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Influence of Graded Levels of Progesterone in Ovariectomized Rats upon Placentomata Formation Measured by Total DNA.* (26659)

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Weichert(1) was first to produce placentomata (deciduomata) reaction in ovariectomized rats. Astwood(2) and Rothchild and Meyer(3) suggested its use in assay of progesterone, whereas Evans *et al.*(4) proposed use of this reaction in assay of luteotropic hormone. Use of DNA in quantitative study of mammary gland growth(5) suggested that a quantitative assay of progesterone or luteotropin might be developed under standardized conditions comparing DNA of normal and traumatized horns of rat uteri. It was thought that studies of the various hormones which might influence growth of the placentomata should be conducted in normal animals before hypophysectomized would be used. Present study concerns influence of graded amounts of progesterone (P) on placentomata formation.

Materials and methods. Sprague-Dawley-Rolfsmeier rats fed Purina Lab Chow were maintained in animal room at $78 \pm 1^\circ\text{F}$ until regular estrous cycles were established. Ovariectomy (OE) was performed when rats showed fully cornified smears. Immediately after operation, progesterone was given at level of 1.5 mg/day from day 1-5 during "pretrauma period"(3). Endometrium of one uterine horn of each animal was trauma-

tized on day 6 by inserting needle through cut tubal end of uterus as far as cervix on this side. By withdrawing needle slantwise, antimesometrial aspect of endometrium was scratched 2 times throughout its entire length by sharp needle point. Immediately after operation, P was given daily at levels of 1, 2, 3, and 6 mg respectively from day 6-9, "post-trauma period"(3), whereas control rats did not receive P during this period. Rats were killed on day 10, 24 hours after last injection. Pituitaries, adrenals and uteri were removed rapidly, transferred to ice-cold vials, weighed on Roller Smith balance and put in deep freezer. Uteri were divided before weighing into control and traumatized horn and processed for DNA(6). Traumatized horns were cut in pieces, washed in alcohol before starting alcohol and ether extraction in order to obtain dry-fat-free tissue (DFFT).

Results. P in graded doses stimulated a graded increase in mean total DNA in the traumatized uterine horns. One mg P increased total DNA of traumatized horn markedly as compared to rats which did not receive P during post-trauma period. Six mg P did not further increase total DNA of traumatized horn as compared to 3 mg group (Table I). Decrease of DNA/mg DFFT took place in all groups. Nine of 23 rats receiving no P during post-trauma period had placentoma reactions better than group average of 7% increase of traumatized horn over control horn. A few rats did not show reactions under influence of P. Per cent increase of wet weight, DFFT and DNA of trauma-

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TABLE I. Effect of Graded Levels of Progesterone on Placentoma Formation after Traumatization of One Uterine Horn in Ovariectomized Rats.

Treat- ment*	No. of rats Without Total reaction†	Avg wt of uteri					DNA/mg DFFT horn				
		Body wt, g		Wet wt/100 g horn		DFFT/100 g horn	DNA/100 g horn		Decrease, %	Trauma, mg	Trauma, mg
		Day 1	Day 10	Control, mg	Trauma, mg		Control, mg	Trauma, mg			
None	23	14	236	240							
1	30	4	226	232	42 ± 2	70 ± 5	8.0 ± 4	11 ± 1.7	21 ± 2.2	.42 ± .012	.46 ± .024
2	27	1	224	228	52 ± 2	167 ± 12	9.6 ± 1.2	24 ± 1.6	27 ± 2.2	.46 ± .014	.84 ± .050
3	25	1	223	231	53 ± 3	189 ± 21	9.2 ± .6	27 ± 2.8	27 ± 2.1	.48 ± .033	.96 ± .072
6	19		238	246	52 ± 2	238 ± 32	9.5 ± .4	32 ± 3.0	25 ± 2.1	.49 ± .018	1.20 ± .115
					55 ± 3	244 ± 25	9.7 ± .5	34 ± 2.6	28 ± 3.3	.46 ± .021	1.11 ± .078

DFFT = dry, fat-free tissue.

P = progesterone.

* Pretrauma period: All rats, day 1-5, 1.5 mg P/day. Post-trauma period: Day 6-9, mg P/day.

† When traumatized horn showed less than 7% increase of total DNA as compared to control horn.

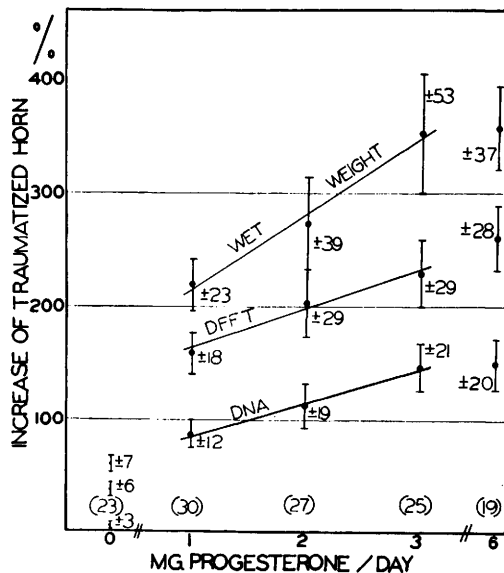


FIG. 1. Percentage weight increase of traumatized uterine horn over control horn under influence of graded levels of progesterone in ovariectomized rats. Number in parenthesis indicates number of animals.

tized horn over control horn is correlated with amount of P given during post-trauma period (Fig. 1). With exception of DFFT in 6 mg group, this level did not further improve reaction. Because of lack of uniformity of placentoma formation, standard deviations are extremely high. Body weight was not affected adversely in spite of 2 operations on day 1 and 6. Pituitary and adrenal weights remained within normal limits.

Discussion. Data presented is first quantitative evidence that graded amounts of P up to 3 mg/day are correlated with extent of placentoma formation in OE rats confirming previous studies based on qualitative data (2). A graded increase of wet weight, DFFT, and total DNA was observed up to 3 mg P. Similarly, the percentage weight increase of traumatized horn over control horn was shown (Fig. 1). It has been reported(7) that polyploidy occurs in growth of placentomata and that these cells cannot reproduce normally. It will be noted, however, that DNA/mg DFFT shows a decrease in every case in traumatized horns as compared to control horns. Since DNA is a nuclear constituent, one would expect DNA to increase/mg rather than decrease if it were to occur

in large numbers unless cytoplasmic volume of polyploid cells increased at a rate faster than in normal cells. To extent that polyploidy does occur, total DNA/100 g would not represent true cell multiplication but rather nuclear multiplication. Since extremely great variations in placentoma reaction were observed in all groups, large number of animals will be required if this technic should be used to develop an assay method. It should be noted that in a few animals given P no reaction occurred whereas increases up to 47% in total DNA occurred in traumatized horn over control horn when no P was given during post-trauma period.

Summary. Sprague-Dawley-Rolfsmeier rats were ovariectomized in estrus, then administered progesterone (P) at rate of 1.5 mg/day for 5 days (pretrauma period). Endometrium of one uterine horn of each animal was scratched throughout its entire length by sharp needle. During post-trauma period, days 6 to 9, P at levels of 1, 2, 3, and 6 mg was injected daily. On day 10 each uterus was separated into control and trau-

matized horn, weighed and processed for DNA determination. The traumatized horns showed a graded increase in wet weight, DFFT, and DNA/100 g B.W. over control horns when 1, 2, and 3 mg/day of P were administered during post-trauma period. Six mg/day did not increase reaction further. Considerable variation in reaction occurred in each group requiring a considerable number of animals for use as assay method.

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Influence of Progesterone, Estradiol Benzoate, and Thyroxine in Ovariectomized Rats upon Placentomata Formation Measured by Total DNA.* (26660)

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A procedure was previously described by which a quantitative estimation of placentomata reaction might be made using DNA as an index(1). During posttrauma period increasing levels of progesterone (P) (1 to 6 mg) were injected. The DNA of traumatized horn was then compared with control

horn. It was shown that 3 mg/day of P had optimal effect. In a study of growth of mammary gland of rat, it was shown that estradiol benzoate (EB) at level of 1 μ g/day synergized with P at level of 3 mg/day to promote optimal growth as measured by DNA (2). The present study was conducted to determine if this level would synergize with P to promote placentomata reaction.

In the strain of rats used, the thyroxine secretion rate (TSR) has been determined. TSR varies from 0.25 μ g to 2.0 μ g/100 g BW with mean of about 1.0 μ g. When 3.0 μ g/100 g BW of L, thyroxine (T_4) was administered with 1 μ g EB + 3 mg P, T_4 was with-

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