ides into lower molecular dialyzable compounds, with a decrease in viscosity(6,7). Recently, aortic arteriosclerosis was produced in the dog after localized irradiation with X-rays(5) and by irradiation with electrons(8). It has been proposed that the naturally occurring process and sequences in which aging and possible nutritional factors were etiological had been accentuated and accelerated by irradiation(5). The present study shows that in the period of 20-28 weeks after completion of the irradiation, the rats fed the high fat diet and treated with X-rays showed pronounced coronary atherosclerosis in 38.8%, while those fed only the high fat diet developed atherosclerosis in 22.7%. In the previous study, however, in a period of 8-15 weeks post-irradiation, 26.6% of the irradiated rats had marked coronary atherosclerosis but there was none in the unirradiated group(1). This indicates that time is a necessary factor in the development of marked coronary atherosclerosis as a result of a high fat diet, and that irradiation accelerates the process.

Summary. Forty-two albino Wistar rats, average weight 133 g, fed on a supplemented high fat diet, were divided into 2 groups. One group was treated with 2500 r (X-rays) to the thorax, and the other kept on the high fat diet, but not irradiated. The rats were killed 20 to 28 weeks after completion of irradiation. Marked atherosclerotic lesions were observed in the coronary arteries of 38.8% of the irradiated group and in 22.7% in the group on the high fat diet alone. In the pulmonary arteries similar changes were noted in 25% of the irradiated group and none in the unirradiated group. Previous studies showed that in 8-15 weeks post-irradiation marked coronary lesions were present in 26.6% of the irradiated group and none in the unirradiated. It is suggested that X-irradiation accelerates the process that develops with diet and with time.

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Effect of Steroidal Anti-Progestins on Implantation of Fertilized Eggs of Rats and Mice.* (27866)

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The implantation of fertilized mammalian eggs is one of the most vulnerable steps in the processes of reproduction. An exact, harmless way of preventing implantation of eggs by interfering with the necessary preliminary changes in the uterus is still under investigation in many laboratories. The progestational proliferation or preparation of the endometrium, necessary for implantation of eggs, depends upon a delicate balance between estrogens and progesterone(1). The possibility of preventing implantation by altering estrogen levels or inducing adequate hormonal imbalance has been raised by Hartman(2). Investigations on the mechanism of implantation of fertilized eggs and the hormonal requirements of the process(3,4,5,6,7,8,9) lead to the general conclusion that either anti-estrogens or anti-progestins may effectively inhibit implantation. Estrogen in adequate dose prevents the action of progesterone, and

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its administration soon after ovulation may cause "tube blocking" of the ova(10,11) or expulsion of ova from the uterus(12); its administration at a later stage prevents implantation(13,14).

Emmens, Cox and Martin(15,16) recently described the anti-estrogenic properties of a number of compounds, including members of the stilbenediol series, MER 25, and certain 19-nortestosterones. MER 25 has been shown to interfere with pregnancy in the rat (17,18,19). The work of Segal and Nelson (19) has clearly shown that the effect of this compound is due to interference with normal segmentation of the egg in the tube, and is not dependent upon adverse changes in the uterus. Among other non-steroidal compounds, the action of ergotoxine and ergocornine in preventing pregnancy in rats and mice has been reported by Shelesnyak(20) and Carlsen *et al.*(21).

An interesting observation made by Bruce (22,23) is that the mere presence of strange males prevents implantation of fertilized eggs in mice. Parkes(24) has described the Bruce effect as a neurohumoral block to implantation.

The most potent of the implantation inhibitors tested thus far as experimental antifertility agents have been the estrogens, estradiol and diethylstilbestrol. Estradiol was found to be most effective at time of implantation, and its action was believed to be upon the uterus(18). The effect of estrone in preventing implantation as well as in causing fetal death has also been recorded(14).

The action of estrogens is generally taken as anti-progestational, and we have reported that certain neutral steroids lacking demonstrable estrogenic activity can act as anti-progestins in inhibiting the effects of progesterone on the rabbit endometrium. Furthermore, even among substances with estrogenic activity, the anti-progestin effect is not necessarily correlated quantitatively with the degree of estrogenic activity(25,26). Accordingly, we have taken several of these estrogenic and non-estrogenic anti-progestins and studied their implantation-inhibiting activity.

Methods. All experiments were performed on female, Sprague-Dawley rats, weighing 200-250 g, and white Swiss mice, weighing 25-35 g. Animals were allowed to mate with males of known fertility. Two male rats were put into each cage of 5 females, but only one male was kept in each cage of 5 female mice. It has been reported earlier(27) that 2 male mice generally fight with each other instead of mating. Mating and the first day of pregnancy were ascertained by finding sperm or vaginal plugs in rats, and vaginal plugs in mice. The compound tested was injected from the first day of pregnancy generally for 3 days unless otherwise stated. All compounds were taken up in sesame oil, and injected subcutaneously in the dorsal region of the neck, using 24- or 25-gauge hypodermic needles to avoid any leakage of the hormone. With 2 daily injections, the second injection was made subcutaneously on the dorsal side of the lumbar region. Each injection was given in a volume of 0.2 ml oil. Control animals received the vehicle (0.2 ml) only. All animals were autopsied between days 10 and 12 after mating. The number of implanted fetuses was counted and observed. Any animal having at least one or more implanted fetus was considered as pregnant. Statistical analysis of the number of pregnant and non-pregnant animals was made using Mainland and Murray's 4-fold contingency test tables (28). The average number of fetuses in pregnant animals in the experimental series

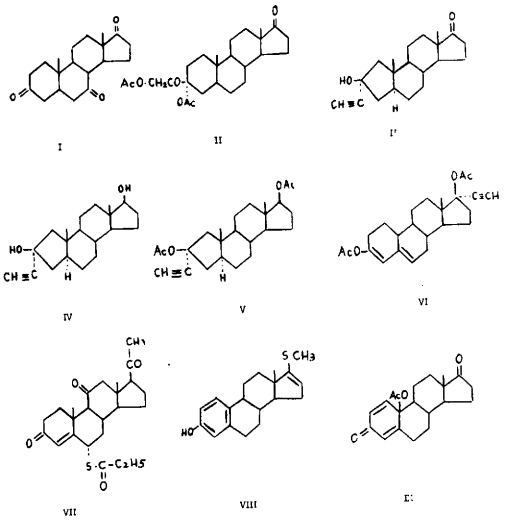

FIG. 1. Anti-progestins tested as implantation inhibitors.

TABLE I. Effect of Steroids on Implantation of Fertilized Eggs of Rats and Mice.

Compound No.	Animal	Dose (mg/day for 3 days)	No. of animals	No. of animals pregnant	P_p*	Litter size (Mean ± S.E.)	P_l*
Control	Rat	.2 ml oil	13	13	—	11.5 ± 1.81	—
	Mouse	.1 ml oil	12	10	—	10.2 ± .56	—
I	Rat	.5	6	6	NS†	11.2 ± 2.37	NS
	"	2.5	6	5	NS	11.4 ± 2.43	NS
	Mouse	.5	6	3	NS	11.0 ± 1.15	NS
	"	2.5	9	7	NS	9.7 ± 1.86	NS
II	Rat	.5	13	10	NS	7.9 ± 1.12	<.01
	"	2.5	5	5	NS	11.8 ± .67	NS
	Mouse	.5	10	10	NS	10.1 ± 1.12	NS
III	Rat	.5	6	6	NS	11.2 ± .75	NS
	"	2.5	5	2	NS	11.5 ± 1.48	NS
	Mouse	.5	9	5	NS	7.4 ± 1.96	<.01
	"	2.5	12	0	<.01	—	<.01
IV	Rat	.02	7	5	NS	11.0 ± 1.05	NS
	"	.1	7	5	NS	9.2 ± .49	<.01
	"	.5	7	0	<.01	—	<.01
	"	2.5	8	0	<.01	—	<.01
	Mouse	.02	10	2	<.05	11.0 ± 0	NS
	"	.1	10	1	<.01	8.0 ± 0	NS
	"	.5	10	1	<.01	3.0 ± 0	<.01
	"	2.5	10	0	<.01	—	<.01
V	Rat	.5	7	4	NS	9.0 ± 1.29	NS
	"	2.5	8	7	NS	10.4 ± .57	.05
	Mouse	.5	10	0	<.01	—	<.01
VI	Rat	.1	7	6	NS	7.17 ± 1.72	<.01
	"	.5	12	2	<.01	9.0 ± 3.0	.05
	"	2.5	5	0	<.01	—	<.01
	Mouse	.1	10	0	<.01	—	<.01
	"	.5	8	0	<.01	—	<.01
	"	2.5	8	0	<.01	—	<.01
VII	Rat	.5	6	6	NS	11.7 ± 1.56	NS
	"	2.5	5	5	NS	10.6 ± 1.9	NS
	Mouse	.5	8	8	NS	12.4 ± .74	NS
	"	2.5	10	5	NS	10.6 ± 1.21	NS
VIII	Rat	1.0	4	0	<.05	—	<.01
	Mouse	.1	8	0	<.01	—	<.01
IX	Rat	1.0	5	0	<.01	—	<.01
	Mouse	.1	8	0	<.01	—	<.01

* Compared with control.
† Not significantly different from control at 5% level.

was compared with those of the controls according to the procedure laid down by Dunnett(29). All animals, before and after mating, were maintained on uniform laboratory conditions and fed a standard Purina laboratory diet with drinking water *ad libitum*.

Results. The structural formulae of the 9 steroid compounds tested in rats and mice are presented in Fig. 1.‡ The number of animals used and the results obtained with each compound are shown in Table I.

Statistical comparisons are made for percentage of pregnancies (P_p) and mean litter seize (P_l). These data demonstrate that at the dosages used: a) compounds I, II and VII failed to reduce significantly the percentage of pregnancies in rats and mice; b) these same 3 compounds similarly failed to reduce litter size significantly, except in a single instance with compound II in rats; c) compounds III and V showed no very signifi-

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TABLE II. Effect of Steroidal Compounds Injected on Different Dates after Mating upon the Implantation of Fertilized Eggs of Rats and Mice.

Compound No.	Animal	Dose (mg)	Injection 1st day after mating					Injection 3rd day after mating				
			No. of animals	No. of animals pregnant	P_p*	Litter size (Mean ± S.E.)	P_1*	No. of animals	No. of animals pregnant	P_p	Litter size (Mean ± S.E.)	P_1
Control	Rat	oil	8	7		10.6 ± .57		11	9		9.78 ± .79	
	Mouse	oil	11	9		13.3 ± .66		10	8		10.57 ± .65	
IV	Rat	1.5	4	1	NS†	2.0	<.01	4	3	NS	5.69 ± 2.73	<.01
	Mouse	.1	9	4	NS	7.5 ± 1.19	<.01	6	4	NS	12.0 ± 1.08	NS
VI	Rat	.5	9	4	NS	8.5 ± 2.79	<.05	5	5	NS	9.8 ± 1.59	NS
	"	1.0	4	0	<.01	— —	<.01	—	—	—	— —	—
	"	1.5	12	0	<.01	— —	<.01	4	3	NS	11.33 ± 2.34	NS
	Mouse	.1	11	1	<.01	— —	<.01	8	0	<.01	— —	<.01
	"	.5	6	0	<.01	— —	<.01	8	0	<.01	— —	<.01
VIII	Rat	1.5	6	0	<.01	— —	<.01					
	Mouse	.1	8	0	<.01	— —	<.01					
IX	Rat	1.0	4	2	NS	2.5 ± .86	<.01					
	Mouse	.1	7	1	<.05	2.0	<.01					

* Compared with control.

† Not significantly different from control at 5% level.

cant effect in rats, but were both effective in mice as inhibitors of implantation; d) compounds IV, VI, VIII, and IX were consistently effective in both species, numbers IV and VI showing significant activity at the same minimal dosage level (0.5 mg/day) in the rat, and probably also (below 0.5 mg/day) in the mouse. The data on compounds VIII and IX are inadequate for even an approximate estimate of their relative potencies.

In Table II the effectiveness of compounds IV, VI, VIII and IX is assessed on the basis of a single injection given on either the first or the third day following mating. With compounds VI and VIII a single injection (of 1 to 1.5 mg) on day 1 significantly inhibits implantation in the rat. The single experiments in the rat with compounds IV and IX did not yield a significant reduction of the number of pregnancies, but in each instance the litter size of the animals carrying fetuses is significantly reduced. Again, in the mouse compounds VI, VIII and IX (0.1 mg doses) significantly reduced litter size. When injections are made on the third day, neither compounds VI nor IV reduce the number of pregnancies significantly in the rat, but the litter size in rats receiving compound IV is significantly reduced. However, on day 3, compound VI was completely effective at both of the dosages used in the mouse. This suggestion of species difference in the activity of these 2 antiprogestins requires further investigation.

In view of the effectiveness of compounds IV and VI when given in a single injection on day 1, we have made an examination of their effectiveness by oral administration in the rat and mouse. The data presented in Table III demonstrate that significant reduction in incidence of pregnancy is attained with 15-mg doses of these 2 compounds, and that at lower dosages the litter size is significantly reduced in the rat. In the mouse, at the dosages used neither compound significantly reduced the percentage of animals pregnant, but, again, litter size was significantly below that found in control animals.

In view of the demonstrated activity of these substances as antiprogestins, one would imagine that progesterone and/or other pro-

TABLE III. Effect of Administration by Gavage of Compounds IV and VI on Implantation of Blastocysts of Rats and Mice.*

		Rat					Mouse				
Compound No.	Dose (mg)	No. of animals	No. of animals pregnant	P_p†	Litter size	P_l†	No. of animals	No. of animals pregnant	P_p†	Litter size	P_l†
Control	Oil	13	10	—	10.7 ± .67§	—	8	6	—	12.7 ± .66§	—
IV	2.5	—	—	—	—	—	9	7	NS‡	7.6 ± 1.67	<.01
	5.0	6	3	NS	6.0 ± 1.44	<.01	—	—	—	—	—
	15.0	7	2	<.05	5.5 ± 4.5	<.01	—	—	—	—	—
VI	.5	—	—	—	—	—	7	5	NS	7.6 ± 1.12	<.01
	5.0	6	4	NS	6.5 ± 2.4	<.01	—	—	—	—	—
	2.5	—	—	—	—	—	9	3	NS	2.7 ± .88	<.01
	15.0	5	1	<.05	3.0	<.01	—	—	—	—	—

* Fed by feeding tube attached to a glass syringe on first day of pregnancy in a volume of 0.4 ml and 0.2 ml in rat and mouse, respectively. Control animals received same quantity of oil only by the same procedure.

† Compared with control.

‡ Not significantly different from control at 5% level.

§ Mean ± S.E.

gestins should antagonize their effects. We have attempted this reversal using progesterone and 3 quite active synthetic progestins as antagonists to compound VI. The data of a number of such experiments with rats and mice are presented in Table IV. The effects of a standard dose of compound VI are seen in the control animals. In only one instance has this effect been significantly reversed, and that is when 10 mg of progesterone per day for 3 days was administered to rats receiving compound VI. The single litter surviving following a similar dosage regimen carried on for 9 days had a probably significantly reduced number of young (6 fetuses), but only one out of 6 pregnancies endured. In not a single instance in the mouse have we been able significantly to reverse the effects of compound VI. Atlhough compounds X, XI and XII have progestational activities which are respectively 2½, 30, and 6 times that of progesterone(30), they have not proven effective in the dosages used in either species.

On examining the implantations in the uteri of animals receiving compounds IV and VI, we were struck by the fact that there were practically no dead or resorbed embryos even though the total number of implantations might be markedly reduced. It would appear, therefore, that in preventing implantation these compounds might act upon the developing eggs as lethal agents rather than as anti-progestins at uterine implantation sites. We have made an examination of this possibility by administering compound VI on the first day of pregnancy in a dosage of 1.5 mg per rat. It should be noted that this has proven consistently to be an implantation-inhibiting regimen (Table II). On the fourth day of pregnancy, the fertilized eggs were flushed and collected from the uterus and from the region of the utero-tubal junction. Comparison was made with ova from a control series receiving the vehicle alone. All of the ova were in 8- to 16-cell stages with intact zona pellucidae (Fig. 2, 3). The rat blastocysts as such, therefore, do not appear to be damaged.

The possibility of damage to the implanted blastocyst was tested by administering compound VI at a dosage of 1.5 mg per rat on the eighth day of pregnancy. These animals were allowed to continue their pregnancy until term and 5 out of 6 delivered normal young in due course. The mean litter size was 9.0 ± 0.72. A group of 5 control animals was injected on the eighth day of pregnancy with the solvent; 4 of them bore normal young with a mean litter size of 10.8 ± 1.55. Thus there would seem to be no significant effect upon the implanting blastocyst using a dosage that effectively inhibits implantation when administered on day one of pregnancy. In view of the relative ineffectiveness of this

TABLE IV. Attempts to Reverse Implantation-Inhibiting Activity of Compound VI.*

Compound	Dose (mg/day for 3 days)	Animal	No. of animals	No. of animals pregnant	P_p†
Control	.2 ml oil	Rat	6	0	—
	.2 ml oil	Mouse	6	1	—
Progesterone	2.0	Rat	4	0	NS‡
	4.0	"	6	0	NS
	10 mg/day for 9 days	"	6	1	NS
	10 mg/day for 3 days	"	9	4	.05
Progesterone + estradiol	10 mg .5 μg for 3 days	Rat	6	0	NS
	Idem	Mouse	11	0	NS
X§	.5 mg/day for 3 days	Rat	6	0	NS
	1.5 mg/day for 3 days	"	5	0	NS
	Idem	Mouse	7	0	NS
XI‖	.5 mg/day for 3 days	Mouse	6	1	NS
	1.5 mg/day for 3 days	"	6	0	NS
	4.5 mg/day for 3 days	"	6	0	NS
XII¶	1.5 mg/day for 3 days	Mouse	7	0	NS

* Compound VI was administered for 3 days (0.5 mg/day) or on first day of pregnancy (1.5 mg) in rats, and on first day of pregnancy in mice (0.5 mg).

† Compared with control.

‡ NS = Not significantly different from control at 5% level.

§ 17α-Ethyl-19-nortestosterone.

‖ 6-Methylene-17-acetoxyprogesterone.

¶ 17α-Ethyl-ethin-19-nortestosterone.

dosage on day 3 (Table II) damage to free ova at any of these stages appears to be ruled out as a significant mechanism.

We have made one attempt to determine if the effects of compound VI endure beyond the pregnancy during which it is administered. Twelve of the animals receiving 1.5 mg of compound VI on day 1 of pregnancy were found, by palpation on the 13th and 14th day, to be non-pregnant. They were then allowed to mate as before with fertile males, and 9 mated successfully within 2 weeks. Autopsy performed on the 9th to 10th day after the successful mating revealed implantations containing an average of 10.0 $\pm$ 1.24 fetuses. Six animals of proven fertility were used as controls, and 5 of them mated within 2 successive estrous cycles. All 5 were found to contain fetuses, and the average was 11.2 $\pm$ 0.44. Thus it appears evident that the duration of effect of compound VI is limited, and certainly does not extend beyond the duration of a pseudopregnancy.

Discussion. Of the 9 anti-progestins tested as implantation inhibitors in rats and mice, 3 have been found to have some estrogenic activity; their approximate potency relative to estrone as standard in the Rubin assay with immature mice is II-1%, VIII-0.1% and IX-3%, but they act in this assay as "impeded" estrogens, *i.e.*, with a non-standard dosage-response curve. They may be regarded as physiological antagonists. However, compound IV which is definitely not estrogenic is implantation inhibiting at approximately similar doses and at somewhat higher doses the closely related compounds III and V are significantly active. As anti-progestins in the rabbit, VIII is active at a dose of 0.4 mg or less, IX at a dose of 0.025 mg or less, whereas the minimum effective dose (MED) of IV is 1 to 2 mg, of III 5 to 10 mg(25), and the MED of VI is not less than 2 mg(31). Thus there is suggested a quantitative dissociation between anti-progestational and anti-implantation activity. However, until we can define more accurately the mechanism of the inhibitory effect herein described, comparisons on the basis of standard assay activity serve only to suggest a rather specific mechanism for this particular effect. This mechanism may involve a direct

effect on the ova or an indirect effect exercised *via* some inhibitory action on the ovaries.

Summary. A method for determining the implantation frequency in rats and mice is described: animals found to have mated are autopsied on days 10 to 12 after established mating and the number of implanted fetuses counted. Among animals receiving injections of sesame oil on days 1, 2 and 3 after mating, 13 of 13 rats contained implantations with an average number of 11.5 ± 1.8 fetuses, and 10 of 12 mice were pregnant with an average of 10.2 ± 0.56 fetuses. Significant departures from these frequencies of pregnancy were observed in both rats and mice receiving, on days 1, 2 and 3 in sesame oil as a vehicle, the anti-progestins: 2β,17β-dihydroxy,2α-ethinyl-A-nor(5α)-androstane (IV); 3β,17β-diacetoxy,17α - ethinyl - 19 - nor - $\Delta^{3,5}$ - androstadiene (VI); 17β-thiomethyl,$\Delta^{1,3,5}$-16-estratetrene (VIII); and 3,17-dione,19-acetoxy-$\Delta^{1,3}$-androstadiene (IX). Among 5 other antiprogestins tested (Fig. 1 & Table I) 3 did not significantly reduce the frequency of implantation (I, II and VII) at any dose used in either rats or mice, and 2 (III and V) were active by this criterion in mice but not in rats. In several instances a non-significant reduction in occurrence of implantation was, however, accompanied by a significant reduction in number of implanted fetuses *e.g.*, at low doses in rats receiving IV and VI. A single injection on day 1 after mating of adequate amounts of compounds IV, VI, VIII and IX is effective for implantation inhibition. Injection on day 3 of doses inhibitory when administered on day 1 leads to much less effective implantation inhibition in several instances (Table II). Two compounds (IV and VI) found effective by injection are also effective when given by gavage to rats and mice but higher doses are required for significant effect by this oral route. The implantation inhibiting effect of one antiprogestin (VI) has been clearly overcome in one instance in rats by the concomitant administration of progesterone alone at high dose, but 3 synthetic steroids administered at high progestational doses are ineffective. Examination of early blastocysts from rats receiving sterilizing dosages of compound VI administered on day 1 revealed no abnormalities or indication of lethal effect. This same compound administered at a consistently effective implantation inhibiting dose failed to

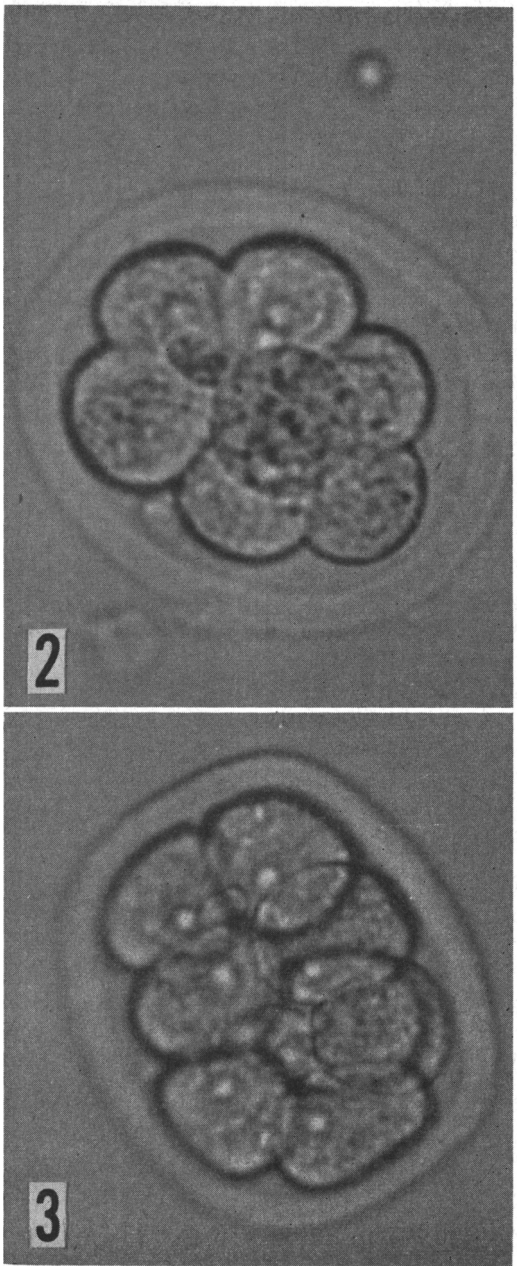

FIG. 2. Four-day uterine ovum from control rat injected with sesame oil on day 1. Magnification 400 ×.

FIG. 3. Four-day uterine ovum recovered from female receiving 1½ mg of compound VI on day 1. Magnification 400 ×.

be embryocidal or abortion-inducing administered to pregnant rats following implantation. The mechanism of the anti-implantation effect remains to be established.

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Effects of Aldosterone on Contractile and Electrical Properties of Driven Isolated Rabbit Atria.* (27867)

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Aldosterone has been reported to produce a marked increase in left ventricular work index in the rat heart-lung preparation(1). This cardiotonic action could be demonstrated with aldosterone concentrations as low as 10^{-9} to 10^{-12}M. It was not clear from these experiments, however, whether or not this effect on ventricular work was due in part to a direct positive inotropic action on the myocardium. In preliminary experiments designed to test the possibility of a direct action of this steroid on cardiac contractility, we were unable to demonstrate any effects on driven isolated rabbit atria(2), although simultaneously and independently, Tanz reported that d,l-aldosterone could produce a direct positive inotropic effect on isolated cat heart preparations(3). These latter findings recently have been more fully described, in-

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