Influence of Lactogenic Hormone and Estradiol Benzoate upon Placentoma Formation in Intact Rats Measured by Total DNA.* (26894)

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Data on influence of progesterone in stimulating placentoma formation in ovariectomized rat as measured by total DNA have been presented(1). Further, the synergistic influence of estradiol benzoate and thyroxine was observed(2). In normal estrous rat, hormone which would maintain secretory activity of the corpus luteum (progesterone) and produce placentoma should be considered luteotropic. The present study is concerned with the influence of lactogenic hormone (LGH), estradiol benzoate (EB) and combinations of the two upon placentoma formation in intact rat as measured by DNA in order to determine their luteotropic action.

Material and methods. Sprague-Dawley-Rolfsmeyer rats fed Purina Lab Chow were maintained in animal room at 78 ± 1°F until regular estrous cycles were established. When rats showed full estrous smears, 1 mg/day of lactogenic hormone (LGH) was injected for 5 days (pretrauma period). On day 6, one uterine horn of each animal was traumatized as described(1). During post-trauma period, days 6-9, groups received treatment indicated (Table I). On day 10 uteri were separated and processed for DNA (3). Ovine lactogen containing 15 IU/mg was used. Contamination by ACTH, TSH, FSH, LH, and growth hormone was believed to be minimal.

Results. In this study rats which responded by placentoma formation were separated from those not responding. Without treatment during the post-trauma period, 1 of 8 rats responded (Table I). When 1 μg EB was administered, only 1 of 7 rats responded, indicating that EB at 1 μg level has no influence in stimulating placentoma formation.

Lactogenic hormone at each of 2 levels, 1 or 2 mg (15 or 30 IU) stimulated placentoma formation in 5 of 17 rats. In those which responded, DNA/100 g BW of traumatized horns showed significant increases over control horns with 2 mg level exceeding 1 mg level.

When 0.5 μg EB was added to 1 mg LGH, only 1 of 6 rats responded and when EB was increased to 1 μg, 6 of 16 rats showed placentoma formation. Addition of EB to 1 mg LGH increased neither % showing placentoma nor mean DNA/100 g BW of traumatized horns.

In all cases where EB was administered, wet and dry weight of control horns was increased in comparison with those not receiving estrogen. DNA/mg DFFT in traumatized horns was always less than in control horns.

Discussion. In previous experiments, it was shown that administration of 3 mg progesterone (P)/day stimulated maximum placentoma formation as measured by total DNA/100 g BW in traumatized horn of uterus of ovariectomized rats(1). A further increase in DNA/100 g BW in traumatized horn was observed by synergism of P, 6 mg, + EB, 2 μg, + thyroxine, 3 μg/100 g BW (2). The same levels of these 3 hormones have been shown to stimulate increased growth of mammary gland measured by DNA(4). These observations further confirm the suggestion that placentoma formation is primarily influenced by P and that other hormones acted synergically.

In present experiment, normal rats were used to determine what hormones might stimulate P secretion by ovary, and P so pro-

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TABLE I. Effect of Graded Levels of Lactogenic Hormone and Estradiol Benzoate on Placentoma Formation after Traumatization of One Uterine Horn of Intact Rats.

Treatment*	No. of rats: No reaction	No. of rats: Reaction	Wet wt/100 g horn: Control, mg	Wet wt/100 g horn: Trauma, mg	Wet wt/100 g horn: Increase, %	DFFT/100 g horn: Control, mg	DFFT/100 g horn: Trauma, mg	DFFT/100 g horn: Increase, %
None	7		66 ± 5	83 ± 7	27 ± 8	11.8 ± .8	14 ± 1.2	16 ± 5
		1	48	559	1,072	9.3	75	705
EB: 1 μg	6		112 ± .9	151 ± 14	38 ± 12	18.8 ±1.3	22 ± 1.6	16 ± 9
		1	92	284	209	15.6	42	169
LGH: 1 mg	12		59 ± 3	79 ± 4	37 ± 6	10.9 ± .6	14 ± .1	27 ± 5
		5	50 ± 1	387 ± 70	673 ±148	10.0 ± .4	52 ± 8.4	411 ±71
LGH: 2 mg	12		63 ± 5	90 ± 7	44 ± 8	11.7 ± .9	14 ± 1.1	24 ± 5
		5	52 ± 4	495 ± 66	860 ±126	10.2 ± .9	68 ± 9.0	583 ±94
EB: .5 μg LGH: 1 mg	5		107 ±21	140 ± 22	35 ± 10	17.8 ±3.4	21 ± 2.9	19 ± 5
		1	99	585	500	16.9	83	391
EB: 1 μg LGH: 1 mg	10		94 ± 8	120 ± 9	32 ± 7	15.2 ±1.2	18 ± 1.2	17 ± 5
		6	92 ± 6	376 ± 50	332 ± 88	15.5 ±1.2	53 ± 7.1	263 ±74

Treatment*	No. of rats: No reaction	No. of rats: Reaction	DNA/mg DFFT horn: Control, μg	DNA/mg DFFT horn: Trauma, μg	DNA/mg DFFT horn: Decrease, %	DNA/100 g horn: Control, mg	DNA/100 g horn: Trauma, mg	DNA/100 g horn: Increase or decrease, %
None	7		42 ± 2.6	34 ± 2.0	17 ± 2.0	.48 ± .026	.47 ±.039	−3 ± .92
		1	56	23	60	.52	1.70	226
EB: 1 μg	6		29 ± 1.1	28 ± 1.5	4 ± 2.5	.54 ± .042	.61 ±.070	12 ± 9.41
		1	28	25	13	.44	1.04	134
LGH: 1 mg	12		45 ± 1.7	36 ± .9	18 ± 2.6	.48 ± .026	.49 ±.024	4 ± 4.54
		5	44 ± 1.9	29 ± 1.3	34 ± 1.0	.44 ± .024	1.48 ±.206	237 ±42.80
LGH: 2 mg	12		45 ± 1.6	32 ± 1.1	28 ± 3.6	.53 ± .045	.46 ±.044	−10 ± .65
		5	45 ± 4.9	26 ± 1.4	41 ± 5.0	.44 ± .044	1.75 ±.201	313 ±58.5
EB: .5 μg LGH: 1 mg	5		31 ± .5	26 ± .6	15 ± 3.5	.55 ± .102	.54 ±.076	2 ± 7.98
		1	47	30	38	.800	2.45	206
EB: 1 μg LGH: 1 mg	10		37 ± .6	33 ± .8	10 ± 3.0	.56 ± .048	.58 ±.044	6 ± .56
		6	33 ± .9	27 ± .6	18 ± 2.7	.51 ± .038	1.42 ±.191	195 ±59.60

LGH = Lactogenic hormone. EB = Estradiol benzoate. DFFT = Dry fat-free tissue.

* Pretrauma period: All rats daily, day 1-5 (6), 1 mg LGH. Posttrauma period: Daily, day 6-9 (7-10).

duced in turn stimulate placentoma formation. Since P showed a graded response measured by DNA, it was thought that level of P secretion might be measured.

It has been suggested that estrogen directly or indirectly *via* pituitary and luteotropic hormone secretion would maintain corpora lutea and P secretion. In present experiment 1 μg EB failed to stimulate placentoma formation.

Evans *et al.*(5,6) were first to present evidence indicating that of various pituitary preparations, their lactogenic preparation containing 30 IU/mg at 1 mg level produced 4 responses in 4 animals and 2 responses in 4 animals at .5 mg level. These observations led to the suggestion that lactogenic and luteotropic hormones were identical.

Present experiment indicates that 1 or 2 mg containing 15 or 30 IU lactogenic hormone stimulated placentoma reaction in about ⅓ of animals (5 of 17), but larger amount increased DNA/100 g to greater extent than smaller dose. The DNA/100 g BW of those responding to higher level of lactogen exceeded DNA of highest level of P administered.

The lactogenic preparation used has been shown by Ferguson and Wallace(7) to contain 17 components by starch gel zone electrophoresis with 3 components making up 70, 22, and 1% of preparation. Pigeon assays indicated 21, 2, and 105 IU/mg lactogen present in these preparations, respectively.

While lactogenic preparation used stimulated marked placentoma formation in about

⅓ of rats, in the light of above observations, authors believe it is premature to identify luteotropic action with lactogen.

It may be pointed out, also, that 1 μg EB used in present experiment stimulated increase in lactogen in 5 days equal to peak level observed on day 16 of pregnancy(8), yet EB had no effect on placentoma formation. If lactogen is luteotropic hormone, it is strange that its concentration is low in early pregnancy when its need for maintenance and secretory activity of corpora is great.

Summary. Sprague - Dawley - Rolfsmeyer rats showing full estrous smears were administered 1 mg/day of ovine lactogen containing 15 IU/mg for 5 days. On day 6, one uterine horn was traumatized and 1 μg EB and 1 and 2 mg lactogen (LGH) were administered on days 6 to 9. On day 10, horns of uteri were separated and processed for DNA. EB had no influence on placentoma formation; whereas, both levels of LGH stimulated placentoma in ⅓ of rats with graded reaction as measured by DNA. EB, .5 μg or 1 μg, with 1 mg LGH improved neither % response nor total DNA in comparison with LGH alone. While lactogenic preparation stimulated placentoma formation, reasons are advanced why it is premature to identify luteotropic action with lactogen.

1. von Berswordt-Wallrabe, R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., submitted.

2. ———, *ibid.*, submitted.

3. Webb, J. M., Levy, H. B., *J. Biol. Chem.*, 1955, v107, 213.

4. Moon, R. C., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 536.

5. Evans, H. M., Simpson, M. E., Lyons, W. R., *ibid.*, 1941, v46, 586.

6. Evans, H. M., Simpson, M. E., Lyons, W. R., Turpeinen, K., *Endocrinology,* 1941, v28, 933.

7. Ferguson, K. A., Wallace, A. L. C., *Acta Endo.*, 1960, v35, suppl. 51, 293.

8. Grosvenor, C. E., Turner, C. W., *Endocrinology,* 1960, v66, 96.

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Plasma Cholesterol Concentrations in Cockerels and Dogs Treated with Bile Acid Binding Polymer and Cholesterol Synthesis Inhibitors. (26895)

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Plasma cholesterol concentrations in animals and men can be lowered by feeding the bile acid binding polymer, cholestyramine resin (MK-135)†(1,2), or the hepatic cholesterol synthesis inhibitors, benzmalecene† (3,4,5,6), and triparanol (MER-29)†(7,8). In men the average maximum blood cholesterol lowering achieved with practical doses of these compounds is about 20%. Larger doses are either inconvenient, or produce no greater effect. Bile acid binders and synthesis inhibitors presumably lower cholesterol levels by different mechanisms, and thus combinations of the 2 might produce greater cholesterol lowering than can be attained with either alone. The present work is a study of the action of combinations of cholestyramine resin with benzmalecene and with triparanol in normocholesterolemic cockerels and dogs.

Methods. Cholesterol was determined by the method of Abell *et al.*(9) in plasma prepared by mixing 2.0 ml of whole blood with 0.35 ml of citrate-dextrose (ACD) solution. (Serum cholesterol concentrations may be

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† Cholestyramine resin is the generic name for a bile acid binding quaternary ammonium anion exchange resin with styrene-divinylbenzene skeleton. Benzmalecene is the generic name for N-(1-methyl-2,3-di-p-chlorophenylpropyl)-maleamic acid (α-isomer). Both are experimental developments of Merck Sharp & Dohme Research Laboratories, Rahway, N. J., and West Point, Pa. Triparanol is the generic name for 1- [(4-diethylaminoethoxy)phenyl] -1-(p-tolyl)-2-(p-chlorophenyl)ethanol, a product of Wm. S. Merrell Co. Cincinnati, Ohio.