values for intrauterine $p0_2$ were obtained from data of Mitchell and Yochim (20).

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