control of reproduction (*19*) and carbachol can alter pineal enzyme activity (*3*). A direct action of carbachol on the pineal gland appears unlikely because the effects of carbachol on reproductive function occurred concomitantly with an alteration of the circadian rhythm of activity, and the pineal gland does not play a major role in the regulation of the activity rhythm (*20*). An alternative possibility is that carbachol-induced changes in SCN activity lead to an alteration in pineal function and, ultimately, gonadal activity.

Although it has long been recognized that the photoperiod can influence seasonal reproductive cycles in animals, only now are some of the neural events that mediate the effects of light on reproductive activity being elucidated. Our results suggest that acetylcholine may play a key role in the mechanism by which a circadian clock is involved in photoperiodic time measurement. Our experimental approach, in which a discrete stimulus (for example, electrical or chemical) is presented at a specific circadian time to induce a photoperiodic response, may prove valuable in determining the neurochemical and neurophysiological events that enable an organism to measure the seasonal change in day length.

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6. The 1-second light pulse was presented 8 hours after lights-off to animals exposed to LD 6:18 for 11 weeks [D. J. Earnest, *Biol. Reprod. Suppl.* **24**, 102A (1981); ——— and F. W. Turek, *ibid.*, in press].
7. The hamsters [Lak:LVG(SYR)] were obtained at 9 weeks of age from Lakeview Hamster Colony, Newfield, N.J.
8. Revolutions of the running wheel were recorded through a microswitch as a pen deflection on an Esterline Angus Event recorder, and daily records kept for each animal.
9. After anesthetizing the hamsters with sodium pentobarbital (6 mg per kilogram of body weight), a circular piece of the skull (2 mm in diameter) was removed and a 22-gauge stainless steel cannula was placed in the brain at the following stereotaxic coordinates with the head held 5 mm above horizontal: 1.6 mm anterior to bregma, 1.5 mm to the right of the midsagittal sinus, and 3.2 mm ventral to the dura mater. Dental cement was applied to the base of the cannula and three anchoring screws in the skull to stabilize the cannula in a fixed position.
10. At this time the testes were large in all animals (mean testis width, 12.2 ± 0.1 mm). The testes of sexually mature hamsters weigh about 3000 mg [F. W. Turek and S. H. Losee, *Biol. Reprod.* **18**, 299 (1978)].
11. A 2-μl injection of either physiological saline or a solution of 0.01*M* carbachol dissolved in saline was injected under light ether anesthesia by a Hamilton microliter syringe. The length of the guide cannula was such that the needle extended 0.5 mm ventral to the tip into the lateral ventricle. A red safelight (Kodak filter 1; 0.5 to 1.0 lux) was used for injections during the dark.
12. Frozen brain sections at a thickness of 60 μm and sections were examined under a light microscope to verify placement of the cannula tip; in all animals, the cannula was observed to extend into the lateral ventricle.
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Physalaemin: An Amphibian Tachykinin in Human Lung Small-Cell Carcinoma

Abstract. *Immunoreactivity to the amphibian peptide physalaemin was characterized from extracts of a human lung small-cell carcinoma by immunological, chemical, and pharmacological means. Tumor-related peptide cross-reacted with three antiserums to physalaemin to yield 1.1 to 1.6 nanomoles per gram of tissue. Physalaemin and tumor peptide had similar retention times on high-performance liquid chromatography after chemical and enzymic modifications that included pH changes, oxone oxidation, use of a hydrophilic ion-pairing reagent, and digestion with trypsin and pyroglutamate aminopeptidase. Both physalaemin and the tumor peptide produced a contractile response of isolated guinea pig ileum at threshold concentrations of approximately 100 to 150 picograms per milliliter. These data suggest that small-cell carcinoma of the lung contains a physalaemin-like peptide that has structural and biological homology to its amphibian counterpart.*

Small-cell carcinoma of the lung (SCCL) exhibits heterogeneity among its constituent cells (*1*), which is reflected in its variable biochemical properties, ectopic (inappropriate) production of peptide hormones (*2, 3*), and chromosomal abnormalities (*4*). Most notable among the hormones detected in extracts of SCCL and at elevated levels in the plasma of patients with this tumor were adrenocorticotropin (*2, 3*), prolactin, oxytocin (*2*), calcitonin, and β–human chorionic gonadotropin (*3*). Immunoreactivity to the amphibian peptide bombesin has been found in all SCCL's resected from patients (*5*) or maintained as tumor-derived cell lines (*6*) or propagated in nude mice (*7*). The immunoreactive substance appeared to have structural homology with the amphibian peptide (*7*) rather than with the larger bombesin-like peptides (*8*). Another amphibian peptide, physalaemin, was detected in SCCL by a radioimmunoassay and by immunohistochemistry (*7*). This group of related peptides (tachykinins) includes substance P and has potent pharmacological effects on extravascular smooth muscle, blood

Table 1. Reverse-phase HPLC retention times of amphibian physalaemin and SCCL physalaemin immunoreactivity. Values are given as means ± standard deviation, with the number of separate experiments in parentheses; N.D., not determined.

Conditions	Retention time (minutes)			
	Physalaemin		Tumor immunoreactivity	
	Oxidized	Reduced	Oxidized	Reduced
HFBA, *p*H 2.3	27.2 ± 0.8 (3)	30.2 ± 0.2 (7)	27.9 ± 0.6 (5)	30.5 ± 0.4 (5)
plus trypsin	33.3*		33.0*	
Acetate, *p*H 4.2	29.5	32.8 ± 0.1 (2)	29.5 ± 0.1 (2)	32.7 ± 0.1 (2)
Phosphate, *p*H 7.0	N.D.	33.1 ± 0.1 (2)	29.4	33.1

*The tryptic fragment lacks a thio-containing residue and hence is neither oxidized nor reduced.

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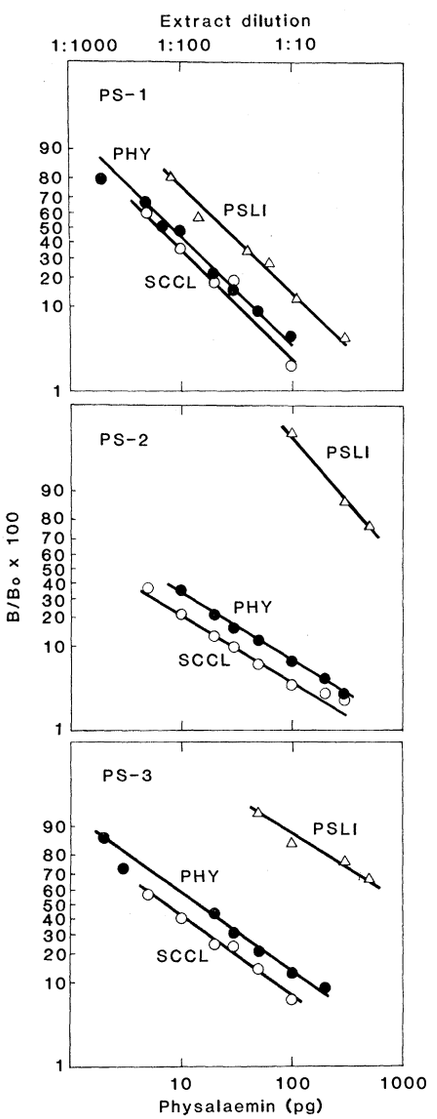
pressure, and exocrine glands (*9*). We have further examined the physicochemical and pharmacological properties of tumor physalaemin immunoreactivity and now present evidence that a peptide in SCCL resembles the amphibian undecapeptide (*9*).

Human SCCL was propagated in nude mice and extracted as described (*7*). Reextraction of the lyophilized extract in 1.0*N* formic acid at 50° to 60°C yielded 1.1 to 1.6 nmole of tumor peptide per gram of tissue, which was 1000-fold higher than that obtained by solubilization in dilute acetic acid (*7*). Three distinct antiserums to physalaemin bound tumor extracts and physalaemin in a parallel manner (*10*) (Fig. 1). These antiserums differed in their cross-reactivity with other tachykinins (*10*) and in their recognition of mammalian physalaemin-like immunoreactivity (PSLI) (Fig. 1). Tumor extract and PSLI were equally recognized by antiserum PS-1, whereas antiserums PS-2 and PS-3 cross-reacted poorly with PSLI (maximum extent, 1 percent and 4 percent, respectively). The responses of the tumor extracts to the antiserums used as distinct molecular probes suggest that the tumor peptide contains an antigenic recognition site similar to that of physalaemin, but different from that of PSLI.

Tumor physalaemin immunoreactivity was resolved by reverse-phase high-performance liquid chromatography (HPLC) (*11*) into two distinct peaks (Fig. 2) regardless of the ionic composition or *p*H of the mobile phase (Table 1). The peak of immunoreactivity occurred in a substance that eluted at 32.7 minutes at *p*H 4.2, coinciding with the retention time of synthetic physalaemin (Fig. 2b). Heptafluorobutyric acid (HFBA) (*12*) decreased the retention time by approximately 2.5 minutes (about one column volume) relative to acetate at *p*H 4.2 (Table 1). The retention time in acetate at *p*H 4.2 was the same as that in phosphate at *p*H 7.0. This indicates the absence of a histidine residue, since the imidazole ring, being charged under acidic conditions, is neutralized at *p*H 7, which would make the molecule more hydrophobic. Physalaemin lacks histidine residues (*9*).

Chemoselective oxidation of the SCCL extract with 1.1*M* excess oxone for 2 minutes at 0°C (*13*) increased the amount of immunoreactivity in the peak at 29.5 minutes, with a proportional decrease in the one at 32.7 minutes (Fig. 2c). This decrease in the retention time of tumor immunoreactivity corresponds to the appearance of oxidized physalaemin at the same retention time (Fig. 2d and Table 1).

The sulfoxide derivatives of peptides with an amidated COOH-terminal methionine residue elute earlier than the unoxidized peptide on HPLC (*14*). The action of pyroglutamate aminopeptidase (*15*) yielded extensive losses of immunoreactivity for both SCCL material and physalaemin, suggesting that our antiserum



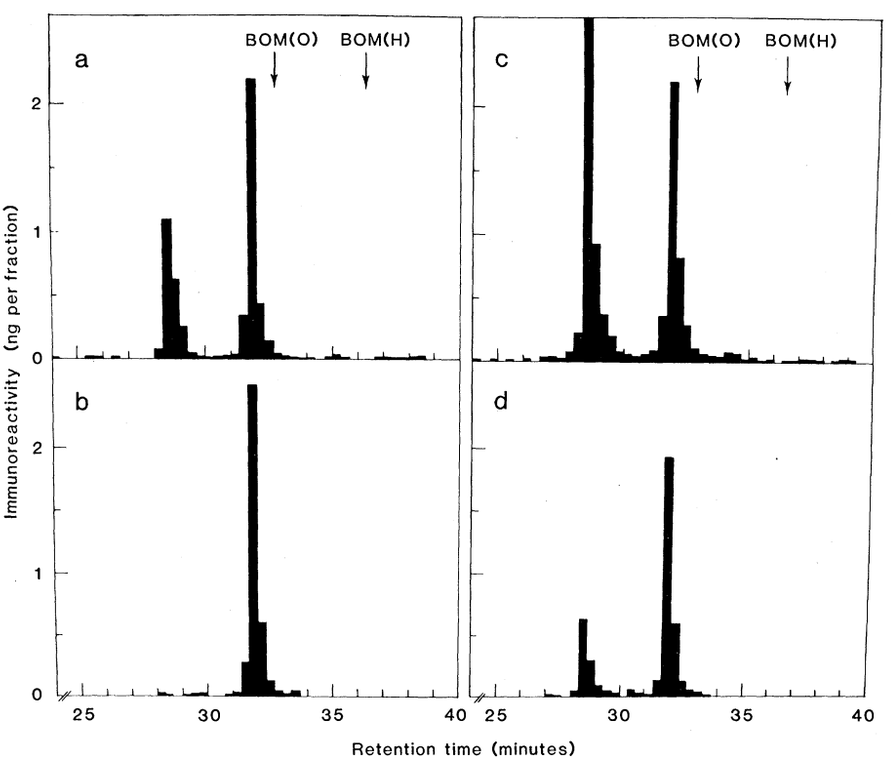


Fig. 1 (left). Immunological cross-reactivity of SCCL (○), mammalian physalaemin-like immunoreactivity (PSLI) (△), and physalaemin (●) with physalaemin antiserums PS-1, PS-2, and PS-3. PSLI was the pooled substance of the peak area from a gel filtration column (*10*). The data points are averages from two to three separate experiments; *PHY*, physalaemin. B/B_0 is the amount of labeled antigen bound by antibody in the presence of unlabeled peptide, divided by the total amount of antigen bound by antibody. Fig. 2 (right). Reverse-phase HPLC analyses of SCCL immunoreactivity and amphibian physalaemin at *p*H 4.2 as detailed in (*11*). (a and c) Untreated and oxone-treated tumor extract, respectively. (b and d) Synthetic physalaemin and the oxone-treated peptide, respectively. *BOM(O)* and *BOM(H)* represent the retention time of oxidized and reduced bombesin markers (*7*), respectively.

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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11. For HPLC, a Laboratory Data Control system fitted with a Rheodyne injector and a Spherisorb ODS 5-μm column (25 by 0.46 cm) was used. Linear gradients were from 0 to 100 percent solvent B for 40 minutes at a flow rate of 1 ml/min, with fractions collected each 20 seconds. Solvent A was 0.01*N* HFBA, *p*H 2.3, or 0.02*M* ammonium acetate, *p*H 4.2, or 0.02*M* sodium phosphate, *p*H 7.0. Solvent B contained the anions of solvent A in 60 to 80 percent CH_3CN. Controls against cross contamination were carried out (*7*).
12. Hydrophilic ion-pairing reagents produce shifts in the retention times of underivatized peptides on reverse-phase HPLC and improve peak symmetry [H. P. J. Bennet, C. A. Browne, S. Solomon, *J. Liq. Chromatogr.* **3**, 1353 (1981)].
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16. The organ bath (2 ml) contained guinea pig ileum in Tyrode's solution or Medium 199, with constant aeration (5 percent CO_2 in O_2) at 37°C. The volume of agents added to the bath ranged from 5 to 35 μl in bovine serum albumin (1 mg/ml). The ileum was attached under 0.5 g of resting tension to a transducer (Gould UC3).
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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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