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Estrogens and Transport of Ova in the Rat.* (29441)

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The effectiveness of estrogens in interrupting pregnancy in the rat and mouse was first demonstrated by Parkes and Bellerby(1) and Smith(2). Later Burdick and his associates (3,4,5,6) reported that large amounts of estrogens accelerated ova transport while small amounts caused retention of ova in the Fallopian tubes where they ultimately degenerated. Dreisbach(7) has been successful in interrupting pregnancy in rats with a single subcutaneous injection of 0.4 mg/kg estrone up to the eleventh day post coitum (p.c.) He has further shown that as little as 20 μg/kg was effective during the period of tubal transport, while 100 μg/kg was necessary to prevent implantation after the ovum was free in the uterus. Edgren and Shipley(8) found that by single injection estrone was most effective in interrupting pregnancy in badger rats on day 3 of gestation (ED50 = 4.2 μg, compared with 13 μg on day 1) whereas from day 7 on, doses up to 160 μg were ineffective. Greenwald(9) reported that the effect of 10 μg estradiol cyclopentyl propionate (ECP) administered to rat on day 1 was to increase tubal and uterine motility so that the fertilized ova were expelled from the uterus by 48 hours p.c. The effectiveness of 5 μg of ECP in accelerating ovum transport varied from animal to animal.

In the rabbit, Pincus and Kirsch(10) found ova retained in the Fallopian tubes after administering estrone and related compounds during preimplantation stage. Noyes *et al*(11) lost many transferred rabbit eggs in estradiol-treated hosts, 23% passing into the vagina 10 to 78 hours after transfer. Black and Asdell(12) gave 2.5 mg estradiol to mated rabbits, but do not record, in contrast, any retention of eggs in the Fallopian tubes. Greenwald(13) reported that ovum transport in the rabbit is accelerated with 25 μg ECP whereas with 250 μg ECP retains ova at the ampullary-isthmic junction for as long as 5 days. These results were confirmed in a subsequent study(14) which indicated that the mechanism of interruption of pregnancy in rabbits by ECP depended on the dose level of the hormone.

Deanesly(15) observed the expulsion of guinea pig eggs from the Fallopian tubes when a very small quantity of estradiol benzoate is given immediately after mating. She has further shown that once the fertilized eggs have passed normally into the uterus, implantation is not prevented by as much as 30 or 50 μg of estradiol benzoate in the guinea pig, but post implantation development may be affected.

In this paper we describe the effects upon implantation and ovum transport in the rat (Sprague-Dawley) of estrone administered before implantation. The methods of pregnancy timing, of hormone administered and implant determination are those described previously(16).

Estrone and implantation. Various dosages of estrone (E_1) in 0.1 ml sesame oil were injected subcutaneously during the preimplantation stage (day 1 through 3) as indi-

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TABLE I. Estrone (E_1) Dosage and Implantation of Fertilized Eggs in Rats.

Total dose of E_1 (μg)	Day of injection	No. rats	No. pregnant	Mean No. of implantations
Oil control	1	5	4	10.7
1.0	1	3	2	14.5
10.0	1	4	2	4.5
10.0	3	5	1	9.0
15.0	1	7	2	3.0
20.0	1	5	0	.0

cated in Table I. Day 1 is ascertained by finding sperm or plug in vaginal swab. All animals were sacrificed on the 9th or 10th day after mating, and the implantation sites and number of implanted fetuses were observed.

The data demonstrate a reduction in number of pregnant animals and in implanted fetuses with as little as 10 μg/rat of estrone given on day 1 or day 3. With 20 μg/rat, all 5 animals lost their conceptus.

Effects of estrone (E_1) on tubal transport of fertilized eggs. Various doses of E_1 were administered as before at different times after establishment of pregnancy. Eggs were flushed and collected from the Fallopian tubes and also from the uterine horns a few hours or days after injection. Results are presented in Table II. It has been our experience with large numbers of pregnant rats that fertilized eggs do not pass on to the uterine horns until day 5 of pregnancy. The data presented in Table II show that as much as 20 μg/rat of E_1 administered on day 1 expels the eggs from the tube to the uterus (Group VI) in about 20-24 hours on day 2 when they should normally be in the tubes, after which they probably pass out of the reproductive tract (Groups VIII and IX). Further data (Groups VII and X) show that tubal eggs do not pass on to the uterine horns within 15 hours after E_1 administration. Data presented in Group XI do not, however, give any evidence that a large quantity of E_1 (250 μg/rat) blocks the fertilized eggs at the utero-tubal junction. We could not recover a single egg on day 4 either from the tube or utero-tubal junction or from the uterus of rats given either 20 μg or 250 μg of E_1 on day 1 or day 3.

Attempted inhibition of estrone action on ovum transport. In view of the demonstrated activity of E_1 on implantation (Table I) and on the tubal transport of eggs (Table II), progestin counteraction has been attempted. The data of a number of such experiments are presented in Table III. The effect of a standard dose of E_1 (20 μg/rat) is seen in control animals (Group I). It is evident from these results (Groups II through VII) that as much as 20 mg of progesterone, 3 mg of 2 very potent synthetic progestins could not reverse the action of E_1. In standard assay in the rabbit, the progestational activity of these 2 synthetic progestins, mentioned in Groups IV and VI, has been estimated to be about 20 times more potent than standard progesterone(17).

Summary. Inhibition of implantation in the rat may be partially accomplished by

TABLE II. Effects of Estrone (E_1) on Movement of Rat Ova.

Group No.	Total quantity E_1 (μg)	Day of injection	Day of ovum collection	No. rats	Total No. eggs Tubal	Uterine	Remarks
I	Oil control	1	4	8	80	0	Normal
II	" "	1	2	5	55	0	"
III	" "	1	5	6	0	61	"
IV	1.0	1	4	3	33	0	"
V	10.0	1	4	3	20	0	"
VI	20.0	1	2*	10	6	10	Movement accelerated
VII	20.0	1	2†	8	60	0	*In situ*
VIII	"	1	4	5	0	0	Expelled
IX	"	1	3	5	0	0	"
X	100.0	2	2‡	4	41	0	*In situ*
XI	250.0	1	4	6	0	0	Expelled
XII	20.0	3	4§	7	0	0	"

* 20-24 hr after E_1 inj. † 12-15 hr after E_1 inj. ‡ 6 hr after E_1 inj. § 24 hr after E_1 inj.

TABLE III. Attempts to Reverse the Implantation-Inhibiting Action of Estrone.*

Group No.	Compound	Dose (mg)	No. of rats	No. pregnant
I	Oil control	.1 ml	5	0
II	Progesterone	5 mg/day for 4 days from day 1	2	0
III	"	5 mg/day for 3 days from day 4†	6	0
IV	Synthetic progestin‡	1.0 mg on day 1	5	0
V	" "	1.0 mg/day for 3 days from day 1	5	0
VI	Synthetic protestin§	1.0 mg on day 1	5	0
VII	" "	1.0 mg/day for 3 days from day 1	4	0

* E_1 was administered (20 μg/rat) on day 1 of pregnancy.
† E_1 (20 μg/rat) given on day 4.
‡ 1, 2α-Methylene-6-chloro-6-dehydro-17α-hydroxyprogesterone-17-acetate.
§ 3β, 17α-Diacetoxy-6α-methylpregn-4-3en-20-one.

a single dose of 10 or 15 μg of estrone per rat administered on day 1 of pregnancy; a single dose of 20 μg per rat appears to cause complete inhibition. Doses of 20 μg per rat or higher cause a premature passage of tubal ova into the uterus with later expulsion from the uterus. This loss of ova and consequent lack of implantation cannot be counteracted by daily preimplantation doses of 5 mg progesterone, or 1 mg of the two synthetic potent progestins:

(1) 1,2α-methylene-6-chloro-6-dehydro-17α-hydroxyprogesterone-17-acetate;

(2) 3β-17α-diacetoxy-6α-methylpregn-4-en-20-one.

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Hypercoagulability and Thrombosis: Effect of Injected Thrombokinase and Adenosine Diphosphate on Established Microthrombi in Rabbits.* (29442)

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There has been an increasing tendency to implicate blood hypercoagulability in the pathogenesis of thrombotic disease. Although intravascular clots have been produced by a combination of increased blood coagulability and stasis, as in the studies of Wessler (1), there have been no reports claiming the production of structured thrombi similar to those found in human disease(2) resulting from alterations of the blood hemostatic components alone.

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