The Influence of Estradiol, Progesterone, and Relaxin on the Peroxidase Activity in the Uterus

During earlier investigations[1,2], it was noticed that the peroxidase activity in rat uterus[3,4] varied considerably from one animal to another and also that the pattern depended upon the test method. The peroxidase activity of the pregnant uterus and the effects of estradiol, progesterone, and relaxin on the apparent peroxidase activity in the non-pregnant uterus have therefore been studied.

Material and methods. White mice weighing 24–26 g were used. The uterus was homogenized with water (equal results with 150 *mM* KCl), 1 ml/100 mg, and debris removed by glass wool. The extracts were assayed for nitrogen with the micro-Kjeldahl procedure and for peroxidase with guaiacol[5] at pH 7·1 and 4·9 (GP) and with mesidine[6] at pH 4·9 (MP). The activity is expressed as the inverse of the time needed for a given increase in absorbance, 0·050 with guaiacol and 0·020 with mesidine. A Beckman DU spectrophotometer and 3 ml cells were used.

Six spayed animals were given 1·5 µg estradiol cyclopentyl propionate[7] subcutaneously once five days prior to sacrifice. Another six animals received in addition 2 mg relaxin[8] in 20% polyvinylpyrrolidone[9] 6 h before sacrifice. In a third series, estradiol benzoate[10] (1 µg subcutaneously per day × 3), progesterone[11] (2 mg subcutaneously per day × 3), and relaxin (single subcutaneous injection 6 h before sacrifice) were given as shown in Table I.

The pregnant mice were sacrificed on day 18–20 of gestation.

Tab. I. 'Guaiacol peroxidase' activity in homogenates of uteri from spayed mice, given various combinations of estradiol, progesterone, and relaxin (mean ± SD).

Treatment	Number of animals	Wet weight mg	Nitrogen in extract mg/ml	Activity $1/t_{0.05}$ min^{-1} mg N^{-1}
Estradiol[7]	6[c]	79±8	1·16 ± 0·14	195 ± 63[a]
Estradiol[7] + relaxin	6[c]	109±11	1·20 ± 0·04	166 ± 76[a]
Peanut oil + relaxin	1	20	1·54	0[a]
Estradiol[10]	5	83±6	0·91 ± 0·07	14·2 ± 4·4[b]
Estradiol[10] + progesterone	5	45±1	Not det.	0[b]
Estradiol[10] + relaxin	4	119±21	0·82 ± 0·12	10·4 ± 1·4[b]
Estradiol[10] + progesterone + relaxin	4	62±8	Not det.	0[b]
Peanut oil	4	21±1	Not det.	0[b]

[a] Assayed at pH 7·1. [b] Assayed at pH 4·9. [c] Assayed in pairs.

Results. GP was found in the uterus from spayed animals given estradiol, but the attempts to demonstrate MP in the same homogenates gave negative results (0·2 ml). Only occasionally and after several minutes was a faint pink colour seen in the mesidine test. Controls showed that GP retained part of its activity at the pH and the peroxide concentration used for MP. Extracts from animals given estradiol + progesterone oxidized neither guaiacol nor mesidine. No change in the peroxidase pattern was brought about by relaxin under our experimental conditions.

The MP reaction with homogenates from pregnant uteri was consistently much more intensive than the faint one occasionally seen in estradiol-treated animals (Table II), but the test for GP was consistently negative (0·2 ml, pH 7·1 and 4·9). The MP activity was not caused by a local effect of the placenta.

Tab. II. 'Mesidine peroxidase' homogenates of uteri from pregnant mice. Two organs were used for every homogenate, and 0·05 ml taken for the assay.

Tissue	Nitrogen in homogenate mg/ml	Activity $1/t_{0.020}$ min^{-1}mg N^{-1}
Bilateral pregnancy:		
Whole uterus	0·85	6·5
	0·67	6·3
Placental insertions	1·18	11·5
Residual tissue	1·41	6·9
Unilateral pregnancy:		
Pregnant horns	1·11	10·8
	1·25	9·6
Non-pregnant horns	0·81	10·0
	1·05	6·6

Both GP and MP activities are peroxidatic in nature since no colour appeared unless hydrogen peroxide was added, but there seems to be some reciprocity in their occurrence. GP may be identical with the peroxidase of rapidly proliferating tissues[4]. The nature of MP is at present left open.

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Zusammenfassung

Es wird gezeigt, dass die Uterusperoxydase aus zwei Komponenten besteht, die unter bestimmten Hormoneinwirkungen getrennt auftreten und mit verschiedenen Methoden nachweisbar sind.

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[2] G. Bloom and K. G. Paul, Exper. *15*, 22 (1959).

[3] F. V. Lucas, H. A. Neufeld, J. G. Utterback, A. P. Martin, and E. Stotz, J. biol. Chem. *214*, 775 (1955).

[4] A. P. Martin, H. A. Neufeld, F. V. Lucas, and E. Stotz, J. biol. Chem. *233*, 206 (1958).

[5] A. C. Maehly and B. Chance, Meth. biochem. Analysis *1*, 357 (1954).

[6] K. G. Paul and Y. Avi-Dor, Acta chem. scand. *8*, 649 (1954).

[7] ECP® (Upjohn).

[8] Prepared from pregnant sow's ovaries according to E. H. Frieden and F. L. Hisaw, Arch. Biochem. *29*, 166 (1950) and assaying approximately 30 GPU per mg. We are indepted to Dr. R. L. Kroc, Warner-Chilcott laboratories, for arranging this assay.

[9] Periston® (Bayer).

[10] Ovex® (Leo).

[11] Progestin® (Pharmacia).