PS-1 also recognizes the NH_2-terminal residue of physalaemin. Physalaemin contains one trypsin-susceptible bond, between the lysine and phenylalanine residues (*9*); cleavage at this point gave an HPLC peak for a substance that eluted at 33.3 minutes (Table 1) and that coincided with the tumor peptide fragment.

The immunoreactivity in the peaks of SCCL purified by HPLC was assessed for pharmacological action on the contraction of isolated guinea pig ileum (*16*). The concentration required to elicit a near-threshold response was similar to that of the amphibian peptide (100 to 150 pg/ml); the tumor peptide and the amphibian peptide were both more potent than histamine and were refractory to atropine and to the histamine blocker pyrilamine maleate.

Our data demonstrate that the substance with physalaemin immunoreactivity found in SCCL extracts immunologically, chemically, and pharmacologically resembles the undecapeptide originally purified from amphibian skin (*9*) and differs from PSLI identified in mammalian tissue (*10*). The correspondence in the retention times of both the oxidized and unoxidized forms of tumor peptide and synthetic physalaemin suggests the absence of any significant structural differences between these peptides, since HPLC can resolve underivatized peptides that differ in the isomeric configuration of only one amino acid (*17*) and in their ionic and hydrophobic properties (*12, 18*). The common identity of numerous peptide hormones in evolutionary dissimilar organisms (*19*) also points to the stability, fidelity, and invariance of selected regions of the genetic code over time and the specificity of membrane receptors—for at least 300 million years in the case of the amphibian peptide physalaemin.

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Vaginal Stimulation: An Important Determinant of Maternal Bonding in Sheep

Abstract. *The immediate induction of the full complement of maternal behavior in nonpregnant ewes primed with estrogen and progesterone has been obtained after 5 minutes of vaginal-cervical stimulation. A similar period of such stimulation given to recently parturient ewes, after the development of selective bonding to their own lambs, reversed their rejection behavior of alien lambs and produced a state of plasticity in maternal behavior, such that ewes receiving vaginal stimulation would accept and adopt alien lambs. These findings implicate vaginal-cervical stimulation as playing a role in the onset of maternal behavior.*

In a number of mammalian species, the onset of maternal behavior of nonparturient females depends to some extent on ovarian hormones, particularly estrogen (*1*). In no species, however, is the full complement of maternal behavior reproduced as rapidly and reliably as it is after parturition. In 50 percent of nonpregnant ewes, priming with progesterone and estrogens induces acceptance of alien newborn lambs (*2*), but this acceptance is in general not immediate, requiring as long as 2 hours of exposure to the lamb. Moreover, the most determinant criterion used in these studies was acceptance of the lamb at the udder. Even when all the components of maternal behavior are observed (licking, low-pitched bleats, absence of aggressive head butts, acceptance at the udder), they may not necessarily arise in the order observed at parturition.

We now report on the immediate induction of the full complement of maternal behavior in nonpregnant ewes treated with progesterone and estradiol and stimulated vaginally for 5 minutes. We further report how vaginal stimulation enhanced maternal care by parturient ewes of alien lambs presented to them about 1 hour after parturition, before selective nursing of their own young was established. As selective behavior is established rapidly in sheep [after periods of contact ranging from 30 to 120 minutes (*3*)], we also tested the effects of vaginal stimulation 2 to 3 hours after parturition and found that vaginal stimulation in-

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duced the recurrence of maternal behavior to alien lambs.

We used multiparous Prealpes de Sud ewes, housed permanently in sheds on zero grazing management. Reproductive cycles were synchronized at estrus, and parturition was induced with an intramuscular injection of 16 mg of dexamethasone (2 mg of Dexadreson per milliliter of Intervet) given on day 144 after mating (normal pregnancy, 146 days). This method of inducing parturition is thought to mimic the normal endocrine events of lambing and has not been found to affect maternal behavior (*4*). Alien newborn lambs were obtained from the 150 ewes of the same flock. Licking times were recorded with a stop-watch, and aggressive behavior, type of bleats emitted by the dam (low or high pitched), and behavior during suckling were recorded.

In experiment 1, 20 nonpregnant multiparous females were treated with progesterone (vaginal sponges of fluoregestone acetate) for 12 days followed by a single subcutaneous injection of 25 mg of 17β-estradiol 12 hours after the withdrawal of vaginal sponge. Testing for maternal behavior was performed in individual pens 12 to 24 hours after the estradiol injection. Half the sheep received vaginal stimulation for 5 minutes (with a vibrator 20 cm long and 5 cm in diameter), after which they were immediately provided with a newborn lamb (less than 5 mintues after its birth). The remaining ten sheep, matched for age and previous maternal experience, were handled in the same manner and also provided with a newborn lamb, but they were not vaginally stimulated before the introduction of the lamb. After 1 hour, lambs were removed and the reaction of ewes noted. Then nonstimulated ewes, which had not shown maternal behavior, underwent vaginal stimulation for 5 minutes, after which the lamb was reintroduced and behavior observed for a further 30 minutes.

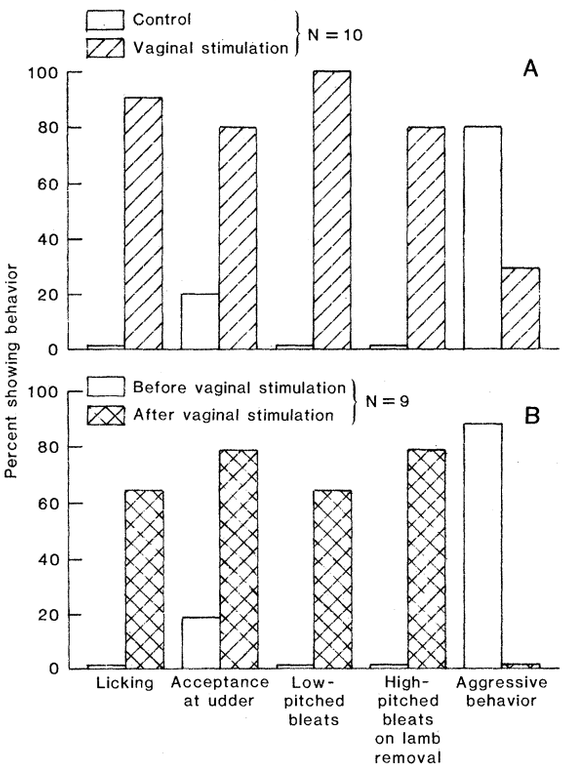

Fig. 1. Effect of vaginal stimulation on the maternal behavior of nonpregnant ewes. (A) The two groups differed significantly on each behavior (one-tailed exact probability, $P < .05$ in all cases). (B) The change of behavior of the same control ewes after vaginal stimulation at the end of the initial test. One of the ten control females did not undergo vaginal stimulation and was excluded from this study (McNemar test for the significance of changes, $P < .05$ in all cases).

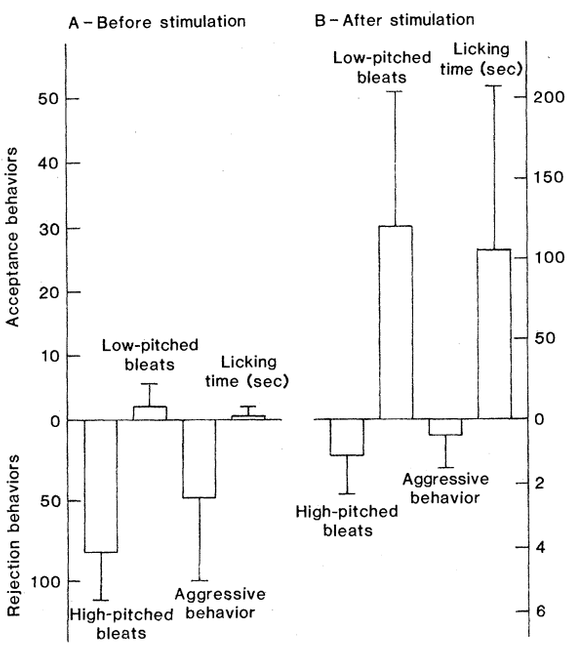

Fig. 2. Effect of vaginal stimulation on adoption of alien newborn lambs by ewes that had established selective behavior. After vaginal stimulation, rejection behaviors gave way to acceptance behaviors. High-pitched bleats [t (22) = 5.45, $P < .001$] and aggressive behavior decreased ($U = 37$, $P < .05$), and low-pitched bleats [t (22) = 4.21, $P < .001$] and licking increased [t (22) = 3.58, $P < .01$].

After the birth of a single lamb, a parturient ewe can be persuaded to adopt an alien lamb if it is newborn and if the fostering occurs soon after the birth of her own lamb (*2*). In experiment 2, seven parturient ewes were given a newborn lamb in addition to their own young, soon after parturition (64.3 ± 35 minutes). Animals were observed over a period of 20 minutes for the amount of attention paid to the two lambs, as measured by duration of licking. Although their own lambs were at this stage nearly dry, ewes spent approximately equal amounts of time licking both the newborn alien (5.6 ± 2.8 minutes) and their own older lamb (3.8 ± 2.3 minutes)

[t (12) = 1.13, $P > .28$]. At no time was the alien preferred, and toward the end of the test period more attention was being paid to her own lamb. A further seven parturient ewes were given an additional newborn alien lamb (85.7 ± 44 mintues after parturition) after 5 minutes of vaginal stimulation. In this case vaginal stimulation consisted of inserting into the uterus a rubber bladder, which was then inflated and maintained in place for 5 minutes before being expelled. Not only did the total amount of licking attention paid to lambs increase significantly from 8.4 ± 2.6 minutes without stimulation to 14.8 ± 3.1 minutes after stimulation [t (12) = 3.78, $P < .01$], but throughout the test period the ewe preferred the newborn alien almost to the complete exclusion of her own lamb. Ewes now spent considerably more time licking the alien lambs (13.6 ± 3.0 minutes) than they did their own (0.9 ± 0.5 minutes) [t (12) = 10.79, $P < .001$).

In experiment 3, 12 ewes had their own lambs removed 2 hours after parturition (mean, 135 ± 30 minutes) and were given a newborn alien. All these ewes had by this time developed a selective bond with their own lamb: their behavior to the newborn alien was aggressive and disturbed, with high-pitched bleats and little licking (Fig. 2A). These ewes were stimulated for 5 minutes as in experiment 2, and their behavior with the alien lamb was again observed. Only two ewes remained aggressive, and nine showed intense licking accompanied by the emission of low-pitched bleats (Fig. 2B).

In addition to the obvious application of these findings to animal husbandry, these studies illustrate the importance of vaginal stimulation for maternal behavior in hormonally primed ewes. In a behavior of such complexity, the part played by endocrine status and sensory cues from the lamb are most important (*2*). Nevertheless, the maternal adoption of infants achieved in previous experiments has generally lacked the full complement of maternal behavior and has required long exposures to the lambs. The mechanism by which vaginal stimulation permits the immediate expression of maternal behavior in sheep is not known. However, a discussion of the importance of vaginal stimulation for maternal care in goats suggested a role for oxytocin (*5*), the release of which may be of some importance, since its injection directly into the cerebral ventricles stimulates maternal behavior of nonpregnant rats (*6*). Vaginal stimulation may also act independently of the release of oxytocin on structures controlling the expression of maternal behavior.

In the development of a selective mother-infant bond to the exclusion of all other infants, maternal olfaction in ewes is essential. Suppression of the sense of smell before parturition leads to a loss of this selective behavior and to the acceptance of any lamb at suckling (*2, 7*). Moreover, anosmia in nonparturient rats facilitates the onset of their maternal behavior (*8*). The possibility exists, therefore, that vaginal stimulation may in some way influence the olfactory system to facilitate maternal behavior. This possible explanation seems more realistic in light of the similarities between these findings and those reported for mice (*9*). In mice some neural mechanism is set in motion by vaginal stimulation (mating), which ensures that the continued presence of the stud male's odor during a critical period after mating does not block his own pregnancy. In mice, this requires the presence of an intact noradrenergic nerve supply to the olfactory bulbs enabling a "print-now" mechanism to confer recognition of the stud male. Although the context is different, both phenomena are characterized by a critical period activated by vaginal stimulation (parturition in this case) that imparts recognition by the olfactory system. Although we can only speculate on this mechanism at the present time, it is possible that vaginal stimulation in sheep may also activate an olfactory print-now mechanism crucial to mother-infant bonding.

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