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Effect of Estrogen and Progesterone on Uterine Acid and Alkaline Phosphatase and β -Glucuronidase Activity in Mated Ovariectomized Rats. (32133)

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The role of uterine enzymes in the process of implantation has been the subject of numerous investigations but still remains poorly understood(1). Confusion exists because many of the uterine enzyme activities may be altered differently by hormone administration, by treatment with deciduomatogenic agents or by the process of nidation itself(2-8).

The search for enzymological evidence of the hypothetical "estrogen surge" required for implantation, which is thought to occur on day 3 following mating in the rat, has also contributed to contradictory findings [an increase in β -glucuronidase on the day prior to implantation reported(9) as evidence of an "estrogen surge" could not be confirmed by us(2)]. Manning *et al*(2) described a technique which permitted simultaneous evaluation and segregation of endocrine and nidatory effects on uterine enzymes in the same animal. Thus rats were surgically prepared with unilateral section and ligation of the fallopian tubes ("USLT" rats). When mated, such animals became unilaterally pregnant, permitting measurement of enzyme activities in the uterine horn subjected to the influence of fertilized ova in comparison with the sterile

contralateral horn. Using USLT rats, it was found(2) that during the implantation process a large increase in alkaline phosphatase activity occurred at the implantation sites of the pregnant horn. Enzyme concentration in the inter-implantation areas as well as in the sterile horn remained similar to that observed *in uteri* from non-pregnant animals. It was therefore postulated that the increased alkaline phosphatase activity resulted from an interaction of the blastocyst with the hormonally conditioned endometrium rather than from direct hormonal influences on the uterus(2).

In the present experiments, USLT rats were bilaterally ovariectomized one or four days following proven mating. Exogenous 17β -estradiol and/or progesterone in doses which succeeded or failed to induce nidation were studied for effects on uterine alkaline and acid phosphatase and on β -glucuronidase in the fertile as compared with the sterile horns.

Material and methods. Mating, tissue separation and homogenation, as well as unilateral tubal ligation and section were carried out as previously reported(2). Occurrence of copulation was determined within 3 hours by vaginal smear and this time was designated as day zero of pregnancy.

Ovariectomies were done under ether anesthesia. The 17β -estradiol (General Biochemicals, 0.1 or 1.0 $\mu\text{g}/0.25$ ml sesame oil) and progesterone (U.S.P., 5.0 mg/0.25 ml sesame oil) were injected subcutaneously.

All animals were killed by decapitation 8 days after proven mating. Alkaline phosphatase(10), β -glucuronidase(11) and acid phosphatase(12) assays were carried out, respectively, on 0.1, 1.0 and 0.2% homogenates. Because of differences in water content of the various uterine samples, results were expressed as units/g of dry tissue.

The data were evaluated statistically by analysis of variance and the "F test" for significance.

The animals were treated according to the "treatment schedule" in Table I.

Results. A) Occurrence of implantation. The results are reported in Table I. Implantation sites (IS) were found on day 8 of pregnancy in the non-ligated (NLT) but not in the ligated (LT) uterine horns of intact control rats. No IS were observed in either horn of untreated rats which had been ovariectomized on days 1 or 4. Seven daily injections of 17β -estradiol, 1 μg , progesterone, 5 mg, or their combination in rats which had been ovariectomized on day 1 failed to induce implantation. The IS were found, however, on day 8 in animals which had been ovariectomized on day 1 and treated with 7 daily doses of 5 mg progesterone combined with 17β -estradiol at a dose of 0.1 μg on days 1, 2 and 3 and 1.0 μg on days 4-7. Implantation also occurred in all rats which had been ovariectomized on day 4 and injected on days 4-7 with either progesterone (5 mg/day) or a combination of progesterone (5 mg/day) and 17β -estradiol (1 $\mu\text{g}/\text{day}$) but not with 17β -estradiol alone. The IS, where present, were always confined to the NLT and varied from 4 to 8 in control as well as treated animals.

B) Enzyme levels. Alkaline phosphatase. Enzyme activity was 10-17 \times greater ($p < 0.001$) in implantation sites (IS) than in inter-implantation areas of non-ligated horns (NLT) or in ligated horns(LT). There were no statistical differences between NLT and LT within groups nor were there differences

in enzyme levels in IS between groups (Table I). The enzyme reaction in the NLT and LT of estrogen-treated animals (Groups 3 and 8) however was significantly greater (1.5-2.0 \times) than that of the intact or ovariectomized controls (Groups 1, 2 and 7) or that of the ovariectomized rats treated with progesterone or a combination of estradiol and progesterone ($p < 0.05$).

Acid phosphatase. All IS (Groups 1, 6, 9 and 10) demonstrated an enhanced enzymatic reaction (1.1-1.5 \times ; $p < 0.05$) while NLT and LT were unaltered. No other appreciable changes in acid phosphatase activity were noted (Table I).

β -Glucuronidase. The β -glucuronidase activity in the NLT and LT of the estrogen-treated animals and in the LT of Group 9 (ovariectomized on day 4 and treated with progesterone) were significantly higher (1.3-1.6 \times ; $p < 0.05$) than in the NLT and LT of the ovariectomized controls. No other changes were recorded (Table I).

Discussion. On the eighth day of pregnancy in USLT rats which had been ovariectomized on either day 1 or day 4 of gestation and suitably maintained by hormone injections, the alkaline phosphatase activity at the IS was increased 10-17 fold while that of the LT and NLT was unaltered. The enhanced alkaline phosphatase activity at this IS is not a function of circulating hormones since the enzyme was not increased in the contralateral LT of the same animals or in either horn of mated non-pregnant USLT animals which were treated either with the estrogen or progesterone. Furthermore, in progesterone-treated, ovariectomized, mated USLT animals which were injected with implantation-preventing doses of 17 -estradiol, no changes in the phosphatase were observed. Blastemic irritation of the endometrium is probably not alone responsible for the stimulated phosphatase reaction because Finn and Hincliffe (4) were unable to enhance the reaction in ovariectomized mice with intrauterine injections of a deciduoma-producing agent. This same agent however, did increase the enzyme in pseudo-pregnant animals(4). These findings clearly support our original hypothesis that the rise in alkaline phosphatase in im-

TABLE I

Group	Ovariectomy	Estradiol-17 β 0.1 μ g/ day on days	Progesterone 5 mg/ day on days	Rats with IS* rats per group	Alkaline phosphatase \S units/g dry wt		Acid phosphatase \parallel units/g dry wt		β -Glucuronidase \ddagger units/g dry wt				
					IS*	NLT*	LT*	IS*	NLT*	LT*	IS*	NLT*	LT*
1	none	—	—	5/5	2545 \ddagger	220	230	38159 \ddagger	23137	23915	34365	33104	29959
2	day 1	—	—	0/5	—	249	213	—	29920	26920	—	30638	24629
3	day 1	—	—	0/6	—	320 $\#$	336 $\#$	—	31822	33635	—	37674 \ddagger	35787 \ddagger
4	day 1	—	1-7	0/5	—	195	237	—	24372	29512	—	25056	32447
5	day 1	—	1-7	0/5	—	110 $\#$	173	—	20355 \ddagger	23305	—	26297	30547
6	day 1	1-3	1-7	5/5	2578 \ddagger	188	150	29184 \ddagger	25477	26085	36730	30675	27841
7	day 4	—	—	0/5	—	217	193	—	26732	26555	—	26266	28529
8	day 4	—	—	0/5	—	311	393	—	32179	34010	—	44332 \ddagger	42892 \ddagger
9	day 4	—	4-7	8/8	2475 \ddagger	256	246	40285 \ddagger	27254	28956	35048	33220	35904 \ddagger
10	day 4	—	4-7	9/9	2486 \ddagger	150	172	32414 \ddagger	21446 \ddagger	23338	31656	26718	26078
\pm	SD				336	73	82	5275	6010	5820	5175	7170	7660

* IS = Implantation sites; NLT = non ligated horn exclusive of implantation sites; LT = ligated horn.

\ddagger = $p < 0.05$ as compared to ovariectomized controls.

$\#$ = $p < 0.05-0.01$ as compared with LT and NLT from same rats.

\S 10 units of alkaline phosphatase activity liberate 1 μ g of phenolphthalein in 1 hr at pH 9.6 and 37°C from phenolphthalein diphosphate under standard condition.

\parallel One unit of acid phosphatase activity liberates 1 μ g of α -naphthol from sodium- α -naphthyl acid phosphate in 30 min at pH 5.2 and 37°C under standard condition.

\ddagger One unit of β -glucuronidase activity liberates 1 μ g of phenolphthalein in 1 hr at pH 4.5 and 38°C from phenolphthalein monoglucuronide under standard condition.

plantation sites occurs when the blastocyst interacts with a suitable hormone-conditioned endometrium(2).

The relationship of estrogen and progesterone to the stimulation of alkaline phosphatase in the proposed interaction of the blastocyst with a suitably hormone-primed endometrium is now more clear. In USLT rats, which were ovariectomized on day 1 or day 4 of pregnancy and treated with estradiol, a statistically significant increase ($1.5-2.0 \times$) in alkaline phosphatase activity occurred in both the LT and NLT. These results agree with numerous earlier reports that estrogens stimulate uterine alkaline phosphatase in immature and castrated animals(3,5,6,7,8). However, when a combination of estrogen and progesterone was administered to mated ovariectomized USLT animals, no enhanced phosphatase reaction was detectable in either the inter-implantation areas, the NLT or the LT whether or not implantation occurred. It would appear, therefore, that the uterine alkaline phosphatase activity which is slightly stimulated by the estrogen is inhibited by progesterone. The enzyme in the implantation site, however, which is derived from the decidua(4,13,14) is probably a different alkaline enzyme because it is not influenced by estradiol nor depressed by progesterone. In this regard, Manning *et al*(2) failed to detect any change in the enzyme levels in either LT or NLT of rats which could be attributed to an "estrogen surge" anticipated on day 3 of pregnancy(13,14,15,16). In addition, it is doubtful that the estrogen could be responsible for the prolonged and progressive rise in the alkaline phosphatase(2) since a single injection of 17β -estradiol (a short acting estrogen) induces implantation in experimentally delayed rats(15). Based on these results, we would alter our original proposal as follows: estrogen may be a necessary stimulus for nidation but the rise in alkaline phosphatase is dependent on the interaction of the blastocyst with a progesterone-conditioned endometrium. This hypothesis is supported by the finding that pregnant animals which were ovariectomized on day 4 of gestation and maintained on progesterone alone demonstrated the enhanced alkaline phosphatase ac-

tivity and normal implantation sites on the eighth day of pregnancy.

The β -glucuronidase activity was unaltered in all USLT animals with normal 8 day nidation sites (Groups 1, 6, 9 and 10) in agreement with our earlier report(2). Progesterone treatment alone was also without effect, while 17β -estradiol administration to day 1 or day 4 ovariectomized rats increased the glucuronidase $1.3-1.6 \times$. This increase, which has been previously observed in ovariectomized rats treated with estrogens(7,8) was blocked by concomitant administration of progesterone. These findings are interesting in view of the postulated role of β -glucuronidase in steroid metabolism(17,18).

The source of the stimulated acid phosphatase activity at the IS (groups 1, 6, 9 and 10) may be the granulocytes, which become hyperplastic near the nidation sites of pregnant rats(19). A similar cell was described in the monkey endometrium and was found to contain high levels of acid phosphatase(20, 21).

Summary. The influences of ovariectomy and hormone replacement therapy on nidation and on uterine phosphatases and β -glucuronidase were studied on the eighth day after mating in rats with unilateral section and ligation of the fallopian tubes ("USLT" rats). No implantation sites were found in either horn of untreated USLT animals which had been ovariectomized on day 1 or day 4. Implantation did occur in the non-ligated uterine horns of intact and ovariectomized USLT rats when the latter were injected daily with suitable doses of 17β -estradiol and/or progesterone. In all of these animals alkaline phosphatase activity was 10-17 times greater in implantation sites than in inter-implantation areas or in the ligated (non-pregnant) horns. A slight rise in acid phosphatase activity was also observed in implantation sites, whereas β -glucuronidase activity was not influenced by nidation. In ovariectomized USLT animals treated only with the estrogen (not implanted), uterine alkaline phosphatase and β -glucuronidase activities were significantly higher than in similar rats which received progesterone in addition. The data support the view that the alkaline phosphatase in

the implantation sites is not the same enzyme as that which is increased by estrogens.

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Selenium-75: An Autoradiographic Study of its Disposition in Cartilage And Bone.* (32134)

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Both *in vitro* and *in vivo*, L-phenylalanine- C^{14} and L-leucine- C^{14} are incorporated into the protein moieties of the proteinpolysaccharides and collagen of cartilage(1,2). Through the use of these radioactive compounds it was possible to study autoradiographically the turnover of the protein portion of the epiphyseal plate of the rat concurrently with a study of the turnover of the S^{35} -labeled chondroitin sulfate(2). Since Se^{75} derived from $^{75}SeO_4^{=}$ is also fixed to the protein moieties of the proteinpolysaccharides of cartilage rather than to

the chondroitin sulfate(3), it was of interest to study autoradiographically the fate of Se^{75} in rat epiphyseal cartilage and bone and to compare its disposition to that of $^{35}SO_4^{=}$ and the C^{14} -labeled amino acids.

Materials and methods. $Na_2^{75}SeO_4$ was prepared from $Na_2^{75}SeO_3$ (Oak Ridge National Laboratory, Oak Ridge, Tenn.) as described elsewhere(3). Sixteen 10-day old rats were injected intraperitoneally with 50 μc of $Na_2^{75}SeO_4$. After 4, 24, 48, and 72 hours, the rats were killed, and their tibiae and humeri removed. In another type of experiment, slices of costal cartilage from freshly killed calves were incubated for 2 hours at $37^\circ C$ in a solution of salts which contained 50 μc of $Na_2^{75}SeO_4$ (3). After incubation, the slices of cartilage were rinsed with water, a 0.5% solution of Na_2SeO_4 , and

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