BRIEF COMMUNICATION

A METHOD FOR THE DIRECT OBSERVATION OF
OVULATION INHIBITION IN THE MATURE RAT

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Present methods used for the detection of ovulation in the mature rat are time-consuming and often unreliable, but direct observation of the ovulatory follicles and corpora lutea on the ovary is complicated in the rat by the short oestrous cycle and persistent corpora lutea from previous cycles (Pl. 1, Fig. 1). Harper (1964) described a method for the identification of mature functional corpora lutea in the rat but this is of little use for the determination of ovulation occurring during prolonged administration of possible anti-ovulation compounds since, in this method, it is impossible to assess the age of the observed corpora lutea. Holmes & Mandl (1962), by serially sectioning ovaries and measuring relative areas of follicles and corpora lutea, obtained an index for ovarian activity which is very accurate but extremely time-consuming. Greep & Jones (1950) sectioned ovaries and determined the age and activity of the corpora lutea histochemically.

The procedure used by Long & Evans (1922) for investigating the age of corpora lutea in the rat by injection of vital dye, has now been adapted to detect the occurrence of ovulation by direct observation of the ovarian surface.

The compounds are administered daily for 15 days successively to mature female rats. On each of the 5th to 8th days inclusive, 3 ml of a 1% filtered solution of Dianil Blue '2R' (G. T. Gurr Ltd) is injected intraperitoneally, all corpora lutea formed during this time or already present thereby being stained blue. The animals are killed on the 16th day when the presence of colourless or pink corpora lutea on the ovaries indicates that ovulation has occurred during the 9th to 15th days (Pl. 1, Fig. 2). A wholly blue ovary demonstrates that ovulation has been prevented (Pl. 1, Fig. 3). This is because the dye is incorporated into the lipid granules of mature corpora lutea and not into corpora lutea developing after administration of the dye.

Ethinyl oestradiol (3:17 β -dihydroxy-17 α -ethinyl-oestra-1:3:5(10)-triene) and megestrol acetate (17 α -acetoxy-6-methylpregna-4:6-diene-3:20 dione) have been used to demonstrate the effectiveness of the method for the detection of inhibition of ovulation by a synthetic oestrogen and progestin respectively. Mature specific-pathogen free rats derived from the Sprague-Dawley strain were dosed orally with varying doses suspended in an aqueous medium (sodium carboxymethyl cellulose) for 15 days. The administration of the vital dye produced a marked diarrhoea in those animals given high doses of ethinyl

oestradiol, often leading to death. This diarrhoea proved highly contagious. Examination of the faeces of affected animals revealed an excessive growth of the normal commensal bacteria of the gut. As a prophylactic, aureomycin (50 mg/l) (Cyanamid Ltd) was added to the drinking water of the rats, starting 1 week before and continuing throughout the time of experiment. This treatment was completely successful. The rats dosed with megestrol acetate or the dose-suspending vehicle alone did not develop these effects if completely segregated from those dosed with ethinyl oestradiol. The contagious nature

TABLE 1
EFFECT OF ETHINYL OESTRADIOL AND MEGESTROL
ACETATE ON OVULATION IN THE MATURE RAT

<i>Compound</i>	<i>Dose (mg/kg/day)</i>	<i>% rats ovulating*</i>	<i>Mean No. of ovulations/ rat ovulating</i>
Ethinyl oestradiol	0.1	60	5
	0.3	10	6
	1.0	0	0
Megestrol acetate	3	40	7
	9	20	10
	27	0	0
Aqueous medium		80	14

* No. of rats/group = 10.

TABLE 2
EFFECT OF ETHINYL OESTRADIOL AND MEGESTROL
ACETATE ON PREGNANCY IN THE MATURE RAT

<i>Compound</i>	<i>Dose (mg/kg/day)</i>	<i>% rats pregnant*</i>	<i>Mean No. of foetuses/ pregnant rat</i>
Ethinyl oestradiol	0.1	50	10
	0.3	10	3
	1.0	0	0
Megestrol acetate	3	20	5
	6	20	4
	12	0	0
Aqueous medium	—	80	9

* No. of rats/group = 10.

of the increased bacterial growth led to the inclusion of the aureomycin in the drinking water as a standard procedure in the experimental method.

The effective doses for completely preventing ovulation with ethinyl oestradiol and megestrol acetate were 1 and 27 mg/kg/day respectively (Table 1).

The overall effect of these steroids on all the stages of reproduction up to and including fertilization has also been examined. Female SPF rats were given various doses of each steroid orally for 7 days consecutively. On the morning of the 8th day, two males were put into each cage of five females, daily dosing

PLATE I

FIG. 1. Unstained ovary from normally cycling 8-week-old rat. $\times 8$.

FIG. 2. Ovary from normally cycling 8-week-old rat killed 7 days after receiving four concurrent intraperitoneal injections of 3 ml of 1% Dianil Blue 2 R. $\times 8$.

FIG. 3. Ovary from 8-week-old rat which has received fourteen daily oral doses of 27 mg/kg/day of megestrol acetate and four daily intraperitoneal injections of 3 ml of 1% Dianil Blue 2 R over the 5th to 8th days of the oral dosing. $\times 8$.

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being continued until the animals mated, as determined by the presence of spermatozoa in the vaginal smear, or for a further 7 days, whichever was the shorter period. Females remaining unmated after 7 days with the male were not used further in the experiment. Mated females were removed from the males immediately after detection and were killed 10 days later and the number of live foetuses present *in utero* were counted.

The values obtained for the detection of ovulation inhibition using the intraperitoneal dye method and those obtained in the studies on inhibition of pregnancy by dosing before fertilization are complementary for ethinyl oestradiol. Complete inhibition of ovulation or pregnancy is obtained at the same dose level. The apparent difference in effective doses obtained with megestrol acetate is probably an artefact due to different dose ratios. However, dissimilarities are present below the effective doses and these may indicate additional mechanisms involved, e.g. changes in secretion of the reproductive tract.

The new method for directly observing ovulation in the rat should greatly assist in the examination of synthetic compounds for fertility control.

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