antigen is of relatively low molecular weight (~25,000) and, with brief centrifugation, is found on top of the gradient; however, after overnight centrifugation through a 5- to 20-percent sucrose gradient, the group-specific antigen clearly separates from the degraded RNA.

These data support the concept of virolysis by antibody and C′ because intraviral components are released by their combined action. That antibody to an "internal" virion antigen was nonlytic, confirms the specificity of the antiserum and the true internal localization of the group-specific antigen. These observations would appear to provide the basis for a relatively rapid technique for detecting envelope antibody to C-type viruses and, by inhibition methods, the corresponding antigen. Contributions of host cell antigens to the viral envelope may also be evaluated by this method.

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10 March 1970

Copulatory Behavior Can Inhibit Pregnancy in Female Rats

Abstract. *If female rats received genital stimulation soon enough after their male partners had ejaculated, sperm transport and subsequent pregnancy were inhibited. Manual stimulation by the experimenter or five intromissions by a male rat were sufficient stimuli to reduce the number of sperm found in the uterus and to reduce the number of uterine implantation sites.*

During copulation the male rat mounts and dismounts from the female a number of times. On some of these mounts he inserts his penis into the vaginal orifice; on the final insertion of a series he ejaculates sperm and an enzymatic coagulate, the vaginal plug, into the female's vagina. The amount of vaginal stimulation the male's intromissions provide during copulation is correlated with probability of pregnancy: the more often he intromits, the more probable it is that the female will become pregnant (*1*, *2*).

There are two major prerequisites for normal pregnancy: (i) fertilization of the eggs and (ii) secretion of hormones, such as progesterone, which permit the implantation and maintenance of the embryos in the uterus. Because this hormonal response can be initiated artificially, without the introduction of sperm, it has been called pseudopregnancy. The male's copulatory behavior promotes both pseudopregnancy and fertilization.

Fertilization depends upon the male's copulatory behavior because the intromissions before ejaculation facilitate the passage of sperm through the tightly closed cervix into the uterine lumen (*2*). The effects of such copulatory behavior on sperm transport and pseudopregnancy have been verified in other laboratories (*3*).

When taking vaginal smears, we noticed that if too much vaginal stimulation had been given to a female rat after copulation the sperm normally found in her uterus were absent. If the male rat's copulatory behavior could have such an effect, this interference with sperm transport would be a major influence on the occurrence of successful pregnancy in the rat. The experiments reported here deal with this inhibitory effect of vaginal-cervical stimulation following ejaculation, and the implications of this phenomenon for successful pregnancy.

One hundred and thirty Sprague-Dawley female rats were housed in community cages and had continuous access to food and water. To facilitate behavioral observations, we maintained the colony room on a reverse light cycle: 14 hours of lights off, 10 hours of lights on; the mid-point of the dark portion was 1:30 p.m.

To determine the number of sperm present in the uterus after copulation, we anesthetized each female and performed a laporotomy. Approximately 2 ml of Liqui-Nox was mixed with 10 ml of water; 0.1 ml of this soap solution was injected into each uterine horn, the fluid was mixed, and the entire fluid contents of the uterus were withdrawn. Sperm counts were made on a hemocytometer (*4*).

In the first experiment, 43 female rats were allowed to copulate with males until each ejaculated. The male was then removed. Normally, approximately 90 percent of such females would have become pregnant (*1*, *2*). In this experiment, however, we took the females out of the cage at various times after each had received the ejaculate, and manually stimulated the cervix with the barrel of a plastic syringe. One hour after the ejaculation, each female was killed and her uterine horns were examined for sperm.

Females were stimulated at times ranging from a few seconds up to almost an hour after ejaculation (Table 1). Cervical stimulation inhibited sperm transport most effectively if it came within 15 minutes after ejaculation. Although the median number of sperm found in the uterus 1 hour after mating was 446×10^5, this amount was reduced by a factor of four when the cervical stimulation came within 15 minutes after ejaculation; 44 percent of the females in this group had no sperm whatever in their uteri. During this initial quarter hour, inhibition was greatest during the first 4 minutes after ejaculation. (The average sperm count with stimulation during the first 4 minutes was 2.7×10^5; with stimulation between 4 and 15 minutes, the average was 272×10^5. This difference was significant at $P < .005$ by the t-test.) Sperm transport was more likely to occur if stimulation followed ejaculation by more than 4 or 5 minutes.

Since sperm counts were always made 1 hour after the male ejaculated and since the time of cervical stimulation

therefore varied between a few seconds and almost an hour after ejaculation, it was possible that sperm would have been present if the female had been examined later. To determine whether genital stimulation after ejaculation had permanently excluded sperm from the uterus and would thus have prevented fertilization, the females were treated as described above except that they were not killed after mating. Instead, they were housed in individual cages for the next 20 days. Then they were dissected, and the number of uterine implantations were counted. Two females did not become pseudopregnant and were excluded from the data (*5*). Although females who copulate and received no vaginal stimulation after ejaculation have, on the average, 13.5 implantation sites, females who received the genital probing within 15 minutes after copulating had an average of only 2.4 implantation sites; 82 percent of these females had no fetuses at all (Table 2). After 15 minutes the stimulation was much less effective in decreasing the litter size. Since the females included in this study all became pseudopregnant, the decreased litter size was probably due to some interference with the process of sperm transport and fertilization and not with pseudopregnancy; cervical stimulation after ejaculation probably excluded sperm from the uterus permanently.

In the first experiment, the experimenter stimulated the rat's cervix after copulation. Could the male's intromissions also have this inhibitory effect? In another experiment, females copulated until they received an ejaculation; at various times after the ejaculation they were placed with a second male who was allowed five intromissions, but no ejaculation. Sperm counts were taken 1 hour after the ejaculation of the first male (Table 1).

When the five intromissions came within the first 15 minutes after ejaculation, the mean sperm count was less than one-tenth the control value. Approximately 29 percent of the females had no sperm in their uteri. Again this inhibitory effect of the intromissions diminished after 15 minutes. A relatively small number of intromissions, therefore, are able to interfere with a preceding ejaculatory series. Behavior can disrupt pregnancy.

To explore the functional ramifications of this phenomenon, we allowed albino females to mate with either an albino male or a pigmented male. Each female was then placed with three males of the other coat color. These males were allowed to intromit until one ejaculated. The female was removed and housed in an individual cage until she gave birth. The offspring of these matings identified the fathers: pups sired by albino males had light skins and unpigmented eyes whereas pups sired by pigmented fathers had dark skins and black eyes. To control for differences in fertility of the two types of males, both types of mating sequences were used: (i) albino first, pigmented second; and (ii) pigmented first, albino second (Table 3). In the case of matings with an albino male first, and a pigmented male second, if the second copulatory bout began within 15 minutes after the first ejaculation, 66 percent of the offspring were pigmented (products of the second mating). The most likely mechanism for this effect is that the second male's intromissions caused the expulsion of the first male's sperm from the female's reproductive tract (*6*). The second male then ejaculated, and his sperm entered the uterus. The second mating had functionally canceled the first.

After 15 minutes, the effect of this

Table 1. The effect of genital stimulation after ejaculation on uterine sperm content. A given female's sperm count was reduced if the probability of obtaining such a count was $< .05$, given the control group as the standard.

Time between ejaculation and stimulation (min)	Females (No.)	Sperm count $\times 10^5$ (mean)	Females with sperm passage (%)		
			Normal	Reduced	None
		Manual stimulation			
Control	14	446	86	14	0
0-15	25	99*	16	40	44
15-30	7	288†	57	28	14
30-45	6	202‡	50	33	17
45-60	4	464	100	0	0
		Stimulation from five intromissions			
0-15	7	39*	28.5	43	28.5
15-30	4	464	100	0	0
30-45	6	363	100	0	0
45-60	6	341	66	0	33

* $P < .001$; † $P < .1$; ‡ $P < .05$.

Table 2. Implantations as a function of genital stimulation.

Time between ejaculation and stimulation (min)	Females (No.)	Implantations (average No.)	Females with implantations (%)		
			Normal	Reduced	None
Control	8	13.5	100	0	0
0-15	11	2.4*	18	0	82
15-30	5	14	83	17	0
30-45	6	6	83	17	0
45-60	7	8.8	57	14	28

* $P < .01$.

Table 3. Effect of different mating sequences on paternity of offspring. Statistical evaluation by the binomial test.

Time between ejaculation and intromission (min)	Females (No.)	Pups (No.)	Offspring produced by ejaculation (%)	
			First	Second
		Albino first, pigmented second		
0-15	5	62*	34	66
15-30	5	59	54	46
30-45	4	57†	88	12
45-60	5	60†	77	23
		Pigmented first, albino second		
0-15	6	57†	26	74
15-30	3	31†	8	92
30-45	3	37†	3	97
45-60	4	43†	16	84

* $P < .025$; † $P < .001$.

inhibition began to decrease. In the case of the pigmented first, albino second matings, however, the offspring were primarily albino (second mating) even when the stimulation came as late as an hour after the first mating. The long-lasting inhibitory effect in the pigmented first, albino second mating may have been the result of less hardy sperm being ejaculated by the highly inbred pigmented male (*7*). In a competitive situation, therefore, it appears a male can cancel the effects of a previous male's copulation if the second male begins intromitting soon enough.

We previously demonstrated that the copulatory intromissions were necessary to initiate both sperm transport and the hormonal conditions of pregnancy (pseudopregnancy) (*1*, *2*). We now suggest that copulatory behavior has a disruptive effect on pregnancy if the intromissions occur too soon after a prior ejaculation. There are other situations in which reproductive processes are blocked: the odor of strange males inhibits implantation in female mice (*8*); sperm seem rejected by the bursa copulatrix of drosophila females when an interspecific mating has occurred (*9*); prolonged auditory stimulation reduces fertility in rats (*10*); and social crowding reduces reproductive success in rats and house mice (*11*, *12*).

The phenomenon discussed here, the inhibitory effect of copulation, may be related to the social structure of rodents. After ejaculation, there is a period during which the male is sexually unresponsive (*13*). This interval varies between 4.6 and 11.9 minutes, depending on which ejaculatory series is being considered. (In this study, the female was most susceptible to inhibitory cervical stimulation during the first 4 minutes after a male's first ejaculation). Although precise data are needed in order to describe the nature of rodent copulatory behavior in settings more natural than the laboratory, it may be that the normal pacing of sexual behavior and the periods of sexual refractoriness ensure the tranquility necessary for sperm transport.

It is, however, already known that under conditions of crowding, laboratory rats show persistent social pathology (*12*). One type of behavioral abnormality is a kind of pansexual behavior in which males mount at a much higher rate than usual. In these crowded colonies, reproduction decreases; part of this decrease may result from the mechanism described in this report. Even among normal colonies, colonies of rats with low social rank have more males than groups of higher social rank; and the low ranked groups have a reduced number of pups born (*14*). One of the mechanisms responsible for the decreased reproductive performance in low-ranking normal groups, as in the abnormally crowded groups, might be copulatory interference with sperm transport. In short, sexual behavior not only stimulates pregnancy, but under certain circumstances inhibits it.

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8 April 1970

Partial Reversion in Yeast: Genetic Evidence for a New Type of Bifunctional Protein

Abstract. *Two kinds of phenotypic expression in purine biosynthesis result from recessive mutation to* ade12 *in baker's yeast. The mutants are adenine-specific, blocked in the conversion of inosine 5′-phosphate to adenylosuccinic acid; their response to inhibition of pathway activity by adenine is considerably reduced. Allelic partial reversions can restore prototrophy without correcting the regulatory defect imparted by the primary mutation. The separation of the two properties of the locus by allelic mutation supports the hypothesis that the locus specifies a protein of two independent functions, enzymatic and regulatory.*

A new physiological role for proteins was established with the identification and isolation of protein repressors as regulators of metabolic activity. Our studies on the genetic regulation of purine biosynthesis in yeast now raise the possibility that a single protein may combine within itself the dual roles of enzyme and repressor.

Single-event mutations at the *ade12* locus (*1*) of *Saccharomyces cerevisiae* produce two distinct phenotypic effects. (i) There is a loss of adenylosuccinate synthetase (AS), the penultimate step in adenosine 5′-phosphate (AMP) biosynthesis, as indicated by (a) the appearance of a nutritional requirement for adenine that is not satisfied by hypoxanthine, (b) the presence of inosine as a characteristic accumulant in cell extracts, and (c) growth-dependent excretion of hypoxanthine (*1*). (ii) The normal regulation of purine biosynthetic activity is modified toward constitutive synthesis. This effect of *ade12* is demonstrable (*1*) in the presence of either of the red pigment-forming, adenineless mutations, *ade1* or *ade2* (*2*, *3*). Strains that are mutant only at *ade1* or *ade2*, or both, are white when grown with excess adenine sulfate [75 μg/ml in GBHA (*4*)], although they routinely develop pigment at the customary supplemental levels of adenine (10 to 20 μg/ml), or on yeast extract–peptone medium (YEP) (*4*). With the addition of the *ade12* mutation to make the combinations *ade1 ade12* or *ade2 ade12*, the cells turn pink, then