m by 0.32 cm, inside diameter) containing 3 percent OV-17 on Gas Chromosorb Q. After sample injection, the column was maintained isothermal at 60°C for 2 minutes followed by an increase of 10°C per minute until 300°C was reached. The flash heater was maintained at 220°C and the separator at 300°C. Helium flow was 26.4 ml/min.

7. *S*-methyl thioacrylate was prepared by the dicyclohexyl carbodiimide coupling of acrylic acid and methanethiol in methylene chloride. This compound consumed 1 mole of HBr to form *S*-methyl 3-(bromo)thiopropionate (M^+, m/e = 182, 184, 186) and 1 mole of bromine to form *S*-methyl thio-2,3-(dibromo)thiopropionate (M^+, m/e = 260, 262, 264, 266). This compound also reacted with methanethiol to form the β-methanethiol addition compound. The *S*-methyl 3-(methylthio)thiopropionate was also prepared from 3-thiopropionic acid by methylation with methyl iodide in aqueous base followed by formation of the thiol ester by dicyclohexyl carbodiimide coupling in methylene chloride with methanethiol.
8. *S*-Methyl thioacrylate and *S*-methyl 3-(methylthio)thiopropionate gave the following mass spectra m/e (relative intensity) 104 (2.5), 103 (3.0), 102 (45), 75 (6.0), 55 (100), 47 (11), 45 (12); and 152 (3.5), 151 (2.5), 150 (36), 104 (44), 75 (61), 61 (100), 47 (35), 45 (27), respectively. Under the described GC conditions, the former compound had a retention time of 2.6 minutes and the latter 11.6 minutes. Dimethyl sulfone had a retention time of 8.3 minutes.
9. Dimethyl trisulfide was prepared from the corresponding Bunte salt [B. Milligan, B. Saville, J. M. Swan, *J. Chem. Soc. (Lond.)* (1963), p. 3608. Tetrahydrothiophene was prepared by the method of C. S. Marvel and W. W. Williams [*J. Am. Chem. Soc.* **61**, 2714 (1939)].
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Immunological Tolerance: Transmission from Mother to Offspring

Abstract. *Mice born and raised by mothers made specifically unresponsive to heterologous erythrocytes by prior treatment with soluble extracts of these cells become themselves tolerant. Tolerance is achieved through nursing since normal neonates fostered by tolerant animals become tolerant, while animals born to tolerant mothers but nursed by normal mice are fully responsive.*

Since theoretical considerations led Burnet and Fenner to postulate that exposure to antigens in prenatal or perinatal life might make an animal unresponsive to the same antigen presented to it later in life, the conceptual framework of immunological tolerance has broadened to include a variety of conditions in which unresponsiveness can be induced not only early in the developmental history of an animal but in adult life as well. While a distinction may be made between transplantation tolerance as originally defined by Billingham, Brent, and Medawar and tolerance to protein or polysaccharide antigens, that distinction has become operationally clouded as the complex interplay among such factors as transplantation antigens, T cells, B cells, and serum antibody and antigen-antibody complexes has been analyzed for the various systems collectively considered as manifestations of immunological unresponsiveness or tolerance (*1*).

Over the past 3 years work from this laboratory has described a system for inducing specific unresponsiveness to heterologous erythrocytes by use of a high-speed supernatant derived from red blood cells lysed in hypotonic solution. Sheep hemolyzate supernatant (SHS) or horse hemolyzate supernatant (HHS) derived, respectively, from sheep red blood cells (SRBC) or horse red blood cells (HRBC), when injected into adult mice, severely reduced the subsequent response of such animals to antigenic challenge by the appropriate intact erythrocyte (*2*). We have reported that the material is nonantigenic as defined by its inability to elicit spleen antibody-forming cells (PFC), its failure to boost a primary response previously induced by intact erythrocytic challenge, and its failure to increase over a 4-week period the serum antibody titer as measured by hemagglutinin or hemolysin titrations. Tolerance was found to be specific and long lasting. Cell transfer studies showed that the tolerance is not expressed by spleen cells from tolerant animals following transfer into normal irradiated hosts, but rather that tolerance is systemic in nature, presumably dependent on serum-mediated blocking of the immune response (*3*). In vitro studies confirmed that cells from tolerant animals respond to erythrocytes in undiminished fashion when placed in cell or organ culture. On the other hand, serum from tolerant animals specifically prevented the in vitro immune reaction of normal spleen cells to the appropriate challenge erythrocyte (*3*).

Our experiments were undertaken to determine the effect on the developing mouse embryo and neonate of treatment of the mother with tolerogenic material obtained from sheep or horse erythrocytes. We used BALB/c mice for all our work. Both SHS and HHS were prepared as described previously and checked for lack of antigenicity prior to use (*2*). Timed pregnancies were obtained by examining daily for presence of vaginal plugs, the day of appearance of a plug being designated day 0. Birth usually occurred 19 days later. To observe the effect of tolerogen administration during pregnancy 0.5 ml of HHS or SHS was administered on days 3, 4, and 5 of development, that is, during the period of embryo implantation and prior to formation of embryonic blood cells or placentation. Other pregnancies were initiated at various intervals after injection of tolerogen. Since we ascertained that there was no significant antigenic competition or cross-reactivity at the PFC level between HRBC and SRBC administered simultaneously, antigenic challenge was achieved by intraperitoneal injection of 0.2 ml of a 1 : 1 mixture of 25 percent SRBC and HRBC 4 days prior to assay of spleen PFC. The normal BALB/c mouse yields about twice as many PFC to SRBC as to HRBC when this regimen is used, and the ratio of PFC response of SRBC/HRBC was used as a useful measure of immunological tolerance. The PFC were enumerated by the method of Cunningham and Szenberg (*4*).

Our initial results indicated that many but not all litters of mice born after treatment of the mother with SHS during pregnancy showed a subsequently reduced response to SRBC, while similar treatment with HHS resulted in a reduction of the response to HRBC. More striking, however, were the results obtained from litters conceived several weeks after administration of tolerogen to the prospective mothers. To date, all litters born to tolerant mothers 5 to 8 weeks after administration of the tolerogenic material and tested between 25 and 45 days of age showed a profound reduction in response to test antigen, while producing a normal level of response to the control erythrocyte (Table 1).

To determine the prenatal versus postnatal influence of the prior induction of maternal tolerance on the immune competence of the litter, rearing experiments were carried out in which newborn mice

Table 1. Effect of tolerance induction of mother on immune response of young. Interval between tolerance induction and conception was 2 to 5 weeks.

Treatment	Litters (No.)	Assays (No.)	PFC/10^6 spleen cells		Ratio SRBC/HRBC
			SRBC	HRBC	
SHS	14	29	67.8*	717.7	0.09
HHS	7	19	523.8	36.1†	14.51
Saline	6	16	697.1	353.3	1.97

*9.7 percent of normal response to SRBC. †10.2 percent of normal response to HRBC.

Table 2. Effect of tolerance induction of mother or foster-mother on immune response of young.

Treatment of		Litters (No.)	Assays (No.)	PFC/10^6 spleen cells		Ratio SRBC/ HRBC
Mother	Nurse			SRBC	HRBC	
SHS	Saline	4	5	853.4	427.5	2.0
HHS	Saline	3	6	884.9*	264.5	3.3*
Saline	SHS	3	4	33.2†	297.6	0.1
Saline	HHS	4	5	596.2	26.0‡	22.9

* One assay was unusually high for SRBC response. †4 percent of normal response to SRBC. ‡8 percent of normal response to HRBC.

Table 3. Effect of tolerance induction of foster-mother on immune response of normal BALB/c mice: single litter.

Mouse No.	Treatment of foster-mother	PFC/spleen		Ratio SRBC/ HRBC
		SRBC	HRBC	
1	Saline	72,000	20,000	
2	Saline	111,000	50,000	2.4
3	Saline	60,000	28,000	
4	SHS	2,000	11,000	
5	SHS	3,000	98,000	0.04
6	HHS	112,000	1,000	
7	HHS	52,000	1,000	84.0

were exchanged at birth between mothers who had become tolerant and saline-treated mothers. The results indicate that mice born of saline-injected mothers and transferred to SHS-treated foster-mothers become tolerant to SRBC, while mice transferred to HHS-treated foster-mothers become tolerant to HRBC (Tables 2 and 3). In the reverse situation, mice born to tolerant mothers and foster nursed by normal mice give normal responses to SRBC and HRBC (Table 2).

The experiments show that immunological unresponsiveness (tolerance) in mice can be transmitted from mothers to their offspring and that the dominant route of tolerance induction appears to be through the milk. If induction occurs through the placenta as well it is no longer apparent after 4 weeks of foster care by normal mice. While cell transfer through milk has been implicated for some systems of tolerance (*5*), it seems unlikely in the experiments we are reporting since tolerance in our system is normally mediated by serum rather than by cells (*3*). On the other hand, the fact that tolerance appears to be more readily induced several weeks after tolerance induction could signify a temporally related shift from serum or milk blocking factors to suppressor cell formation (*6*); experiments to distinguish between these possibilities are now appropriate.

We now tend, however, to favor the interpretation that in our system tolerance is transferred via blocking factors carried in serum and milk. Among the candidates for such blocking are persistent tolerogen, antibody, or antigen-antibody complexes (*7*); all of these could readily be transmitted both transplacentally and through milk (*5, 8*). Of these possibilities, persistent tolerogen seems least likely since tolerance is more readily transmitted several weeks after treatment than with treatment begun during pregnancy. A gradual release of modified tolerogen, however, cannot be rigorously excluded. While antibody-mediated suppression might seem unlikely because of the absence of a detectable antibody response as measured by induction of hemolysins and hemagglutinins after initial administration of tolerogen (*2*), we do have preliminary observations suggesting that a low level of detectable, red cell specific antibody may appear several months after tolerance induction in the absence of any deliberate de novo antigenic stimulation. Moreover, the production of circulating antibody to a different determinant of the red cell membrane not leading to detectable hemagglutinin or hemolysin production is a possibility that has been discussed extensively (*3, 9*). The most likely explanation, however, seems to be the presence and transfer of antigen-antibody complexes, since these are known to be relatively stable, long lasting, readily transferred from mother to young, and capable of abrogating spleen cell responses to heterologous erythrocytes in vitro as well as to influence the capacity of spleen cells to transfer immune responses in vivo (*7, 8*).

Our experiments focus on a neglected yet important dimension of the development of immunity: the influence of the mother, through the maternal-fetal transfer via placenta and milk, on the spectrum of immunological responsiveness as measured in her offspring (*5, 10*). The transfer (passive?) of tolerance, leading to a persistent suppression of specific immune reactions, leaves the offspring susceptible to postnatal establishment of actively acquired tolerance, a fact which may be of paramount importance in considering the vertical "transmission" of infectious agents or the postnatal susceptibility to cancer-inducing agents. Especially in the light of the extensive evidence suggesting that many aspects of the immune system mature early during embryogeny (*3, 9*), transmission of immunological tolerance may represent a key element in the pattern of immunological responsiveness established during postnatal development. To the extent that much of the period of nursing in the mouse corresponds in man to the third trimester of in utero fetal development (*8*), we must consider transplacental transfer of tolerogenic influences of potential major importance in the ontogeny of human immunological responsiveness, emphasizing that there is every reason to assume that passive immunity and passive unresponsiveness may represent two important parameters of the maternal influence on the ontogeny of the immune response.

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Sexual Cyclicity in Captive Lowland Gorillas

Abstract. *Oppositely sexed pairs of gorillas exhibit some behavior indicative of higher cognitive functioning, such as individual partner preferences and varied copulatory positions, but also mate in a cyclic manner closely related to the degree of female genital swelling. The latter finding is contrary to predictions based on their advanced position in phylogeny.*

Nearly 30 years ago Beach (*1*) proposed that animals with relatively larger forebrains and, consequently, greater cognitive abilities were less dependent on gonadal hormones for the regulation of their sexual behavior than were animals with smaller forebrains. Evidence in support of this hypothesis has appeared over the years, but relatively little systematic data have been obtained on the more advanced species, in particular the higher primates (*2*). In a study of the sexual behavior of gorillas, I obtained evidence contrary to Beach's hypothesis in that the behavior of these apes, close taxonomic affiliates of man, was rather closely related to the phase of the female's sexual cycle.

My primary objective was to evaluate periodicity in sexual behavior and female genital swelling of gorillas for evidence that the behavior was hormone-dependent. The female gorilla does not possess a sex skin as extensive as that of the chimpanzee or baboon, but it does exhibit tumescence of the perineal labia with a cycle (*3*) comparable to that of these other species and presumably under similar hormonal control (*4*). The subjects were four male and nine female lowland gorillas (*Gorilla gorilla gorilla*), ranging in age from 7 to 10 years at the beginning of the study in 1971. The animals were housed in cages consisting of an inner compartment 2.3 m by 2.3 m by 2.1 m high and an outdoor compartment 2.3 m by 4.1 m by 2.7 m high, interconnected by a guillotine door. When they were not being tested, the females were caged in isosexual pairs and the males were caged alone. Oppositely sexed pairs were tested approximately daily throughout the sexual cycle of the female. Before each test the male was confined to the outer compartment of his home cage (the test cage) and the female was introduced into the inner compartment. The test was begun after the door separating the compartments was raised and the female had entered and was confined to the outer compartment. The test was terminated after 30 minutes had elapsed without the occurrence of copulation. The labial tumescence (LT) of the female was rated on a scale of 0, 1, or 2, corresponding respectively to detumescence, minimal tumescence, or maximal tumescence, as previously described (*3*).

I paired the 13 gorillas in 20 different combinations for approximately 2000 tests over a 3-year period. All nine females copulated with at least one male partner, but only seven females from nine pairs copulated on a regular basis and were involved in copulation that culminated in ejaculation. For 4 of the 11 pairs that did not copulate or copulated very infrequently, the females failed to exhibit tumescence of the perineal labia, suggesting hormonal inadequacy as the basis for the lack of sexual activity. However, in the seven remaining infrequently copulating pairs, the female did exhibit cyclic fluctuations in LT. Since the females in these latter pairs did copulate when tested with different males, their differential responsiveness may be an example of individual partner preferences. My interpretation of this finding is that some factor other than hormonal, presumably a higher cognitive variable, exerted an important influence on the sexual behavior of these animals (*5*).

A clearer indication of higher cognitive capabilities in the gorilla was found in the varied copulatory positions taken by the subjects. Of the seven females that copulated regularly, four used the dorsal-ventral position exclusively, one used the ventral-ventral position exclusively, and two used both positions. Of the four males, two used only the dorsal-ventral position and two used both positions. Moreover, a number of variations of the two major positions were also seen. These data indicate that the primary copulatory position for these animals was dorsal-ventral, but that a considerable amount of variability was exhibited within and between positions. This type of variability in copulatory positions contrasts with the relatively invariable, stereotyped performance characteristic of species below the level of the apes and appears to represent a prerogative related to the latter's pronounced degree of encephalization.

Another conspicuous feature of the gorilla's sexual interactions was related to the different roles played by the male and female before copulation. The male was quite unobtrusive in soliciting copulation, his positive responses before copulation consisting primarily of approaches to the female and occasional touches with the back of the hand. The female, on the other hand, was very assertive, backing forcefully into the male, frequently pushing him against a wall, and actively rubbing her genitalia against the male by rhythmically raising and lowering her rump while emitting a soft, high-pitched fluttering vocalization. The male also vocalized during copulation and in a pitch similar to the female's, but his vocalization consisted of a short burst rather than the prolonged refrain of the female.

The most significant finding, however, was the relationship between various measures of sexual behavior and the female's labial condition (Table 1). Since the tests were conducted approximately daily throughout the female cycle, the proportion of tests conducted at each degree of LT reflects the proportion of days each degree of LT was represented in the cycle. Based on a cycle of 31 or 32 days (*3*), LT ratings of 0, 1, and 2 were obtained on 19,

Table 1. Sexual behavior of captive lowland gorillas for different labial tumescence (LT) ratings of the females. The female success ratio is defined as 100 times the number of copulations per test divided by the number of female presentations per test; *N* is the total number of tests.

LT rating	*N*	Presentations		Female success ratio	Copulations		Ejaculations	
		Percent of tests	Per test (median)		Percent of tests	Per test (median)	Percent of tests	Per test (median)
0	705	23	1.6	3	1	0.3	0	0.0
1	342	48	2.1	35	25	1.2	14	1.0
2	102	66	2.8	41	43	1.8	37	1.0
Totals	1149*	34*	2.1†	23*	12‡	1.4	7‡	1.0

*$P < .001$, Friedman two-way analysis of variance (*14*). †$P < .05$, Friedman two-way analysis of variance (*14*). ‡LT = 1 compared with LT = 2, $P < .05$, Wilcoxon matched-pairs signed-ranks test (*14*).