perimental series are treated separately, since the response durations recorded at comparable temperatures in the separate experimental series often differed appreciably. These differences probably reflect nonuniformity in criteria of the four observers in deciding that significant population response had ended, but conceivably also include real differences between the organisms from the separate collections. In any case, calculation of Q_{10} values is meaningful only in comparisons within an experimental series, where the prehistories of the animals were the same and where observer bias, if any, was constant; such values with their standard errors (*1*) appear in Table 1.

The real durations of response, measured from onset of significant activity rather than from application of the stimulus, would be even less temperature-dependent than these values seem to indicate. A major portion of the apparent difference in Fig. 1 is accounted for by the fact that the latent interval between application of the stimulus and attainment of full, maximum response was 2 to 4 seconds at 10° to 15°C against ≤1 second at the warmer temperatures. No allowance was made for this factor in the Q_{10} estimates, although it probably operates on the beach in a manner tending to produce greater uniformity in the distance that an animal is carried by a wave before it reburies, since the factor implies a more uniform duration of real transport than is indicated in Fig. 1.

The Q_{10} values, as calculated, are much smaller than those seen in most behavioral and physiological responses, but are nonetheless appreciably larger than those common in circadian rhythms. Since the times of passage of ocean waves in the uprush zone, to which this response is apparently keyed, are much more irregular than the timing of the daily or the tidal cycle, it is perhaps not surprising that the response is not as thoroughly buffered against environmental temperature as are the longer-period time-measuring systems. The sensitivity to temperature, evident in these data, is still so small that the response duration at all temperatures overlaps most of the ecologically appropriate values.

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References and Notes

1. The term Q_{10} is defined as the ratio between a reaction rate of one temperature, r_T, and the rate at a temperature 10°C lower, r_{T-10}. On the basis of measurements at any two temperatures, $T1$ and $T2$ ($T1 > T2$), an average Q_{10} over the interval can be calculated as the ratio $r_{T1} : r_{T2}$ raised to the power of $10/(T1 - T2)$. When measurements, at different temperatures, of the times required for a reaction to reach a given end point (t_{T1} and t_{T2}) are available, rather than reaction rates themselves, the average Q_{10} can be calculated as the ratio $t_{T2} : t_{T1}$ raised to the power of $10/(T1 - T2)$. Standard deviations of the individually calculated Q_{10} values were estimated as $(c/a^2)(b/a)^{c-1}(a^2s_b^2 + b^2s_a^2)^{\frac{1}{2}}$, in which a is the average duration of response at the higher temperature, b is the average duration of response at the lower temperature, s_a^2 and s_b^2 are the variances of the mean durations at these temperatures, and c is the absolute value of the difference in temperatures, divided into 10.0.
2. C. S. Pittendrigh, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 159 (1960).
3. J. T. Enright, *Comp. Biochem. Physiol.* **7**, 131 (1962).
4. Other components of behavior are involved in the subsequent seaward migration with the ebbing tide. These include an endogenous rhythm of activity synchronized by the tides; and, in conjunction with this rhythm, a different response, of much longer duration, to pressure increases (*3*, for details).
5. For a morphological characterization of this unnamed species, see ref. 7 of J. T. Enright, *Science* **133**, 758 (1961).
6. Supported by NSF grant GB 5471. I thank Michael Lohmann, Roswitha Hauenschild, and Thea Schultze for serving as observers; and E. W. Fager, R. Lasiewski, and M. Lohmann for critically reading the manuscript.

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Nonhormonal Basis of Maternal Behavior in the Rat

Abstract. *Rats were tested for induction of maternal behavior by exposing them to young pups continuously for 10 to 15 days. Nonpregnant intact, ovariectomized, and hypophysectomized females were studied, as well as intact and castrated males. Nearly all the animals exhibited the four main items of maternal behavior and there were only minor differences in the latencies for the onset of maternal behavior among the various groups. It is concluded that all rats have a basic level of maternal responsiveness which is independent of hormonal stimulation.*

From the earliest studies, maternal behavior in the rat has been considered to be dependent upon hormones. It appears during a particular phase of the endocrine reproductive cycle (namely, postparturition), and its onset is closely associated with the onset of lactation, which is known to be under hormonal control. After parturition nearly all females show maternal behavior upon being exposed to pups, but in the single study in which a large number of animals were tested, even after 4 days of continuous exposure, maternal behavior appeared in only 30 percent of nonpregnant females (*1*).

In several mammalian species [mouse (*2*), hamster (*3*), wolf (*4*), monkey (*5*), and human beings] young elicit maternal behavior in nonpregnant females, as well as at the usual time after parturition. This is particularly striking in the mouse. Maternal behavior appears in nonpregnant mice and, since hypophysectomy does not prevent it, it is clearly not based upon hormones (*2*).

We exposed nonpregnant female rats to pups for 10 to 15 days and tested daily for the appearance of maternal behavior. Lengthening the period of exposure to pups proved highly successful in eliciting maternal behavior, and the study was extended to include males, gonadectomized females and males, and hypophysectomized females.

Three groups of nulliparous females of the Charles River strain, 80 to 120 days old, were used: intact ($N = 14$), ovariectomized ($N = 12$), and hypophysectomized ($N = 11$). The hypophysectomized females, which were obtained commercially, were examined by means of daily vaginal smears to confirm the absence of estrous cycling. The operations were performed approximately 2 weeks before the start of testing. In addition two groups of males of the same strain and age-range were used: intact ($N = 13$) and castrated ($N = 12$). Each animal was housed in a large rectangular cage, 45.7 by 50.8 by 41.9 cm, with transparent plexiglass walls, a grid floor, wall feeder, water bottle, and two bins containing hay and coarse wood shavings for nesting ma-

Table 1. Percentage of animals displaying each of the four items of maternal behavior. Number in each group is in parentheses.

Group	Retrieve	Crouch	Lick	Build nest
	Females			
Intact (14)*	93	100	100	100
Ovariectomized (12)	92	83	100	92
Hypophysectomized (11)	100	100	100	100
	Males			
Intact (13)	77	77	85	46
Castrated (12)	83	75	83	67

*Observations made inadveretntly on only 10 of the 14 females of this group.

terial. After 3 days of habituation to the cages, five pups, 5 to 10 days old, were placed at the front of each cage. Measures of latency for the onset of maternal behavior were taken from this time. Retrieving was observed for 30 minutes starting when the pups were first introduced, then observations of crouching over the young (as in nursing), licking, nest-building, and other items of behavior were observed over the next 2 hours for 1-minute periods at 20-minute intervals. These pups remained with the female until the next morning at which time nests were scored, the pups were removed and weighed, and a fresh litter of five pups of the same age-range was placed in the cage and testing was repeated, now with a 15-minute retrieving test. Testing was done for 10 days if an animal displayed maternal behavior on 3 separate days, and for 15 days otherwise (*6*).

Group percentages for the appearance of the four main items of maternal behavior (Table 1) and group latencies for the onset of these items (Table 2) were used to evaluate the effects of pup stimulation on the appearance of maternal behavior. The percentage of nonpregnant females displaying each of the items of maternal behavior was nearly 100 percent, which represents an increase over the 30 percent found when only 4 days of exposure was used (*1*). There was no indication that the ovaries were involved in the induction of maternal behavior. First, induction of maternal behavior had no effect on estrous cycling. The average interval between estrous vaginal smears was 4.7 days (out of a total of 127 female days) among 11 of the 14 females induced to show maternal behavior, as compared to 4.8 days for a group of five control females that subsequently lived in the same cages as the experimental females but were not exposed to pups and therefore, of course, did not exhibit maternal behavior. Second, the ovariectomized females showed maternal behavior with the same high percentages as the intact females. It can be said, in fact, that the induction of maternal behavior in the nonpregnant females was not dependent upon any hormones since all of the hypophysectomized females also showed all four items of maternal behavior.

Males also were induced to show maternal behavior by exposure to pups and although smaller percentages displayed the different items of maternal behavior, only with respect to nest-building did the males differ significantly from the females ($z = 2.14$ to 3.48, $P < .05$ to .01). The two groups of males themselves did not differ (nest-building: $z = 1.50$, $P > .10$).

Table 2. Latency for the onset of each of the four items of maternal behavior, mean day ± standard deviation.

Group	No.*	Retrieve	No.	Crouch	No.	Lick	No.	Build nest
				Females				
Intact	13	6.77 ± 2.81	14	6.36 ± 2.34	14	6.50 ± 0.30	10	5.90 ± 2.07
Ovariectomized	11	7.09 ± 2.97	10	5.20 ± 1.66	12	6.12 ± 2.47	11	3.45 ± 2.11
Hypophysectomized	11	6.18 ± 3.71	11	4.27 ± 2.26	11	3.33 ± 0.52	11	1.64 ± 2.02
				Males				
Castrated	10	6.80 ± 4.02	9	6.00 ± 3.02	11	4.50 ± 2.87	6	2.33 ± 3.59
Intact	10	7.40 ± 4.80	10	5.70 ± 7.55	10	4.55 ± 2.75		

* Positive cases only.

The average latencies for the onset of the various items of maternal behavior in the nonpregnant females were all longer than 5 days. This explains why Wiesner and Sheard (*1*), who used only 4 days of exposure to pups, failed to find a significant proportion of the females becoming maternal. Latencies for retrieving, crouching, and licking were not affected by ovariectomy but removal of the pituitary caused a significant reduction in latencies for the onset of crouching and licking (median test; crouching: chi-square test = 6.82, df = 1, $P < .01$; licking: chi-square test = 9.1, df = 1, $P < .01$). Among the hypophysectomized females nest-building was precipitated in 10 of the 11 animals before they were exposed to young, and among the ovariectomized females 6 of the 11 animals were affected similarly. This effect of hypophysectomy on nest-building has already been reported but the effect of ovariectomy on nest-building is at odds with previous reports (*1*). There were, therefore, significant differences in latencies for the onset of nest-building among the three groups (Kruskal-Wallis one-way analysis of variance: $H = 17.64$, df = 2, $P < .001$).

There were no significant differences in latencies between the two groups of males nor between the males and the three groups of females. Too few intact males built nests consistently to establish a reliable average latency and the few castrated males that built nests consistently represent too small a sample and were too variable to compare with the hypophysectomized females.

After the onset of maternal behavior the various items continued to appear with regularity in all groups of females but the males were somewhat less regular than the females. The maintenance ratio (the ratio of positive tests to tests after onset) for each of the maternal items ranged from .63 to .95 in the females and from .34 to .88 in the males.

Our results show that there is a basic maternal responsiveness in rats which is not dependent upon hormones or sex for its arousal. Further, there are no marked changes in ovarian and pituitary activity during the induction of maternal behavior. It requires however an average of 6 days of exposure to pups to elicit maternal behavior in nonpregnant females while the postparturient female responds immediately to her pups when they are born. From this we conclude that hormonal changes during pregnancy and parturition cause an increase in maternal responsiveness over the basic level found in the nonpregnant female. We have already found such an increase in maternal responsiveness when pregnant females were delivered by Caesarean section at progressively later stages of pregnancy (for example, 10, 13, 16, 19 days). Tested in the same manner as the animals of the present study, these females showed latencies for the onset of maternal behavior that were shorter than those of the nulliparous intact females of the present study, and more significantly, were progressively shorter as pregnancy advanced (*8*). It is likely, therefore, that hormonal stimulation during pregnancy and parturition, acting on the basic maternal responsiveness of the nulliparous female, is an additional factor which contributes to the rapid appearance of maternal behavior at parturition (*9*).

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9. After the present study was completed we discovered independent confirmation of our results in studies by J. Cosnier [*Compt. Rend. Soc. Biol.* **157**, 1611 (1963)] and J. Cosnier and C. Couturier [*ibid.* **160**, 789 (1966)] who used exposures of newborn for 2 hours a day to induce maternal behavior in intact and ovariectomized nonpregnant females. The latencies that they observed appear to be similar to the ones found by our method.

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Altered Response to Pneumococcal Polysaccharide in Offspring of Immunologically Paralyzed Mice

Abstract. *The dose of pneumococcal polysaccharide type III required to induce immunologic paralysis in newborn offspring of immunologically paralyzed mice was one-tenth of the corresponding paralyzing dose for newborn offspring of normal mice. Similarly, immunization of the offspring of the paralyzed mice was accomplished with one-tenth the dose of polysaccharide necessary to immunize normal newborn mice. The altered susceptibility of newborn mice from paralyzed mothers to the induction of both paralysis and immunity was predicted from theories of antibody formation which postulate that the induction of tolerance or immunity is controlled by the concentration of natural antibodies specific for the antigen used.*

The theory of antibody formation proposed by Eisen and Karush (*1*) postulated, as did that by Jerne (*2*), that preformed natural antibody functions as the immunologic recognition system. According to Eisen and Karush (*1*) bimolecular antigen-antibody complexes (AgAb) constitute an effective stimulus for antibody formation and result in immunity. Trimolecular complexes (Ag_2Ab) would not stimulate the antibody-producing cells and would result in immunologic paralysis. The latter complexes are assumed to be degraded and eliminated from the organism. The induction of paralysis or immunity in the animal would depend upon the ratio of the concentration of antigen to that of the corresponding natural antibody present. The paralyzed animal would not have in circulation natural antibody specific for the antigen, since this antibody would have been removed after forming a complex with excess antigen.

Support for this view was provided by the work, of Segre and co-workers (*3*), on the enhancement by specific antibody and by normal γ-globulin of the antibody response in pigs deprived of colostrum. Dawe *et al.* (*4*) presented evidence that the enhancement of immunologic reactivity in rabbits injected with Freund's adjuvant was due, in part, to an increase in concentration of natural antibody. Hemphill *et al.* (*5*) succeeded in terminating immunologic tolerance to human serum albumin in mice by exposing their spleen cells to antigen-antibody complexes, but not to antigen alone. We now present evidence that the susceptibility of newborn mice to the induction of immunologic paralysis and immunity with pneumococcal polysaccharide is controlled by the concentration of natural antibodies specific for the polysaccharide.

Newborn mice acquire maternal γ-globulin both by transplacental transfer and by intestinal absorption from the colostrum during the first days of life (*6*). Since natural antibodies are presumably γ-globulins, the concentration of natural antibodies in the circulation of newborn mice should be the sum of the individual's own antibodies and those passively acquired from the mother. Thus, if a newborn mouse were the offspring of an immunologically tolerant mother it would have in circulation only its own natural antibody with specificity corresponding to the tolerated antigen. The concentration of natural antibody against the tolerated antigen should be lower in the offspring of a tolerant mother than in the offspring of a normal mother.

Adult female white mice (NIH) were immunologically paralyzed by intraperitoneal injection of 500 μg of pneumococcal polysaccharide type III dissolved in sterile saline (*7*). Preliminary experiments indicated that this dose of polysaccharide resulted in immunologic paralysis, as reported also by Felton (*8*). Both normal untreated and treated adult female mice were bred. Within 24 hours after birth, newborn from both paralyzed and normal mothers were injected intraperitoneally with varying quantities of the polysaccharide. The mice were challenged 7 days later with 100 minimal lethal doses (MLD) of the infectious tissue suspension (*7*) to determine the immunizing and paralyzing doses for each group (Table 1).

It was found that offspring of normal mothers were immunized with 0.05 μg and paralyzed with 0.5 μg of the polysaccharide. In contrast, the newborn from mothers paralyzed with the polysaccharide were paralyzed with 0.05 μg of the polysaccharide (a dose which immunized the offspring of normal mothers) and were immunized with 0.005 μg. The differences in the responses of the two groups of mice are consistent with the assumed differences in the quantities of natural antibody available to act as part of the immunologic recognition system. The reduction in the quantity of antigen necessary to induce paralysis or immunity appears to reflect a reduction in the quantity of natural antibody.

The possibility of transplacental transfer of the polysaccharide was also investigated. Pneumococcal polysaccharide present in the mother could pass through the placenta and influence the responsiveness of the newborn. If this were the case, the results for newborn from paralyzed mothers (Table 1) could be explained by the fact that enough of the polysaccharide was transferred to the fetus to reduce markedly the dose of antigen necessary to induce paralysis. It was, therefore, desirable to determine whether transplacental transfer of the polysaccharide took place.