THE EFFECTS OF OESTROGENS AND ANTI-OESTROGENS ON OVUM TRANSPORT IN THE LABORATORY MOUSE

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Summary

Suitable doses of the anti-oestrogens DMS, MER 25, and MRL 37 given to mice on the first 3 days after mating caused retention in the oviduct of all ova (predominantly in the ampulla) until at least day 4, and prevented implantation but did not cause the loss of many ova. MER 25 caused tube-locking of ova in only about 50% of mice, but implantation did not occur although apparently normal blastocysts were present in the uterus on day 4. DMS, the most oestrogenic of the three compounds, markedly reduced the recovery of ova after day 4. The blastocoele in the oviducal ova found in control and treated mice on day 4 was not as well developed as in uterine ova.

The anti-oestrogens did not reverse the tube-locking produced by low doses of oestradiol, but themselves caused retention of some ova in the oviduct. Progesterone did not prevent the tube-locking found in mice given oestradiol or an anti-oestrogen. The three anti-oestrogens are oestrogenic in some vaginal assays, and in their effects on early pregnancy, and it is believed that the effects of these substances on tubal transport are probably due to inherent oestrogenicity, and not to the antagonism of endogenous oestrogens.

I. Introduction

In many laboratory animals the rate of passage of ova through the oviduct is greatly modified by treatment with oestrogens and anti-oestrogens immediately after mating (Greenwald 1957, 1963; Chang 1964, 1966; Chang and Yanagimachi 1965 in the rabbit; Greenwald 1961, 1965, 1967; Banik and Pincus 1964 in the rat; Whitney and Burdick 1936; Burdick, Emerson, and Whitney 1940; Greenwald 1967; Humphrey 1968*a*; Humphrey and Martin 1968 in the mouse). Implantation is prevented since the zygotes are either lost from the reproductive tract or retained in the oviduct for long periods. Chang and Yanagimachi (1965) and Greenwald (1965) noted that the disturbances of tubal transport produced by anti-oestrogenic compounds in the rabbit and rat respectively are similar to those produced by oestrogens.

Oestra-3,17β-diol has multiple effects on tubal transport in the mouse — treatment on day 1 only causes tube-locking of all ova, predominantly in the ampulla, treatment on day 2 causes accelerated transport of ova from the isthmus to the uterus and vagina, whilst multiple injections on days 1–3 cause both tube-locking in the ampulla, and accelerated transport and loss of about 60% of the ova

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(Humphrey 1968*a*). Tube-locking is due to prolonged closure of the ampulla–isthmus junction, while the loss of ova is apparently due to increased motility of the isthmus and uterine musculature. In an earlier paper (Humphrey and Martin 1968) it was shown that in the mouse the anti-oestrogens dimethylstilboestrol (DMS), 1-[*p*-(2-*N*,*N*-diethylaminoethoxy)phenyl]-2-(*p*-methoxyphenyl)-1-phenylethanol (MER 25), and 1-[*p*-(2-*N*,*N*-diethylaminoethoxy)phenyl]-2-(*p*-methoxyphenyl)-1-phenylethane (MRL 37) have a similar effect to oestradiol in causing retention of many zygotes in the oviduct but did not cause loss of ova. The present experiments were an extension of that work to investigate this tube-locking action, and to see if it is an oestrogenic effect.

II. Materials and Methods

General conditions and procedures were as described by Humphrey (1968*a*) and Humphrey and Martin (1968). Pregnant QS mice were grouped in boxes, and randomized within each box, one to each experimental group. In all experiments the compounds, dissolved in peanut oil or dimethylsulphoxide, or the vehicle only in control mice were given by subcutaneous injections at noon on days 1, 2, and 3 of pregnancy or part thereof as outlined later. The day of finding the copulatory plug was called day 1 of pregnancy.

To determine the compounds' overall antifertility effects, mice were killed on day 12, examined for implants, and the numbers pregnant analysed by χ^2 tests (corrected) in a 2×2 table. In determining the effects upon tubal transport, mice were killed on the afternoons of days 4, 5, 6, or 7 and the oviducts and uterine horns separately flushed for ova with Krebs–Ringer phosphate buffer containing 1% bovine serum albumin. The ova in the washings were counted and examined with phase-contrast lighting to establish normality, noting the size of the blastocoele. The ovaries were examined microscopically and the corpora lutea counted. The numbers of ova lost from the tract were calculated from the differences between corpus luteum counts and the total recovery of ova for each mouse. The anti-oestrogens have some luteolytic actions (see Section III) and ovum loss was also estimated from the differences between the numbers of ova recovered in treated mice and the mean corpus luteum count of control mice.

Experiments were performed as outlined in the following schedule:

(1) Mice received varying doses of anti-oestrogens or the vehicle only on days 1–3, and were killed either on day 4, when the tracts were flushed for ova, or on day 12 when the uteri were examined for implants.

(2) The location of ova in the oviducts on day 4 was determined by examination either of histological sections or of straightened, compressed oviducts, as described by Humphrey (1968*a*).

(3) The location of ova in the ampulla on day 4 (see Section III) may be due to prolonged closure of the ampulla–isthmus junction from day 1 to 4, or to retrograde movement of ova from the isthmus to the ampulla on days 2 or 3. This was investigated in an experiment in which mice received 1·0 mg/day of MRL 37 in dimethylsulphoxide or the vehicle only on days 1–3 or part thereof and were killed on days 2, 3, or 4. The location of the ova in the compressed oviducts was determined.

(4) In an attempt to find the duration of tube-locking, mice were given daily injections of an anti-oestrogen or oil only on days 1–3 and were killed on day 5, 6, or 7 and the tract flushed.

(5) Since the anti-oestrogens were luteolytic in some mice, attempts were made to reverse this effect on tubal transport by concurrent administration of progesterone. Mice received daily subcutaneous injections on days 1–3 of an anti-oestrogen or oestradiol and of progesterone or peanut oil at the dose levels shown in Table 5. After killing the mice on day 4, the tracts were flushed for ova.

(6) The possibilities that the anti-oestrogens could prevent oestradiol-induced tube-locking, or that these substances and oestradiol acted by common mechanisms were then investigated. On days 1–3, mice received daily separate simultaneous injections of the vehicles only or doses of oestradiol and either DMS, MRL 37, or MER 25 as shown in Table 6. The distribution of ova on day 4 was determined by flushing the tracts. The low doses of oestradiol and the anti-oestrogens were chosen since they did not cause complete tube-locking and additive interactions between oestradiol and the anti-oestrogens were expected.

(7) MER 25 causes complete tube-locking in only a proportion of mice, perhaps because ova are expelled prematurely from the isthmus to the uterus before day 4. Eight mice per group were given 4·0 mg/day of MER 25 in dimethylsulphoxide or the vehicle only on days 1–3 and were killed and the tracts flushed on the afternoon of day 3.

The ovum and corpus luteum counts were analysed, isolating the variance for differences between boxes and replicates, but these analyses are not included to conserve space. Only statistically significant effects are discussed, the levels of significance being indicated in the text. In all experiments, a double-blind technique was used throughout.

III. Results

(*a*) *Experiment 1*

Table 1 shows that all the compounds caused retention of ova in the oviduct on day 4. Tube-locking of virtually all ova occurred with suitable doses of DMS and MRL 37 but MER 25 in peanut oil at the highest daily dose of 8·0 mg caused complete tube-locking in only 8 of 20 mice (40%) and little or none in the remaining animals. However, MER 25 in dimethylsulphoxide was effective at a daily dose of 4·0 mg which caused tubal retention of all ova in 4 of 7 mice. All of the anti-oestrogens were luteolytic and significantly reduced the corpus luteum counts ($P<0·05$); the ovum losses were thus underestimated, but a comparison of the total recovered with the mean corpus luteum count in control mice (values in parenthesis in ovum loss column) shows that none of the anti-oestrogens causes as marked a loss of ova as occurs after treatment with oestradiol (Humphrey 1968*a*). Greatest loss occurred in mice given DMS.

In both control and treated mice, most of the uterine ova were blastocysts but about 67% of the oviducal ova were still at the morulae stage or the blastocoele was small. Thus the tube-locked ova appear to be slightly retarded in the development of the antrum (see Humphrey 1968*a*). However, there was no increase in the incidence of abnormal ova.

Doses of DMS that caused virtually complete tube-locking of ova on day 4 did not completely prevent implantation ($P<0·05$), indicating that some tube-locked ova reach the uterus and are viable. In contrast, many mice given 4·0 mg/day or more of MER 25 had apparently normal ova in the uterus on day 4 but no implants were found on day 12 in mice given this dose.

In an earlier paper (Humphrey and Martin 1968) it was shown that mice given 2·0 mg/day of MRL 37 in peanut oil had 13% of the ova in the uterus on day 4 and 30% of treated mice had implants on day 12. In the present work, this dose given in dimethylsulphoxide caused complete tube-locking and inhibition of pregnancy. Experiments not tabulated here showed that the differences in tube-locking caused by 2·0 mg of MRL 37 in oil compared with MRL 37 in dimethylsulphoxide were statistically significant.

TABLE 1

EFFECTS OF SOME ANTI-OESTROGENS ON EARLY PREGNANCY IN THE MOUSE

All results are expressed as means. Values in parenthesis are corpus luteum counts on day 4

Treatment on Days 1–3	Daily Dose (mg)	Ova Recovered on Day 4: In Oviduct	In Uterus	Total	Ova Lost by Day 4	No. Mice Pregnant on Day 12 (%)	Mean No. of Implants on Day 12
		10 mice/group				10 mice/group	
Peanut oil	0·1 ml	0·9	11·4	12·3	2·1(2·1)	70·0	9·3
DMS	0·025	4·0	5·7	9·7	2·4(4·7)	70·0	7·6
	0·1	7·2	2·1	9·3	4·0(5·1)	40·0	2·3
	0·4	9·1	0·0	9·1	3·4(5·3)	50·0	2·9*
	1·6	8·0	0·3	8·3	3·1(6·1)	20·0	1·4*
		20 mice/group				11 mice/group	
Peanut oil	0·1 ml	2·5	6·8	9·3	2·8(2·8)	54·6	6·3
MER 25	1·0	1·8	7·3	9·1	1·4(3·0)	54·6	5·5
	2·0	3·4	5·7	9·1	2·3(3·0)	27·3	1·8*
	4·0	3·3	6·2	9·5	0·8(2·6)	0·0	0·0
	8·0	5·7	4·2	9·9	−0·4(2·2)	0·0	0·0
		7 mice/group				12 mice/group	
Dimethyl-sulphoxide	0·1 ml	2·9	7·3	10·1	2·7(2·7)	66·7	6·8
MER 25	0·5	2·4	8·3	10·7	2·4(2·2)	60·0	6·7
	1·0	4·4	7·4	11·9	−2·0(1·0)	16·7	0·8
	2·0	5·4	5·6	11·0	0·7(1·9)	25·0	3·5
	4·0	7·4	1·9	9·3	4·9(3·6)	0·0	0·0
	8·0	5·1	4·6	9·7	2·3(3·2)	0·0	0·0
	16·0	6·4	4·6	11·0	2·0(1·9)	—	—
		10 mice/group				10 mice/group	
Dimethyl-sulphoxide	0·1 ml	2·7	7·9	10·6	2·3(2·3)	80·0	8·3
MRL 37	0·25	5·2	7·3	12·5	−0·6(0·4)	90·0	9·3
	0·5	8·6	2·8	11·4	0·8(1·5)	40·0	1·9
	1·0	10·9	0·0	10·9	0·6(2·0)	0·0	0·0
	2·0	12·3	0·0	12·3	−0·9(0·6)	0·0	0·0
	4·0	8·1	1·6	9·7	−0·8(3·2)	0·0	0·0
	8·0	8·3	0·4	8·7	−0·9(4·2)	0·0	0·0

* Smaller than control implants.

(*b*) *Experiment 2*

Examination of compressed oviducts (Table 2) showed that in mice treated with an anti-oestrogen, most of the ova were in the ampulla (loop 2) of the oviduct on day 4 with others scattered throughout the isthmus (loops 3–8), although MER 25 again caused retention of ova in only a proportion of mice. In oil-treated mice, all but one ovum were in the uterus; the exception was found in the isthmus immediately before the uterotubal junction (loop 8). Serial sections of the oviducts confirmed the tube-locking of most ova in the ampullae of mice given an anti-oestrogen.

TABLE 2

LOCATION OF TUBE-LOCKED OVA ON DAY 4

Seven mice were used per treatment group

Treatment on Days 1–3	Mean Ovum Counts in Oviduct Loop*:								Mean Ovum Counts in Uterus‡
	2†	3	4	5	6	7	8	Total	
Peanut oil (0·1 ml daily)							0·1	0·1	9·5
DMS (400 μg daily)	2·7		1·0	0·3	0·2			4·1	0·0
MRL 37 (1·0 mg daily)	9·0			0·3	1·0	0·1	0·3	10·6	0·7
MER 25 (16·0 mg daily)	1·9				0·6		1·0	3·4	4·6

* Determined in compressed oviducts. † Ampulla.

‡ Determined by flushing with buffer solution.

(c) *Experiment 3*

The results presented in Table 3 show that MRL 37 causes retention of ova in the ampulla from day 2 to 4, apparently by prolonged closure of the ampulla–isthmus junction, but there is no evidence for adovarian movement of ova.

TABLE 3

LOCATION OF OVA IN THE OVIDUCTS OF MICE TREATED WITH MRL 37

Mice received 1·0 mg/day of MRL 37 in dimethylsulphoxide on days 1, 2, and 3 or part thereof. Control mice received dimethylsulphoxide only. 5 mice were used per treatment group

Treatment on Days 1–3	Day Mice Killed	Mean Ovum Counts in Oviduct Loop*:								Mean Ovum Counts in Uterus†
		2	3	4	5	6	7	8	Total	
Control	2	1·6	3·0	3·0	2·4	1·2	0·2	0·4	11·4	0·0
MRL 37	2	4·2	0·6	4·6	0·4	0·8	0·0	0·0	10·6	0·0
Control	3		0·8			1·6	0·6	7·6	10·6	0·0
MRL 37	3	6·6				2·4	0·6	1·4	11·0	0·0
Control	4							0·4	0·4	9·6
MRL 37	4	4·4		1·0		0·8	1·3	0·8	8·6	1·0

* Determined in compressed oviducts.

† Determined by flushing with buffer solution.

(d) *Experiment 4*

The results (Table 4) show that the recovery of ova in the DMS-treated mice was low on all days. MRL 37 produced tube-locking of many ova up to day 7. Again, MER 25 had variable effects on tubal transport but caused marked tube-locking on day 7. The tracts of control mice were not flushed, since implantation commences on day 5. On days 6 and 7 a mean of 9·1 and 7·7 implants respectively were found in the control animals.

TABLE 4

DURATION OF TUBE-LOCKING IN MICE GIVEN ANTI-OESTROGENS

Results are mean ovum counts for 10 mice/group. Anti-oestrogens were administered in 0·1 ml peanut oil on days 1, 2, and 3. Tracts of control mice were not flushed, since implantation commences on day 5. On days 6 and 7 a mean of 9·1 and 7·7 implants respectively were found in the control animals

Treatment on Days 1–3	Day Mice Killed	No. of Ova Recovered: In Oviduct	In Uterus	Total
DMS (400 μg)	5	2·1	0·0	2·1
	6	1·1	1·1	2·2
	7	0·4	0·5	0·9
MRL 37 (1·0 mg)	5	6·6	0·8	7·4
	6	5·0	0·1	6·1
	7	6·3	0·3	6·6
MER 25 (16·0 mg)	5	2·2	4·8	7·0
	6	1·5	4·7	6·2
	7	6·2	1·4	7·6

(e) *Experiment 5*

Table 5 shows that progesterone had no significant effect on tubal transport in control or treated mice. The tube-locking produced by oestradiol and DMS was apparently partly reversed by progesterone but these effects were not statistically significant.

TABLE 5

EFFECTS OF PROGESTERONE ON TUBE-LOCKING IN THE MOUSE

Mice received daily injections of either dimethylsulphoxide (control), 0·1 μg oestradiol, 25·0 μg DMS, 1·0 mg MRL 37, or 2·0 mg MER 25 with either 2·0 mg progesterone or peanut oil on days 1, 2, and 3 of pregnancy. Results are mean ovum counts for 10 mice/group, killed on day 4

Treatment on Days 1–3	No. of Ova Recovered: In Oviduct	In Uterus	Total	No. of Ova Lost
Dimethylsulphoxide+oil	1·7	9·1	10·8	1·6
Dimethylsulphoxide+progesterone	0·3	10·3	10·6	2·6
Oestradiol+oil	4·2	2·0	6·2	2·9
Oestradiol+progesterone	3·0	3·1	6·1	4·7
DMS+oil	3·6	4·5	8·1	3·8
DMS+progesterone	2·7	6·5	9·2	3·1
MRL 37+oil	8·1	1·9	10·0	0·8
MRL 37+progesterone	9·2	2·1	11·3	1·6
MER 25+oil	2·8	7·2	10·0	−1·0
MER 25+progesterone	3·9	6·2	10·1	3·8

(f) *Experiment 6*

Table 6 shows that the anti-oestrogens did not reverse the tube-locking produced by oestradiol, but themselves caused some tube-locking, even at these

small doses. There seemed to be an additive interaction between DMS and oestradiol, but the analyses of variance showed that this was not significant.

TABLE 6

INTERACTIONS OF OESTRADIOL AND ANTI-OESTROGENS ON TUBAL TRANSPORT IN THE MOUSE

Mice received separate injections of oestradiol and either DMS, MER 25, MRL 37, or the vehicles only on days 1, 2, and 3 of pregnancy. Results are expressed as mean ovum counts for mice killed on day 4

Treatment	Anti-oestrogen Dosage (μg)	Oestradiol Dosage (μg)	No. of Ova Recovered: In Oviduct	In Uterus	Total	No. of Ova Lost
			15 mice/group			
Oil	—	—	0·3	9·1	9·4	2·9
	—	0·025	1·0	6·7	7·7	4·7
	—	0·05	2·3	4·9	7·3	4·9
DMS	25	—	1·7	4·9	6·6	5·8
	25	0·025	5·1	2·8	7·9	4·9
	25	0·05	3·9	1·7	5·6	5·1
			8 mice/group			
Dimethyl-sulphoxide	—	—	1·3	5·8	7·0	5·6
	—	0·025	0·3	7·0	7·3	4·9
	—	0·05	2·4	4·8	7·4	4·9
MRL 37	200	—	2·9	6·4	9·3	4·3
	200	0·025	1·6	5·6	7·3	6·8
	200	0·05	2·9	4·1	7·0	4·9
MER 25	2000	—	2·4	6·5	8·9	2·3
	2000	0·025	2·9	6·4	9·3	3·0
	2000	0·05	2·1	7·5	9·6	4·1

(g) *Experiment 7*

None of the mice given either MER 25 or dimethylsulphoxide had ova in the uterus on day 3, so that accelerated transport did not occur. These results have not been tabulated.

IV. DISCUSSION

The three anti-oestrogens tested all caused tube-locking of virtually all ova, predominantly in the ampulla, until at least day 4, although there were distinct differences in the effectiveness of the different substances. MER 25 affected tubal transport in only a proportion of mice, and the tube-locking produced by this substance appears to be a quantal response. The recovery of ova was high on all days in mice given MRL 37 or MER 25, but fell markedly after day 4 in DMS-treated animals.

Thus, treatment with the anti-oestrogens on days 1–3 of pregnancy has identical effects on tubal transport to a single injection of oestradiol on day 1 (Humphrey

1968*a*), but does not cause accelerated transport and loss of ova as occurs following oestradiol on days 1–3. These differences between the effects of multiple injections of an anti-oestrogen or of oestradiol may be because the anti-oestrogens do not affect the contractability of the isthmus, or because they produce prolonged tube-locking so that fewer ova reach the isthmus, or for some other reason.

The retention of ova in the ampulla from day 1 onwards following oestradiol, MRL 37, and possibly the other anti-oestrogens is due to prolonged closure of the ampulla–isthmus junction and retrograde movement of ova from the isthmus to the ampulla does not occur (Humphrey 1968*a*, and the present results).

The simplest explanations for these effects of the anti-oestrogens on tubal transport are: (1) antagonism of the effects of endogenous oestrogens; or (2) direct effects on the oviduct.

Following simultaneous administration of low doses of oestradiol and an anti-oestrogen some tube-locking occurred. There was no evidence of antagonism between the substances; instead there was possibly a slight additive interaction. For these reasons, and because the anti-oestrogens have similar effects on tubal transport to oestrogens, it is believed that the tube-locking effect of these substances is not due to antagonism of endogenous oestrogens.

The effects of these substances on ovum transport may well be a direct effect since they all exhibit some oestrogenicity in some assays. Of the three substances, DMS is the most potent in vaginal smear and vaginal tetrazolium reduction assays, and appears to be a weak (pro)-oestrogen (Emmens 1965*a*; Martin, unpublished data). MER 25 is active in rat vaginal smear tests and mouse tetrazolium assays, and seems to be oestrogenic at least at higher doses (Emmens 1967). MRL 37, a close structural analogue of MER 25, exhibits some oestrogenicity in the vaginal tetrazolium assays and may also be weakly oestrogenic (Pollard 1966; Emmens *et al.* 1967). All these substances resemble oestrogen in that they inhibit the deciduoma reaction in rats and mice (Stone and Emmens 1964*a*, 1964*b*; Humphrey 1968*b*) and produce hypertrophy and secretion of the endometrial glands (Humphrey 1968*b*). However, MRL 37 and MER 25 but not DMS prevent oestradiol-induced implantation in ovariectomized mice (Humphrey 1968*b*) and these two substances exhibit both oestrogenic and anti-oestrogenic properties at the doses used in the present experiments.

Similarly, the anti-oestrogens U-11555A and U-11100A are also oestrogenic (Emmens and Martin 1965; Emmens 1967) and have identical effects to DMS, MRL 37, and MER 25 on tubal transport, the deciduoma reaction, and endometrial histology (Humphrey 1967). The effects of these substances on tubal transport are believed to be due to their oestrogenic, rather than anti-oestrogenic properties (Humphrey 1967).

In the present work, the retention of ova in the oviduct was associated with delayed development of the blastocoele (see also Humphrey 1968*a*) but the zygotes appeared otherwise healthy and developed to zona-less blastocysts. MER 25 has been stated to be zygotoxic in the rat and rabbit (Segal and Nelson 1958; Chang 1959; Schlough and Meyer 1965) but ovum transfer studies have shown that the zygotes recovered from mice treated with oestradiol, DMS, MRL 37, or MER 25 are viable (Humphrey 1968*b*). Similarly, the antifertility agents norethynodrel, U-11100A,

U-11555A, and MRL 41 were believed to act directly on the developing embryos in mice, rats, and rabbits (Pincus *et al.* 1956; Chang 1959, 1964; Davis 1963*a*; Nelson, Davidson, and Wada 1963; Davidson, Schuchner, and Wada 1965; Prasad, Kalra, and Segal 1965; Schlough and Meyer 1965). However, ovum transfer studies have shown that these substances are not zygotoxic but have an effect on the endometrium (norethynodrel, Davis 1963*b*; MRL 41, Staples 1966; U-11100A and U-11555A, Duncan and Forbes 1965; Humphrey, unpublished data). It can be assumed that the loss of ova following treatment with some of these substances is due to abnormal tubal transport, as they all show some oestrogenicity (Emmens 1965*a*, 1965*b*, 1967; Emmens and Martin 1965).

From the present results, it is concluded that the anti-fertility effects of DMS, MRL 37, and MER 25 are partly due to disruption of ovum transport, and these actions are probably due to oestrogenic rather than to anti-oestrogenic properties.

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