

infection would continue and be manifest as a focus.

The biological importance of competence arises from the increased probability of survival which it imparts to the sarcoma genome. We have experimentally observed that on serial 3-day passages of the sarcoma virus in 3T3 cells (when the harvested virus contained few competent virions) the absolute titer of recoverable sarcoma virus rapidly declined to extinction (8).

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References and Notes

1. J. Harvey, *Nature* **204**, 1104 (1964).
2. J. Moloney, *Nat. Cancer Inst. Monogr.* **22**, 139 (1966).
3. R. Huebner, J. Hartley, W. Rowe, W. Lane, W. Capps, *Proc. Nat. Acad. Sci. U.S.* **56**, 1164 (1966).
4. J. Hartley and W. Rowe, *ibid.* **55**, 780 (1966).
5. R. Ting, *Virology* **28**, 783 (1966).
6. P. Sarma, W. Vass, R. Huebner, *Proc. Nat. Acad. Sci. U.S.* **55**, 1453 (1966).
7. T. O'Connor, in *Perspectives in Leukemia*, W. Dameshek and R. Dutcher Eds. (Grune and Stratton, New York, in press).
8. P. Fischinger and T. O'Connor, in preparation. The 3T3 cells were a gift from Dr. Green [G. Todaro and H. Green, *J. Cell Biol.* **17**, 299 (1963)].
9. M. A. Chirigos, K. Perk, W. Turner, B. Burka, M. Gomez, in preparation.
10. M. Pring, *Proc. Nat. Acad. Sci. U.S.* **58**, 607 (1967).
11. The technical assistance of Mrs. Carolyn Moore and Ellis Sheets is gratefully acknowledged.

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Pregnancy Following Coital-Induced Ovulation in a Spontaneous Ovulator

Abstract. Ovulation induced in the immature rat by pregnant mare's serum can be prevented by chlorpromazine. Coitus induces ovulation in such animals, and ovulation so induced may result in pregnancy. Both implantation and pregnancy appear to be normal although the duration of gestation may be prolonged. Decidual response also is produced in the immature rat after coitus-induced ovulation. We suggest that the reflex release of luteinizing hormone may occur in Primates after coitus.

Reflex ovulation following coitus and electrical or mechanical stimulation of the uterine cervix has been demonstrated in both immature and mature rats (1). Hence the necessary neural pathways for induction of reflex ovulation exist in an animal generally considered to be a spontaneous ovulator.

In earlier studies (1) we used immature rats treated with pregnant

mare's serum (PMS) and blocked the PMS-induced ovulation with chlorpromazine; the rats were then permitted to mate or were exposed to either electrical or mechanical stimulation of the uterine cervix. Ovulation occurred in all groups; the criterion for ovulation, and hence endogenous release of luteinizing hormone, was the presence of ova in the oviduct (2). This study was designed to demonstrate that these ova can be fertilized, will implant, and will undergo normal development.

Immature 28-day-old Purdue-Wistar rats were injected between 0800 and 1000 hours with 30 or 45 I.U. (international units) of PMS; 51 hours later each rat was injected with 0.75 mg of chlorpromazine per 100 g of body weight, this dose being sufficient to block the release of luteinizing hormone and prevent ovulation. Control rats received the same treatment except for the injection of chlorpromazine. The animals were then placed with mature male rats overnight, and vaginal smears were taken next morning to determine the presence or absence of sperm. The rats were either autopsied on day 9 after mating to determine whether implantation had oc-

curred, or permitted to go to term or close to term.

Mating occurred in 50 percent of the rats treated with 30 I.U. of PMS. The incidence of mating was increased to 83 and 87 percent in two separate groups by treatment with 45 I.U. of PMS, and dropped to 55 and 58 percent in two groups after treatment with 45 I.U. of PMS and the chlorpromazine (Table 1). Thus chlorpromazine appeared to interfere partially with the female's receptivity of the male, but approximately half of the females still mated.

Implantation of the blastocysts was noted in 100 percent of the mated rats treated with 30 I.U. of PMS and in 75 percent of the rats treated with 45 I.U. of PMS. Treatment with 45 I.U. of PMS and chlorpromazine decreased the percentage of rats with implantation sites to 43 percent, but the difference was not significant ($P > .05$). The percentages that we report of mated rats with implantation sites are comparable to the values obtained by others (3): our averages were 22.6 ± 2.4 percent for rats treated with 30 I.U. of PMS and 25.4 ± 2.4 percent for rats treated with 45 I.U. of PMS.

A number of rats were permitted to go to term or were killed on day 25 after mating if parturition had not occurred. Of those treated with PMS alone, 54 percent became pregnant. Of the 11 rats treated with PMS and chlorpromazine, 55 percent mated and 33 percent became pregnant. The difference is not significant ($P > .1$).

The tendency for fewer rats with implantation sites on day 9 or fewer pregnancies at term following treatment with both PMS and chlorpromazine, than with PMS alone, may reflect a direct effect of chlorpromazine on the ovary. Others have noted that chlorpromazine and similar drugs reduce or inhibit decidualoma as well

Table 1. Implantation and pregnancy after mating in the PMS-treated, immature rat, with and without blockage by chlorpromazine (CPZ) of spontaneous release of luteinizing hormone. Numbers of rats appear in parentheses.

Rats		Mated rats	
Treated with:		Showing sperm in vagina (%)	With implantation (%)
PMS (I.U.)	CPZ		
30(18)	No	50	100
45(12)	No	83*	75
45(12)	Yes	58	43†
45(15)	No	87	54
45(11)	Yes	55	33‡

* One additional rat had blastocysts on day 9 although no sperm were found in the vaginal tract on the morning after mating. † Not significantly different from controls without CPZ ($P > .05$). ‡ Not significantly different from controls without CPZ ($P > .10$).

Table 2. Decidual reaction in the immature rat after treatment with PMS or chlorpromazine (CPZ), or both, and mating. Number of rats appear in parentheses.

Rats		Traumatized horn: normal horn (av. wt %)	
Treated with:		Having decidualoma (%)	
PMS (I.U.)	CPZ		
45(9)	No	100	192.0
45(8)	Yes	88	92.4*

* Significantly different from controls without CPZ ($P < .05$).

as the number of ova produced in superovulated rats (4). Although the reduction in the number of mated rats presenting implantation sites after treatment with chlorpromazine is not significant, the trend is present. This fact led to an experiment in which the left uterine horn was traumatized on day 6 after treatment with PMS (Table 2); 100 percent of the rats showed a decidual response when treated with PMS only and 88 percent responded when chlorpromazine also was given. However, the increase in the weight of the traumatized horn, as compared with the control horn, was 192 percent for the first group and 92 percent for the group receiving chlorpromazine, a difference significant at the 5-percent level. Thus the decreased sensitivity of the uterine endometrium may be a factor in the decreased incidence of implantation in the rats treated with chlorpromazine.

Parturition failed to occur earlier than day 23 of pregnancy; in some rats it occurred as late as day 25. The average period of gestation for our colony is 21 days. On autopsy the rats that failed to deliver by day 25 of gestation contained viable young *in utero*. Comparison of size of fetuses *in utero* from different animals on the same day of pregnancy, as well as the degree of resorption occurring, suggested delayed nidation. Prolongation of gestation by from 2 to 11 days has been reported in immature rats treated with PMS and human chorionic gonadotropin, and in 33-day-old rats treated with PMS alone (3). Thus there appears to be an initial hormonal imbalance that is not conducive to implantation at the normal time. Although it has not been ascertained whether the initial levels of estrogen and progesterone are too high or too low for promotion of nidation, available evidence suggests that production of estrogen may be prodigious (5). Treatment with PMS may cause a greater degree of thecal cell luteinization than is normal, and this possibility could account for greater production of estrogen by the corpora lutea.

These data indicate that the ovum produced by the immature rat after coitus can be fertilized and developed into a normal fetus. Thus reflex ovulation, in response to coitus, can result in pregnancy in a spontaneous ovulator such as the rat. This demonstration of coitus-induced ovulation followed by pregnancy supports our earlier pro-

posal (1) that ovulation and pregnancy may occur in the spontaneous ovulator after coitus. Such occurrence could explain the reports of women's conception just before, during, and immediately after menstruation (6).

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References and Notes

1. C. Aron, G. Arch, J. Ross, *Intern. Rev. Cytol.* **201**, 139 (1966); R. R. Carlson and J. DeFeo, *Endocrinology* **77**, 1014 (1965); J. W.

- Everett, *ibid.* **80**, 145 (1967); F. E. Harrington, R. G. Eggert, R. D. Wilbur, W. H. Linkenheimer, *ibid.* **79**, 1130 (1966); M. X. Zarrow and J. H. Clark, *J. Endocrinol.*, in press.
2. M. X. Zarrow, A. L. Caldwell, E. S. E. Hafez, G. Pincus, *Endocrinology* **63**, 748 (1958).
3. H. H. Cole, *Amer. J. Physiol.* **119**, 704 (1937); *Science* **91**, 436 (1940); H. M. Evans and M. E. Simpson, *Endocrinology* **27**, 305 (1940).
4. V. J. DeFeo, *Anat. Rec.* **127**, 409 (1957); A. Psychoyos, *J. Endocrinol.* **27**, 337 (1963); M. X. Zarrow and K. Brown-Grant, *ibid.* **30**, 87 (1964).
5. D. T. Armstrong and R. O. Greep, *Endocrinology* **76**, 246 (1965); E. G. Rennels and G. G. Guillet, *Anat. Rec.* **145**, 274 (1963); H. M. Teel, *Amer. J. Physiol.* **79**, 184 (1926).
6. B. B. Rubenstein, *Ohio State Med. J.* **35**, 1066 (1959); S. Sevtit, *Lancet* **1946-II**, 448 (1946).
7. Aided by NIH grant HD-02068. The PMS was obtained by courtesy of J. B. Jewell, Ayerst Laboratories, New York.

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Intracerebral Saline: Effect on Memory of Trained Mice Treated with Puromycin

Abstract. *It was shown that puromycin administered to mice 1 or more days after maze-learning blocks expression of memory; the blockage can be removed by intracerebral injections of saline. We present evidence that intracerebral injections of saline are relatively ineffective in restoring memory when puromycin is administered either before or immediately after training; in these two situations puromycin appears to interfere with consolidation of memory.*

We have reported that expression by mice of memory of maze-learning, lost after treatment with puromycin, can be restored by small intracerebral injections of saline (1). More recently we have found sham injections to be ineffective in removing puromycin's blockage of memory. In all these experiments puromycin was injected 1 or more days after training in an amount demonstrated to cause essentially complete suppression of memory for at least 3 months—the longest duration of these retention tests. A single treatment with intracerebral injections of saline, as late as 60 days after the puromycin, proved capable of restoring expression of memory.

Our most recent experiments attempted to unmask whatever memory may be established when training is conducted in the presence of puromycin, or when puromycin is injected immediately after training. Barondes and Cohen (2) observed that when mice were trained to a Y-maze in the presence of puromycin they retained memory of the maze at a high level for less than 45 minutes, and that memory was virtually lost after 3 hours. Agranoff, Davis, and Brink (2) found memory of training in goldfish to be lost when puromycin was given immediately after training; they concluded that puromycin in this situation interferes

with consolidation of memory. There is the possibility in both these instances, as in those that we studied previously, that puromycin blocks expression of memory without alteration of the process that maintains the basic memory trace. We have used intracerebral injections of saline, given several days after training, to test this possibility.

All intracerebral injections, 12 μ l in volume, were made bitemporally (3) in albino mice weighing 28 to 32 g; each injection contained 90 μ g of puromycin or isotonic NaCl, and was made 5 days after the injection of puromycin.

Our usual behavioral procedure (3)

Table 1. Retention of memory by mice treated with puromycin and, except for controls, 5 days later with saline. Group A: puromycin given 5 hours before discrimination-training. Group B: puromycin given 4 to 8 minutes after training. Percentages of savings of trials and errors for the mice that retained memory were, respectively, 94 ± 7 and 96 ± 4 percent (means \pm S.D.); for those with impaired memory, 37 ± 26 and 57 ± 19 percent; for those that lost memory, 1 ± 4 and 1 ± 4 percent.

Group	Memories (No.)		
	Lost	Impaired	Retained
Control A	13	1	0
Saline A	9	5	3
Control B	7	0	0
Saline B	4	9	6