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An Orally Active Compound with Anti-Fertility Effects in Rats.* (24068)

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Inhibition of fertility in rats can be achieved by interfering with normal mechanisms at any one of several vulnerable points in the reproductive process. These include impairment of gametogenesis by compounds having direct action on germ cells(1,2), prevention of ovulation by administering steroids which suppress pituitary production of gonadotrophins(3), withdrawal of progestational support of the endometrium(4), or destruction of developing embryos with anti-metabolites(5). The use of estrogens to terminate pregnancy in rats was reported 3 decades ago by Smith(6). Subsequently, numerous investigations established that high dosages of estrogen cause tubal blockage when administered immediately after fertilization, while later treatment interferes with maintenance of a progestational endometrium(7,8). A type of activity completely different from any of those mentioned above appears to be characteristic of a particular triphenyl ethanol derivative, 1-(*p*-2-diethylaminoethoxyphenyl)-1-phenyl-2-p-anisylethanol.† When female rats are treated for a brief post-copulatory period with an adequate dose of this compound, implantation never occurs. These animals enter a period of pseudopregnancy, characteristic of rats following sterile mating, and upon resumption of estrous cycles, are completely capable of bearing normal litters(9). This report presents experiments leading to these observations. We have attempted to establish the phase of the reproductive process influenced by MER-25, dosage requirements for the compound's anti-fertility effects, and site of its activity.‡

Methods. Fertile female rats of Sprague-Dawley strain were placed with proven male breeders during early evening and vaginal smears were examined for spermatozoa the following morning. The day on which sperm were found in the vaginal smear is considered day 1 of pregnancy. On this basis, days 1-3 and part of day 4 comprise the period of oviducal passage. The remainder of day 4 and day 5 follow tubal exit when blastocysts are free in the uterus and days 6, 7, 8 are the period of implantation. Formulating dosage schedules in relation to these reproductive events, MER-25 was administered by stomach tube on a body weight basis. The effectiveness of systemic administration has been reported(10). Concentrations were established so that the animals uniformly ingested 0.1 ml of olive oil solution/100 g body weight. Body weights and vaginal smears were recorded daily. Eight to 12 days post-coitus, the animals were laparotomized and both uterine horns

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TABLE I. 4-Day Treatment with MER-25, 25 mg per kg Daily Oral Administration.

Treatment days post-coitus	No. of animals	No. of pregnancies	Avg duration pseudo pregnancy (days)
1– 4	20	0	15
2– 5	8	0	15
3– 6	8	0	14
4– 7	8	0	15
			Avg litter size
5– 8	8	8	9
1– 4 (control)	15	15	10
Continuous treatment			
5–21	4	4	9

were examined for implantation sites. Number of animals employed in each experimental group is indicated in the tables. For histological study of developmental events occurring prior to tubal exit, oviducts were recovered from treated and untreated animals, 1, 2, 3 and 4 days after mating, fixed in Bouin's or Zenker's fluid and serially sectioned. To determine if uteri of treated animals retain capacity to form decidua, 3 mated females were treated with MER-25 during the first 4 days post-coitus and then subjected to artificial uterine irritation. On day 11, these animals were sacrificed and the irritated sites sectioned and examined for decidual reactions.

Results. Timed - administration experiments. When treatment with 25 mg/kg body weight was initiated on day of mating and continued throughout normal period of oviducal passage of ova (days 1-4), implantation never occurred (Table I). Laparotomy on day 12 revealed absence of nidation sites. Animals treated on days 1-4 regularly entered a period of pseudopregnancy of 15 days average duration, then resumed normal estrous cycles. Subsequent matings with the same breeding males proved fertile. Gestation was likewise prevented in all instances when treatment included any part of the oviducal period and was continued for 4 days (days 2-5, 3-6, 4-7). When treatment of similar length was initiated after tubal exit of the ova (days 5-8), not a single pregnancy was terminated and the fetuses developed normally. Litter sizes in these cases were not significantly different from controls. It has been possible to administer the compound continuously following period of oviducal passage without impeding gestation, causing fetal resorption, or reducing litter size (Table I).

Daily administrations of 25 mg/kg of the compound for 1, 2, or 3 days, including portions of the period of oviduct passage, drastically reduced fertility, although occasional pregnancies did result (Table II). These occurred mainly when the abbreviated treatment period was initiated immediately after copulation. When begun later in the oviducal period and extended into the period of the free blastocyst, the 3-day or 2-day treatment was essentially 100% effective in preventing implantations. Single, daily ingestions of 25 mg/kg body weight, regardless of day of administration, failed frequently to provide absolute protection against implantation and gestation. That this may merely reflect a dosage phenomenon is indicated by the results of increasing treatment to 2 or 3 administrations of 25 mg/kg during the first post-copulatory day. The b.i.d. treatment significantly reduced the number of successful pregnancies to only 1 out of 8, while the t.i.d. regimen proved 100% effective in 12 cases. For the purposes of a 4-day treatment period, however, the dosage of 25 mg/kg is well above

TABLE II. 3-, 2- or 1-Day Treatment with MER-25, 25 mg/kg Daily Oral Administration.

Treatment days post-coitus	No. of animals	No. of pregnancies	Litter size
1-3	10	3	1, 1, 2
1-2	8	3	7, 3, 1
1	8	6	11, 7, 5, 3, 2, 2
1 (2 × 25 mg/kg)	8	1	6
1 (3 × 25 ")	12	0	
2-4	10	1	11
2-3	8	0	
2	8	3	8, 8, 7
3-5	10	0	
3-4	8	1	6
3	8	3	11, 8, 5
4-6	10	0	
4-5	8	0	
4	8	3	9, 9, —
5-7	5	5	9 (avg)
5-6	8	8	9 "
7-8	4	4	10 "

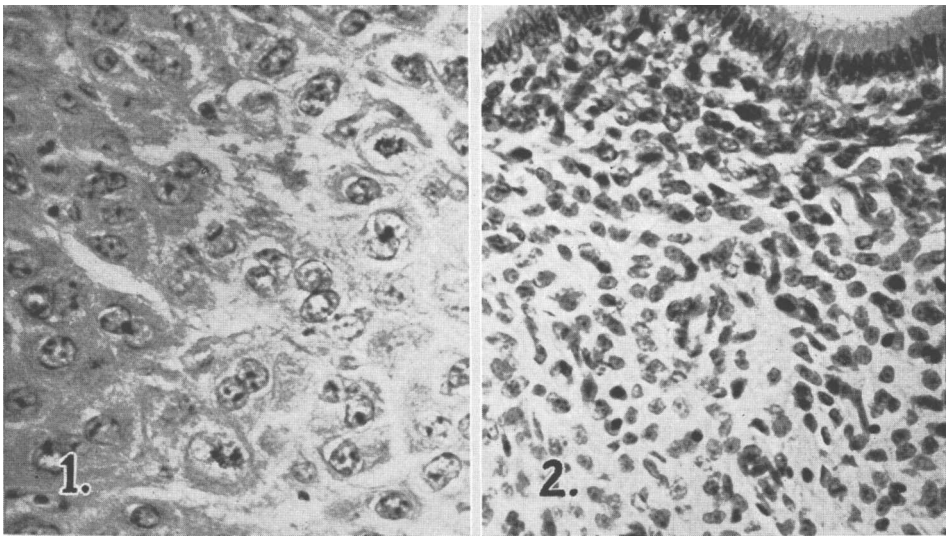

FIG. 1. Cross-section of artificially stimulated region of MER-25 treated rat uterus. Note decidual cells and stromal edema (×340).

FIG. 2. Cross-section of contralateral, unstimulated uterine horn at same magnification as Fig. 1. Histologic appearance is typical of pseudopregnancy.

the minimal effective dose. Absolute prevention of pregnancies was achieved with 20 mg/kg and 15 mg/kg. The dose of 10 mg/kg was effective in most instances, and when this low dosage failed to prevent pregnancy completely, there was a severe reduction in litter size (Table III).

Artificial stimulation of endometrium. Uteri of animals treated daily with 25 mg/kg MER-25 are capable of forming decidual reactions at time of normal implantation. Uterine sites, irritated by needle-scraping, develop typical deciduomata (Fig. 1) within one week after artificial stimulation. Enlarged decidual cells exhibit a high incidence of mitoses; stromal edema and vascularization is pronounced. There were no implantation sites in the undisturbed, contralateral uterine horn of these mated, then treated animals. The histologic appearance is that of normal pseudopregnancy (Fig. 2).

TABLE III. MER-25 Treatment, Days 1-4 Post-Coitus Oral Administration.

Daily dose, mg/kg	No. of animals	No. pregnancies	Litter size
25	20	0	
20	12	0	
15	12	0	
10	12	2	1, 3

Developmental events within oviducts. Gross morphology, average tubal diameters, and histologic characteristics of oviducts from treated animals do not differ from controls. Within 24 hours after mating, sections through newly fertilized eggs of treated animals show no abnormalities. Extrusion of 2nd polar body and fusion of pronuclei appear

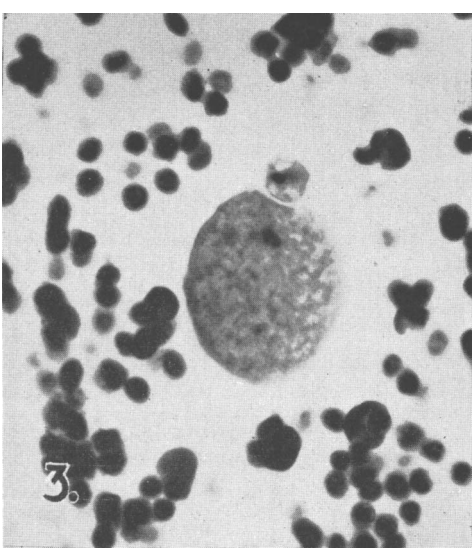

FIG. 3. Tubal ovum within 24 hr post-coitus. Animal was treated with MER-25 12 hr prior to sacrifice. Note normal fusion of pronuclei and extruded 2nd polar body (×530).

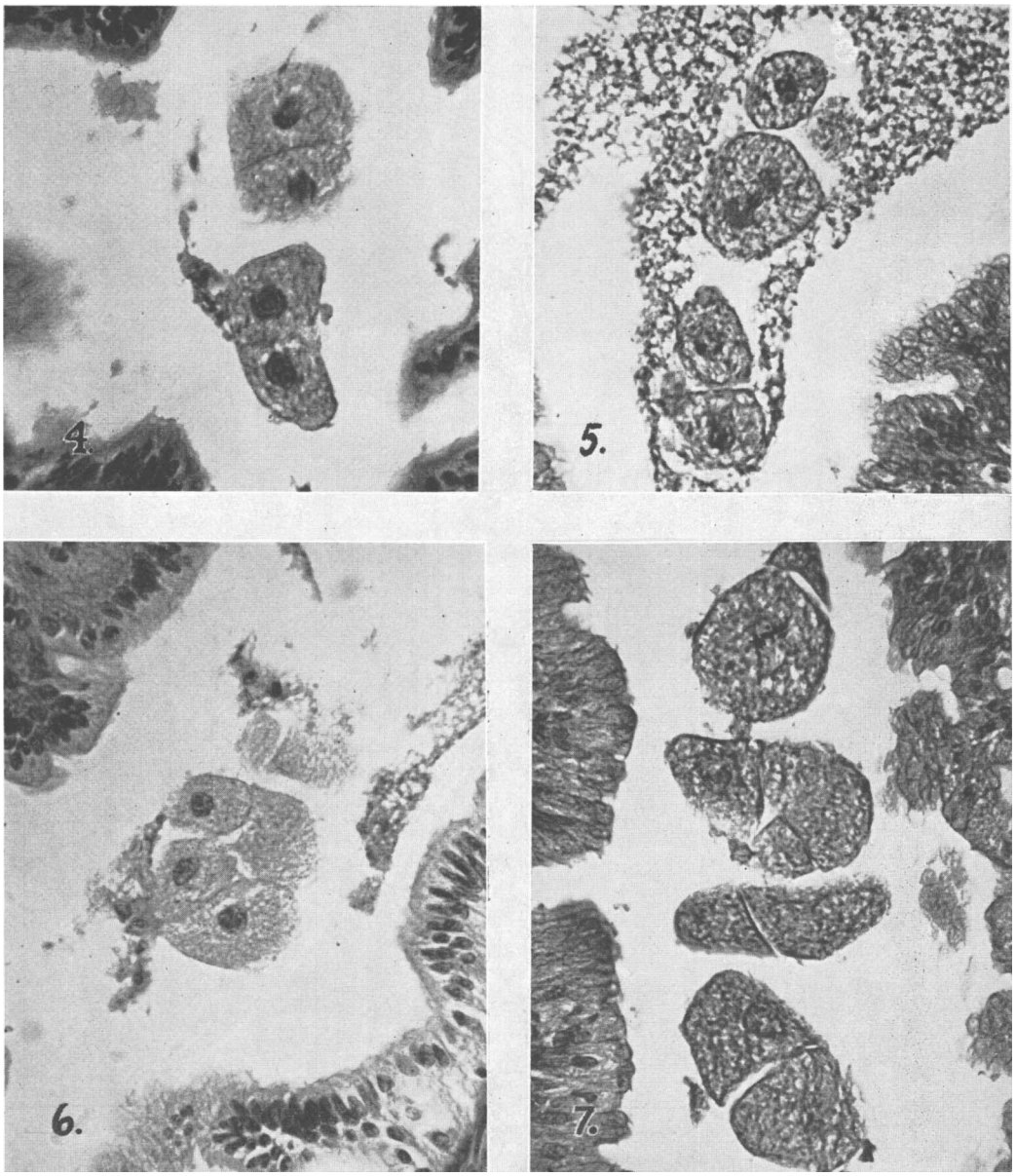

FIG. 4. Section through tubal ova of control animal sacrificed between 24 and 48 hr post-coitus. Each of these zygotes had reached the 4-cell stage (×340).

FIG. 5. Section through tubal ova of MER-25 treated animal sacrificed between 24 and 48 hr post-coitus. Blastomeres are of irregular size. Note arrested spindle apparatus (×340).

FIG. 6. 8-cell morula sectioned within the oviduct of a control animal sacrificed between 48 and 72 hr post-coitus (×340).

FIG. 7. Zygotic fragments found in oviduct of MER-25 treated animal sacrificed between 48 and 72 hr post-coitus. Note abnormal and unequal cleavages and beginning of fragmentation (×340).

to occur in normal fashion (Fig. 3). The beginning of first cleavage can frequently be observed. Between 24 and 48 hours most normally developing ova are in the 2-cell stage or have reached the 4-cell stage (Fig. 4). In treated animals sacrificed between 24 and 48 hours, more frequently than not, there are evidences of abnormal divisions with blastomeres

of irregular size and arrested spindle apparatus (Fig. 5). Between 48 and 72 hours, the normal zygote has reached the 8- or 12-cell stage with daughter blastomeres of equal size (Fig. 6). Treated animals by this time show an apparent degradation of developing ova. Irregular and unequal divisions result in bizarre aggregates of blastomeres and many of the clusters start to fragment (Fig. 7). Pycnotic nuclear material is frequently seen. After the third day, between 72 and 96 hours post-coitus, neither developing eggs nor identifiable remnants of eggs could be found in oviducts of animals treated daily with MER-25. Normal animals sacrificed at the same time may be expected to show well-formed morulae approaching the utero-tubal junction.

Discussion. The period of effectiveness for achieving anti-fertility activity with MER-25 corresponds to the time necessary for passage of fertilized ova through the oviduct and the preimplantation phase of zygotic development *in utero*. Inasmuch as treatment was initiated many hours after mating, fusion of gametes and all phases of the reproductive process leading to this event could not be involved in the observed inhibition of fertility. We cannot rule out, however, the possibility that the compound may also act at a prior vulnerable point in the sequence of reproductive events; this warrants further investigation. It seems well-established, on the other hand, that the influence of MER-25 is exerted prior to and exclusive of the process of implantation.

Throughout these experiments, when length of treatment or dosage factors were not optimal and pregnancies resulted, there was frequently a reduction in litter-size. Ovaries of these animals contained normal numbers of functional corpora lutea and no fresh resorption sites were noted in the uterus. This indicates that a usual complement of ova were released at ovulation and that the reduced litter-size did not result from fetal resorption. It would appear, therefore, that the defect arises in the developing eggs themselves, or in the capacity of the uterus to accept blastocysts for implantation. However, the fact that some blastocysts do implant indicates that the uterus is capable of supporting nidations. This is confirmed by development of deciduomata following artificial stimulation of endometrium of treated animals. In addition, this reaction proves that the compound does not interfere with post-copulatory maintenance of luteal body function, for the decidual reaction could not occur in its absence. That the corpora lutea are functional is indicated also by vaginal smear records of successfully treated animals. Each of the 142 animals treated adequately for prevention of pregnancy entered a characteristic period of pseudopregnancy, a phenomenon that requires maintenance of functional corpora lutea.

It would appear, therefore, that by the time of implantation, there was only a partial survival of ova, below the number released at ovulation and subsequently fertilized. Those blastocysts that did implant developed normally and well-formed fetuses were delivered at term. These offspring showed no signs of anomalies whatsoever and were reproductively normal upon reaching sexual maturity. Fetuses developed normally also in those cases treated continuously following tubal exit (days 5-21), but normal parturition was prevented by MER-25 administration. The mechanism of this interference with parturition is not yet understood and is presently under investigation. It is likely that the effect is on the maternal endocrine balance and is related to estrogen antagonism by the compound. By virtue of extensive pharmacologic testing, Thompson and Lerner(11,12) have established that MER-25 inhibits the peripheral response of various end-organs to both exogenous and endogenous estrogenic stimulation. On the basis of the present observations, the compound's anti-fertility activity cannot be clearly linked with its known anti-estrogenic activity. This mode of action, however, must be considered as a distinct possibility.

It may be concluded that the anti-fertility effect of MER-25 results from annihilation of the zygotes as they descend the tubes or while they are free blastocysts in the lumen of the uterus. The mechanism of this cytotoxic effect remains to be established. Conceivably, the compound may act not on the eggs themselves, but on the maternal duct system in a manner so as to remove necessary support for normal zygotic development.

Summary. When MER-25 is administered orally to female rats on day of mating and continued throughout the normal period of oviducal passage of ova, implantation does not occur. Restricting treatment period to only one day post-coitus can also be effective in preventing nidation. Treated females enter a post-copulatory period of pseudo-pregnancy, then resume normal estrous cycles. The anti-fertility effects of MER-25 appear to be exerted prior to exit of ova from the oviducts. The compound does not act by preventing decidua development, nor by interfering with post-copulatory maintenance of luteal body function. Histologic evidence indicates that ova of females treated with MER-25 undergo degeneration after extrusion of 2nd polar body.

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Serum Cholesterol in Man and the Unsaponifiable Fraction of Corn Oil in the Diet.*† (24069)

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A decrease in serum cholesterol level has been found when corn oil is used in the diet, in substantial amounts in man(1-6) and in the cholesterol-fed chick(7). It has been suggested that much of this effect is due to something besides the fatty acid glycerides in corn oil(8). For these reasons we excluded comparisons involving corn oil in our recent analysis of a large series of fat-feeding experiments(9,10). This analysis indicated that with many fats (butterfat, olive oil, cottonseed oil, sunflower seed oil, sardine oil and mixed fats of ordinary foods), a change in fat content of the human diet results in a change in serum cholesterol level which is predictable from a regression equation, cholesterol rising with increasing saturated and falling with increasing poly-unsaturated fats in the diet: Δ Chol. $= 2.74\ (S_2 - S_1) - 1.31\ (P_2 - P_1)$, (Equation 1) where S and P are percentages of total calories provided by glycerides of saturated and of poly-unsaturated fatty acids, respectively, and the subscripts refer to first and second diets. When data from dietary changes involving corn oil were tested with this equation, the results tended to show that corn oil produced slightly lower serum cholesterol values than predicted. This would conform to the suggestion that corn oil may contain a special cholesterol-lowering substance not accounted for as fatty acid glyceride, presumably in the unsaponifiable frac-

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