hybrid cells were simultaneously tested for the presence of EBV antigens by the indirect immunofluorescence test and by electron microscopy. Cover slip monolayer cultures of the four clones of D98/HR-1 and of D98 cells were prepared. Hybrid and D98 cells were grown in 250-ml tissue culture flasks, treated in a similar manner as the cells in the hybridization experiment, and examined by electron microscopy. The cells were examined for virus antigens and virus particles 7 days after removal of medium containing IdU, a time corresponding to the assay of virus DNA in IdU-treated cells. Cells grown on cover slips were fixed in acetone and examined for EBV antigens with an antiserum against EBV (obtained from K. Traul, Pfizer, Inc.). Simultaneously, the hybrid cells grown in tissue culture flasks were fixed and examined by electron microscopy.

The results of the DNA-RNA hybridization experiments are shown in Table 1. All four clones tested contained DNA of EBV in varying genomic equivalents. Earlier experiments indicated that the D98 human parent cell contained no virus genomes, whereas the HR-1 Burkitt lymphoblastoid cell line, which was actively synthesizing virus, contained at least 680 genomes per cell (*5*). The number of genome equivalents in the D98/HR-1 hybrids ranged from 4 per cell in clone 2 to 18 per cell in clone 3. The number of genome equivalents in clone 1, initially 11 per cell, increased to 97 per cell after treatment with IdU. The IdU-treated cells were positive for EBV antigens by immunofluorescence, with up to 20 percent of cells containing EBV antigens in the nucleus or the cytoplasm. Virus particles were also observed by electron microscopy (*4*).

The data from the DNA-RNA hybridization experiments reveal that the somatic cell hybrids contain EBV genomes, even when the cells express no EBV markers, an observation also true of EBV-negative cell lines NC37 and F265 (*2*). The induction of virus antigens and virus particles with IdU indicates that the latent EBV genome can be induced to replicate. The induction of virus DNA is shown by the increase in hybridizable virus DNA in cells treated with IdU. Complete and incomplete virus particles, detectable by electron microscopy, also appear after IdU treatment.

The EBV genome is repressed in the somatic cell hybrids, although it is expressed in the parental HR-1 cells. This would suggest that information supplied by chromosomes of the parental D98 cells aids in suppressing expression of EBV information. The infectivity and oncogenic potential of the EBV recovered from the somatic cell hybrids following induction by IdU remains to be established.

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References and Notes

1. M. A. Epstein, B. G. Achong, Y. M. Barr, *Lancet* **1964-I**, 702 (1964); M. A. Epstein, G. Henle, B. G. Achong, Y. M. Barr, *J. Exp. Med.* **121**, 761 (1965).
2. B. Hampar, J. G. Derge, L. M. Martos, J. L. Walker, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 3185 (1971); P. Gerber, *ibid.* **69**, 83 (1972); K. Sugawara, F. Mizuno, T. Osato, *Nature* **239**, 242 (1972).
3. R. Glaser and F. J. O'Neill, *Science* **176**, 1245 (1972).
4. R. Glaser and F. Rapp, *J. Virol.* **10**, 288 (1972).
5. H. Zur Hausen and H. Schulte-Holthausen, *Nature* **227**, 245 (1970); M. Nonoyama and J. S. Pagano, *ibid.* **233**, 103 (1971).
6. J. W. Littlefield, *Science* **145**, 709 (1964).
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Sexual Behavior in Rhesus Monkeys after Vasectomy

Abstract. *Vasectomy had no statistically significant effect on the sexual behavior of vigorous adult rhesus monkeys. Of two groups of males matched for level of sexual performance, one was vasectomized and the other underwent sham vasectomy. In sex tests administered within the month after surgery, the groups showed no difference ($P > .05$) in rate of mounting, intromission, or ejaculation, or in any other measure of sexual or sex-related behaviors.*

Fifty years ago hundreds of men in the United States, lured by the hope of experiencing sexual rejuvenation, were vasectomized by overenthusiastic clinicians who used as their rationale the results of poor research (*1*). Vasectomy proved to be no fountain of sexual vigor. In 1970 an estimated 750,000 men were vasectomized in this country as a contraceptive measure (*2*). The number of vasectomies has been rising rapidly despite complaints during the past decade of such side effects as impotency, decreased control over ejaculation, and other psychiatric symptomatology (*3*). However, Thompson and Illsley (*4*) noted that impotency is not a likely consequence of vasectomy and that if it does occur, it is probably only temporary; they conceded, however, that "the problem has not been adequately researched" (*4*, p. 2).

Psychological factors are often cited as being particularly important in determining the advisability of vasectomy as a method of birth control. In addition, numerous questions about the anatomical, neuroendocrinological, and immunologic consequences of vasectomy remain unanswered. Appropriate pre- and postsurgical care and operative techniques need to be standardized if the operation is to be widely adopted as a satisfactory contraceptive measure and if the effects of vasectomy are to be correctly assessed. Not only do we need to know what aspects of sexual behavior change after vasectomy and to what extent, but we must identify which changes are due directly to physiological factors and which to cultural and psychological factors.

To this end we vasectomized a number of adult male rhesus monkeys whose sexual behavior was studied before and after surgery. Despite their resemblance to man, these primates can be expected to be free of such emotions

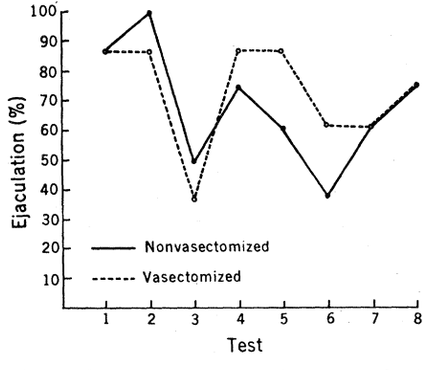

Fig. 1. Mean percentage of vasectomized and nonvasectomized males ejaculating in eight tests (two per week) of sexual behavior after surgery.

as guilt and castration anxiety and the many superstitions that often complicate sexual behavior in man. Moreover, changes in their sexual behavior after vasectomy cannot, we assume, be attributed to economic, social, or cultural factors. Released by vasectomy from the fear of causing pregnancy, many men report increased pleasure and frequency of sexual intercourse. Associating the operation with castration and a loss of manhood, on the other hand, may give rise to castration anxieties and a depressed level of sexual performance (*3*). The fact that we found no statistically significant differences in performance between the control group (after sham vasectomy) and experimental group (after vasectomy) of rhesus monkeys suggests that the changes reported in man are in fact psychological in origin.

All subjects, male and female, were wild-born animals. The males had been in the laboratory at least 2.5 years and had had previous experience in the test situation; the female partners had been in the laboratory for about 5 years and had been ovariectomized for about the same length of time. They were rendered receptive by being injected with estradiol benzoate before being used as test partners.

Twice a week each male had access to a large cage containing an estrogen-primed female. The moment the male entered the cage a clock was started, and the latencies (the time lapses between the beginning of the test and the particular responses) to the first mount, intromission, and ejaculation were recorded. The test lasted for 15 minutes or until the male ejaculated. The frequency of occurrence of various responses was recorded and analyzed in terms of rate per minute. The analysis of rate was used because the duration of tests varied depending on latency to ejaculation.

After two blocks of eight tests spanning a 2-month period were completed, the males were divided into two groups of eight animals each in such a way that no significant differences existed in any measure between the two groups. The vasectomies and sham vasectomies were performed aseptically under general anesthesia. About 1 cm of the vas deferens was removed, and both ends were ligated. Blood was taken from each male just before vasectomy and at weekly and later monthly intervals after surgery for studies of the formation of antibody to spermatozoa (*5*).

One week after vasectomy eight postoperative tests of sexual behavior were administered in a 4-week period according to the same testing procedure used before surgery. Performance in these tests was compared with that in the eight preoperative tests.

Table 1. Mean rates and mean latencies for four components of sexual behavior before and after sham vasectomy (control, C) and vasectomy (experimental, E) in adult male rhesus monkeys. None of the differences between control and experimental males or before and after vasectomy for the two groups are statistically significant ($P > .05$).

Behavior	Before vasectomy		After vasectomy	
	C	E	C	E
Rate (per minute)				
Contact	1.35	1.46	1.19	1.39
Mounting	1.23	1.33	1.09	1.21
Intromission	0.89	1.13	0.78	0.95
Pelvic thrusting	6.27	7.90	5.79	6.75
Latency (minutes)				
First mount	1.60	1.67	2.08	1.72
First intromission	3.15	2.35	2.91	2.47
Ejaculation	5.76	4.83	5.24	4.24

The behavior of the vasectomized and nonvasectomized males showed no statistically significant differences. Males in the control group developed erections in an average of 87.5 percent of the tests before sham vasectomy and in 81.2 percent of the tests afterward. In the experimental group, the percentages were 96.9 before vasectomy and 90.6 after this surgery. A *t*-test (two-tailed) was used to evaluate differences, and $P = .05$ was adopted as the level of statistical significance.

The rate of occurrence of such components of sexual behavior as contacting the female, mounting, intromission, pelvic thrusting, pelvic thrusts per intromission, and ejaculation (Fig. 1, Table 1) did not differ between the two groups, nor were latencies to the first mount, intromission, and ejaculation significantly different. The frequency of other behaviors often associated with sexual behaviors, such as grooming, threatening, yawning, low-intensity threat, and aggression, was alike for vasectomized and nonvasectomized animals. The females made no distinction between the two groups of males in such behaviors as presenting (to contact by the male or at a distance without physical contact), grooming, fear grimacing, threatening, aggression, and sitting next to the male (proximity response).

The behaviors mentioned above have specific meaning in the formal test situation. A contact, for example, is said to occur only when the male places one or both hands on the hips of the female in the area in which the hands are held during a complete adult male mount. Touching the female on any other part of the body, including the external genitalia, is not classified as a contact. Presenting is likewise specifically defined and is comparable to the lordosis response in rodents. A female rhesus who simply stands still is not considered to be in a present posture. The legs and arms must be extended and the tail deviated for the behavior to be classified as a present response. What we record as a fear grimace is a particular facial expression that may be totally unrelated to the experience of fear in man. Additional detailed descriptions of individual behavioral items are omitted here because they are not essential to an understanding of the results of this study.

When the individual's performance before and after vasectomy was compared, statistical analysis by means of the Wilcoxon *T*-test showed no significant changes for any measure of behavior. Mean frequency of ejaculation was lower after surgery, but the difference was not statistically significant ($T = 4$, $P > .05$), and the experimental and control groups did not differ significantly from each other.

Any discomfort that may have resulted from the operation did not interfere with the display of sexual behavior 1 week after surgery, and no differences were evident during the first postoperative month. However, because modification of behavior may not appear until much later when immunological changes (if any) occur, these animals should be checked for long-term behavioral and immunological changes.

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References and Notes

1. C. R. Moore, in *Sex and Internal Secretions*, E. Allen, Ed. (Williams & Wilkins, Baltimore, 1932), pp. 281–371.
2. W. M. Moss, *Fert. Steril.* **23**, 33 (1972). Moss cites the Association for Voluntary Sterilization as the source of the estimate.
3. A. K. Datta, *J. Indian Med. Ass.* **50**, 52 (1968); A. S. Ferber, C. Teitze, S. Lewit, *Psychosom. Med.* **29**, 354 (1967); F. J. Ziegler, D. A. Rodgers, R. J. Prentiss, *Arch. Gen. Psychiat.* **21**, 46 (1969); D. A. Rodgers, F. J. Ziegler, N. Levy, *Psychosom. Med.* **29**, 367 (1967).
4. B. Thompson and R. Illsley, *Int. Planned Parent. Fed. Med. Bull.* **6**, 1 (1972).
5. N. J. Alexander, unpublished results.
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