Postcopulatory Effects of Two Antifertility Agents on Ova Transport and Implantation (34098)

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Inhibition of fertility by measures initiated after mating can be accomplished readily in laboratory animals by the ingestion or injection of many compounds. However, most of these compounds have additional properties which render them unsatisfactory for use in the control of mammalian reproduction. Chemical structure and biological properties of these antifertility compounds, both steroidal and nonsteroidal, have been reviewed extensively (1–3).

Saunders and Rorig (4) reported the antifertility activity of several nitriles administed to rats after mating. The present paper describes additional studies with two of these nitriles and demonstrates that their contraceptive activity is caused primarily by an acceleration of the movement of zygotes through the oviducts and uterus. Thus, the cleaving ova arrive in the uterus prematurely and may be expelled from the reproductive tract, before completion of the normal physiological sequence of events leading to implantation.

Materials and Methods. Mature virgin female rats weighing about 200 g were caged overnight with adult males. Copulation was established by the presence of sperm in vaginal smears taken the next morning between 9:00 and 10:00 AM. The day sperm were found was designated Day 1 of pregnancy. The compounds studied were 2,3-bis (4-hydroxyphenyl) valeronitrile (SC-3402) and 2,-3-bis(4-methoxyphenyl)pent-2-enenitrile (SC-3296). Compounds were dissolved or suspended in corn oil and administered daily subcutaneously on the days indicated. Unless otherwise indicated, animals were autopsied approximately 24 hr after the last injection. The dose that prevented implantation in 50% of the animals treated (ED_{50}) was estimated by the method of Berkson (5).

General implantation tests. Groups of bred animals were treated daily from Day 1 through 7, Day 1 through 3, or from Day 4 through 7 of pregnancy and autopsied on Day 8 of pregnancy; implantation sites were counted and ED_{50} values were determined for each compound. In the strain of rats used in these studies, ova pass from the oviducts into the uterus on Day 4 of pregnancy and all have reached the uterus by 8:00 AM of Day 5. Therefore, the injection on Day 4 was given between 4:00 and 5:00 PM to minimize or eliminate interference with the oviduct phase of transport. All other injections were given during the morning between 8:00 and 9:00 AM.

Ova transport studies. The procedure was similar to the general implantation studies described above except that the period of treatment varied and animals were killed approximately 24 hr after the last injection. At autopsy physiological saline was flushed through the oviducts and the cornua of the uterus; the contents were collected into watch glasses; and the ova present were transferred to a hanging drop preparation for viewing with a microscope at a magnification of 100–800× to evaluate the stage of development and morphological condition of the ova.

Delay of implantation. The procedure was a modification of that described by Barnes and Meyer (6). Animals were injected subcutaneously with 4–8 mg 17α-acetoxy-6α-methylprogesterone (MAP) daily from Day 1 until the termination of the experiment. The ensuing delay in nidation ensured the presence of viable free blastocysts in the uterus for a

prolonged period of time. Test materials were then administered subcutaneously daily from Day 5 through 9. Some groups received further treatment with 1 μg estrone subcutaneously daily from Day 10 to autopsy on Day 15 for the purpose of either inducing implantation of unimplanted viable blastocysts or maintaining a possible pregnancy that may have been established earlier as a consequence of treatment with the test compound. Groups of animals were autopsied on Day 10 or Day 15, implantation sites were noted, uteri were flushed with physiological saline and washings were searched for blastocysts.

Deciduomata responses. Adult (200-g) rats with normal cycles were made pseudopregnant during estrus by cervical stimulation with a vibrating probe (7). The day of stimulation was considered Day 1 of pseudopregnancy and of the test. The establishment and maintenance of pseudopregnancy was confirmed from vaginal smears taken daily after application of the stimulus. On Day 5, a silk thread was inserted into the lumen of the left cornu of the uterus, threaded along its entire length, and left *in situ*. Subcutaneous injections of 100 or 200 μg SC-3402 were given daily in 0.2 ml of corn oil from Day 5 through Day 8. Control animals were similarly treated with vehicle. Each group contained 5–6 rats. Animals were autopsied on Day 9; uteri were removed and weighed. Differences between corresponding horns from control and treated groups were evaluated statistically using Student's *t* as a test criterion.

Results. Effect on implantation. Comparing ED_{50} values resulting from treatment periods of different length (Table I), SC-3296 was two to three times more potent than SC-3402. Anti-implantation activity was greatest when SC-3296 and SC-3402 were administered for the longer treatment period (Day 1 through 7). The ED_{50} was increased two or fourfold when the period of administration was limited to the first 3 days of pregnancy and eightfold when treatment was given between Day 4 and Day 7 of pregnancy. Estrone, on the other hand, was equally potent when administered either from Day 1 through 3 or Day 1 through 7 and only about twice as much was required to produce the same effect on fertility after treatment from Day 4 through 7 of pregnancy.

TABLE I. Comparison of Antifertility Activity after Administration of Compounds Subcutaneously for Varying Periods of Time after Mating.

Period of treatment[a]	Dose (μg) required to prevent implantation in 50% of the animals treated (ED_{50})[b]		
	SC-3296	SC-3402	Estrone
Day 1 through 3[c]	50 (15)	100 (20)	4 (20)
Day 4 through 7[c]	100 (15)	300 (20)	8 (20)
Day 1 through 7[d]	12 (40)	40 (50)	3.5 (40)

[a] Injections on Day 4 were given 4:00 to 5:00 AM and all other injections were given 8:00 to 9:00 AM.

[b] Numbers in parentheses represent the number of animals treated.

[c] Animals were autopsied on Day 8 of pregnancy.

[d] Animals were autopsied on Day 15 of pregnancy.

The antifertility activity of SC-3296 and SC-3402 were also studied in hamsters and rabbits. SC-3402 given daily subcutaneously from Day 1 through 7 of pregnancy prevented implantation in hamsters and rabbits at doses of 200 and 100 μg, respectively. SC-3296 was effective in the rabbit at a dose of 100 μg but had no effect in the hamster at doses of up to 800 μg.

Effect on transport of ova. These data are summarized in Table II. The fertilized ova recovered from control animals treated with corn oil were found only in the oviducts on Day 1, 2, and 3, distributed between the oviducts and uterus on Day 4 and exclusively in the uterus on Day 5 of pregnancy. In comparison to controls, treatment with 2 μg of estrone for 1, 2, or 4 successive days after mating had little or no effect on distribution or subsequent recovery of ova. Similarly, 5 μg of estrone was without effect when administered for 1 day or 2 days in succession but the number of ova, recovered on Day 5, was drastically reduced after 4 days of treatment. When the amount of estrone administered was increased to 10 μg, a slight reduction in the number of ova was observed 24 hr after a single injection on Day 1 and complete, or nearly complete, loss of ova occurred when

TABLE II. The Effect of Estrone, SC-3296, and SC-3402 Injected Subcutaneously after Copulation on Rate of Transport of Ova.

Compound	Treatment: Daily dose (μg)	Treatment: Days of injection	Day of autopsy[a]	No. of rats with ova/no. treated	Recovery of ova: Location (%): Oviduct	Recovery of ova: Location (%): Uterus	Recovery of ova: Total no.[b]
Corn oil	—	1	1	4/4	100[d]	0	35 (8.8)
		1	2	4/4	100	0	41 (10.2)
		1–2	3	4/4	100	0	36 (9.0)
		1–3	4	7/7	4	96	49 (7.0)
		1–4	5	6/7	0	100	56 (8.0)
Estrone	2	1	1	4/4	100[d]	0	39 (9.8)
		1–2	3	4/4	100	0	32 (8.0)
		1–4	5	3/4	4[c]	96	28 (7.0)
Estrone	5	1	1	4/4	100[d]	0	38 (9.5)
		1–2	3	4/4	100	0	28 (7.0)
		1–4	5	2/4	0	100	5 (1.2)
Estrone	10	1	1	4/4	100[d]	0	38 (9.5)
		1	2	4/4	100	0	26 (6.5)
		1–2	3	2/4	100	0	3 (0.8)
		1–4	5	0/5	0	0	0 (0)
SC-3296	16	1	5	6/8	0	100	24 (3.0)
SC-3296	64	1	1	4/4	100	0	52 (13.0)
		1	2	4/4	100	0	30 (7.5)
		1–2	3	4/4	55	45	19 (4.8)
		1–4	5	0/4	0	0	0 (0)
SC-3402	50	1	5	7/8	0	100	41 (5.1)
SC-3402	200	1	1	4/4	100[d]	0	37 (9.2)
		1	2	3/4	100	0	19 (4.8)
		1–2	3	1/4	100	0	1 (0.2)
		1–4	5	1/4	100[d]	0	1 (0.2)

[a] Animals autopsied on Day 1 were killed 8 hr after the single injection; all others were killed approximately 24 hr after last injection.

[b] Numbers in parentheses are the average number of ova recovered per treated animal.

[c] Normal unfertilized ova surrounded by corona radiata cells.

[d] Most ova still surrounded by corona radiata and cumulus cells.

treatment was continued for 2 or more days. After treatment with 64 μg SC-3296 for 2 days, the number of ova recoverable was greatly diminished and no ova were recovered after administration for 4 days. When the dose was decreased to 16 μg per day for 4 days the number of ova found in the uterus was also very small. The administration of 200 μg SC-3402 produced results qualitatively similar to those obtained with 10 μg of estrone. The 50-μg dose of SC-3402, given for 5 days, reduced the number of ova recovered to one-half that of control animals. None of the treated groups differed from control animals either in the percentage of ova classified morphologically as normal or in the distribution of ova according to developmental stages.

The effect of compounds during delay of implantation. In the first experiment (Table III), 60% of the control animals receiving 2 mg MAP only, implanted by Day 10, before the addition of estrone to the treatment schedule. However, these implantation sites

TABLE III. The Effect of SC-3402 and SC-3296 in Mated Intact Rats during Period of Delayed Nidation Induced with 17α-acetoxy-6α-methylprogesterone (MAP). All injections were given subcutaneously.

Daily treatment from Day 5–9 of pregnancy		Day of autopsy	No. rats with sites/no. treated	Total no. sites	Total no. blastocysts recovered[a]
Compound	Dose (μg)				
2 mg MAP daily from Day 1 of pregnancy through day preceding autopsy; 1 μg estrone Day 10–14					
SC-3402	200	10	3/5	5	0
SC-3402	200	15	2/5	2[b]	0
SC-3296	50	10	5/5	55	—
SC-3296	50	15	5/5	54[b]	—
Control	—	10	3/5	6	25 (4)
Control	—	15	5/5	45	—
2–8 mg MAP daily from Day 1 through 9 of pregnancy; uterus ligated on Day 5 at cervix					
SC-3402	200	10	2/8	10[b]	0
Control	—	10	3/7	30	24 (4)

[a] Numbers in parentheses are the number of treated animals from which blastocysts were recovered.

[b] Most implantation sites were in advanced stages of resorption.

were smaller than in controls, the total number was only minimal and nidation was obviously proceeding asynchronously since normal free blastocysts were also recovered from the uterus of each of the rats that had implantation sites. The number of unimplanted blastocysts recovered after flushing on Day 10 was reasonably compatible with the number of sites induced by subsequent estrogen treatments.

Three of five animals treated with 200 μg of SC-3402 during the delay period had only a few implantation sites on Day 10 prior to initiation of estrone treatment but unimplanted blastocysts were not found in the uterus. Resorption of implantation sites was in progress. Thus, nidation appeared to have been initiated but implantation failed in an early stage.

SC-3296 induced nidation before treatment with estrone in all animals. The number of sites was normal, but 40 of a total of 54 sites were in various stages of resorption.

The presence of blastocysts in the uterus on Day 10 in the control group and their absence in groups treated with SC-3402 in the above experiment suggested that blastocysts may have been expelled from the uterus as a consequence of the treatment. In order to explore this possibility, SC-3402 in corn oil or corn oil alone was administered to animals in which implantation was delayed with 2–8 mg/day of MAP and ligatures were placed around the cervical ends of the uterine horns on Day 5. In spontaneously nidating animals implantation sites were normal, but resportion of sites was occurring in animals treated with SC-3402. In animals that were devoid of sites, blastocysts were recovered from all animals given MAP but not from animals receiving MAP plus SC-3402.

Effect on formation of deciduomata. The weight of the traumatized cornu from control animals and rats given 100 μg and 200 μg SC-3402 was 749 ± 48, 623 ± 46, and 404 ± 50 mg, respectively, and the weight of the nontraumatized cornu was 159 ± 5.8, 284 ± 36, and 232 ± 12 mg, respectively. The depression in weight of the traumatized cornu was significantly different ($p<.05$) from the corresponding uterine horn of control animals only with the higher dose of SC-3402. In contrast the weights of the nontraumatized cornu from animals given both levels of

compound were significantly greater ($p < 0.05$) than in controls.

Discussion. Both SC-3296 and SC-3402 were four to five times more effective in inhibiting fertility when the compounds were administered while the ova were still in the oviducts (Day 1 through 3). SC-3296 ($ED_{50} = 12$ μg) was more potent than SC-3402 ($ED_{50} = 40$ μg) during both the oviduct and uterine preimplantation stages of pregnancy. However, the qualitative effects of the two compounds were similar to that of estrone, *i.e.*, both drugs interfered with fertility primarily by acceleration of the movement of ova through the oviduct. This effect was amply shown by the diminution in number of ova recovered on Day 3 of pregnancy and/or their early disappearance from the reproductive tract. The unfavorable consequences arising from premature arrival of cleaving ova into the uterus, has been well documented in the rat and rabbit (8–10). The morphologic condition of ova recovered from the oviducts and uterus from Day 1 through 5 of pregnancy was similar in groups treated with SC-3296, SC-3402, estrone, and the controls. Therefore, none of these compounds had observable direct effects on the developing ovum during the preimplantation stages of embryonic development.

The acceleratory effect of estrogens on transport of ova through the oviduct is well known (11–14). Therefore, the results obtained with the administration of from 5–10 μg estrone were anticipated and are in agreement with those of Banik and Pincus (15). A similar mechanism of action of SC-3296 and SC-3402 was suspected since these compounds had shown weak estrogenic activity in the rat vaginal smear assay (4). Using current estimates of estrogenicity, 7.5 and 0.25% respectively (Nutting, unpublished), to calculate estrone equivalents contained in 64 μg of SC-3296 and 200 μg of SC-3402, acceleratory doses of both compounds, values of 4.8 and 0.5 μg equivalents of estrone, respectively, were obtained. Approximately 10 μg of estrone were required to produce the same degree of acceleration. Therefore, the acceleratory potency of SC-3402 on ova transport was about 20 times greater than can be ascribed to an estrogenic effect while that of SC-3296 appears to be due to the inherent estrogenic activity of the compound. On the other hand, the implantation-inducing activity and estrogenic activity of the two compounds appears to be more closely correlated. In ovariectomized rats treated with progesterone and in intact rats treated with MAP, 0.5 (16) and 1 μg (6) estrone, respectively, induced nidation of delayed blastocysts. Therefore, on the basis of the estrogenic potencies of the two test compounds one would have exptected, as occurred, that 64 μg SC-3296 (4.8 equivalents estrone) would induce implantation in all delayed rats and 200 μg of SC-3402 (0.5 equivalent estrone) would induce it in about one-half of the animals. However, qualitative differences in the ability of the two compounds to induce implantation were evident. Although induction of nidation occurred with both compounds in the delayed-implantation studies, success in terms of the number of sites was far below normal with SC-3402. The absence of ova from these reproductive tracts, or from similarly treated animals with ligated uteri, coupled with the deciduoma-inhibiting activity suggests that SC-3402 triggers the initiation of the nidation process and then antagonizes subsequent progress toward completion of the process.

In conclusion, SC-3402 is an effective post coital antifertility compound in rats, hamsters, and rabbits. In rats, it affects fertility primarily by causing an increase in the rate of movement of fertilized ova through the oviduct. Neither of these compounds demonstrates cytocidal effects during the cleavage stages of development and they do not appear to exert blastocidal effect during normal pregnancy or pregnancy modified by an intervening period of delayed nidation. If initiation of treatment is delayed until the morula have reached the uterus, much larger doses are needed to prevent fecundity. The development of deciduoma is impaired and pregnancy is terminated during the early stages of implantation.

Summary. In rats the ED_{50} values for the antifertility activity of 2,3-bis(4-hydroxyphenyl)valeronitrile (SC-3296) injected sub-

cutaneously from Day 1–3, Day 4–7, or Day 1–7 of pregnancy were 50, 100, and 12 μg respectively and those of 2,3-bis(4-methoxyphenyl)pent-2-enenitrile (SC-3402) given for the same treatment periods were 100, 300, and 40 μg, respectively. Both compounds also prevented pregnancy in rabbits, but only SC-3402 was effective in hamsters. On the basis of the number of ova recovered from rats after flushing the reproductive tract, daily treatment with 5 μg of estrone, 64 μg of SC-3296, and 200 μg of SC-3402 accelerated ova transport. This effect appears to be independent of the weak estrogenic properties of SC-3402 but not of SC-3296. The delay of implantation, produced by 17α-acetoxy-6α-methylprogesterone, was interrupted by 50 μg of SC-3296 injected from Day 5–9 of pregnancy but not by 200 μg of SC-3402 or by 1 μg of estrone after treatment with SC-3402. Blastocysts were absent from flushings obtained from the uteri of the latter two groups even after ligation of the uterus at the cervix. Morphologically, no adverse effects on the preimplantation stages of the developing ova were observed. SC-3402 (200 μg) inhibited the formation of deciduoma in the rat.

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1. Pincus, G., *in* "Preimplantation Stages of Pregnancy" (G. E. W. Wolstenhome, ed.,) p. 378. Little, Brown, Boston, Massachusetts (1965).

2. Jackson, H., *in* "Antifertility Compounds in the Male and Female" p. 109. Thomas, Springfield, Illinois (1966).

3. Saunders, F. J., Physiol. Rev. **48**, 601 (1968).

4. Saunders, F. J. and Rorig, K., Fertility Sterility **15**, 202 (1964).

5. Berkson, J., J. Am. Statist. Assoc. **48**, 565 (1953).

6. Barnes, L. E. and Meyer, R. K., J. Reprod. Fertility **7**, 139 (1964).

7. DeFeo, V. J., Endocrinology **79**, 440 (1966).

8. Chang, M. C., J. Exptl. Zool. **114**, 197 (1950).

9. Dickman, Z. and Noyes, R. W., J. Reprod. Fertility **1**, 197 (1960).

10. Doyle, L. L., Gates, A. H., and Noyes, R. W., Fertility Sterility **14**, 215 (1963).

11. Burdick, H. O. and Pincus, G., Am. J. Physiol. **111**, 201 (1935).

12. Whitney, R. and Burdick, H. O., Endocrinology **24**, 45 (1939).

13. Greenwald, G. S., Fertility Sterility **12**, 80 (1961).

14. Deansley, R., J. Reprod. Fertility **5**, 49 (1963).

15. Banik, U. K. and Pincus, G., Proc. Soc. Exptl. Biol. Med. **116**, 1032 (1964).

16. Nutting, E. F. and Meyer, R. K., J. Endocrinol. **29**, 243 (1964).

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