dogenous leukemia viruses. It might also be expected that mere expression of the two sets of cell genes composing murine sarcoma viruses might result in cellular transformation. However, several criteria show that anaerobically treated cells are not in fact transformed (*15*).

We have established that the sarcoma virus genome is composed (in a recombined form) of two types of normal cellular genetic information; that is, endogenous type-C leukemia virus RNA plus the RNA transcripts of additional specific yet undefined genes. That the resulting sarcoma virus is highly oncogenic compared to its parent leukemia virus suggests that uncontrolled expression of a few cellular genes can directly lead to neoplastic transformation.

Our experiments show that these genes are expressed by normal cells in response to anaerobic stress. These findings are consistent with several possible explanations for their function in the cell. For example, these genes may allow the cells to respond to conditions of curtailed respiration by facilitating fermentation through coding for key enzymes in glycolysis or sugar uptake. This speculation in turn complements Warburg's findings that cancerous tumors in general (*16*), as well as murine sarcoma virus transformed cells (*17*), show increased fermentation, although respiration itself is not suppressed (*18*). Alternatively, induction of endogenous leukemia virus expression may be interrelated with the induction of the MSV-rat sequences, through a more complex series of events.

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Vasoactive Intestinal Polypeptide Occurs in Nerves of the Female Genitourinary Tract

Abstract. *The vasoactive intestinal polypeptide occurs in a richly developed population of nerves that are abundant in the female genitourinary organs. In pigs, cats, rats, and mice these nerves seem to innervate vessels and smooth musculature. Evidence indicates that vasoactive intestinal polypeptide represents a peptide neurotransmitter. Its effects on uterine blood flow and contractility, for example, may be considerable.*

The vasoactive intestinal polypeptide (VIP) isolated by Said and Mutt (*1*) represents a new neuronal peptide (*2–4*). Our immunocytochemical studies have demonstrated that VIP nerves are not confined to the gut but occur also in the brain (*3*) and around cranial blood vessels (*4*). Here we report that nerves containing this vasodilatory peptide can be found intimately associated with vessels and smooth muscle of the female genitourinary tracts of pigs, cats, rats, and mice.

Pigs were obtained at a nearby abattoir, cats were killed with Nembutal, and rats and mice were decapitated. Feline and murine tissue was used for immunocytochemistry and feline and porcine material for radioimmunoanalysis. Samples were taken from ovaries, Fallopian tubes, uterus, vagina, kidneys, ureters, urinary bladder, and urethra. For immunocytochemistry, samples were frozen in melting Freon-22 and freeze-dried, and were then vapor-fixed with diethylpyrocarbonate and embedded in paraffin as described previously (*3*, *4*). Sections (3 μm thick) were cut and the paraffin was removed. Antiserum to VIP [No. 5603-6, see (*5*)] was allowed to react with the sections, and the site of antigen-antibody reaction was revealed by means of the unlabeled peroxidase (PAP) method of Sternberger (*6*). The controls used were those recommended by Sternberger (*6*) and included the application of antigen-inactivated antiserum (containing 30 nmole of highly purified porcine VIP per milliliter of antiserum diluted 1:80).

Specimens for radioimmunoanalysis

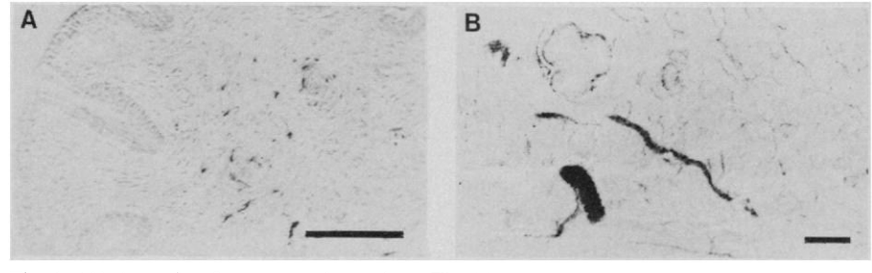

Fig. 1. (A) A section from cat endometrium. The black-stained VIP-containing nerves occur scattered between the glands; some of these nerves are visible around a small artery. (B) A section from cat uterine wall. VIP-containing nerves occur either singly or in large bundles. In the upper left corner three myometrial arterial branches are surrounded by VIP-containing nerves in an innervation-like pattern. The sections were stained with the immunoperoxidase procedure for demonstrating VIP (*6*), and photographs were taken in a Nomarski-type interference contrast microscope to reveal histological details. (Scale bars, 100 μm)

were immediately frozen in liquid nitrogen and stored at −80°C before being assayed. After being thawed, the material was extracted according to Larsson *et al.* (*3*). The same antiserum (No. 5603-6) as was used for immunocytochemistry was used for radioimmunoanalysis (*7*). ^{125}I-Labeled VIP was prepared by a chloramine-T method to a specific radioactivity of approximately 900 μc per nanomole of peptide. Highly purified porcine VIP was used as a standard. Free, labeled VIP was separated from antibody-bound VIP by absorption to plasma-coated charcoal. The lowest VIP concentration to be distinguished from zero (95 percent confidence limit) was 3.3 pmole/liter. The within and between assay reproducibility expressed as coefficient of variation was 0.07 and 0.15 at a level of 44 pmole/liter. All samples were assayed in triplicate in at least three dilutions.

In all organs studied, except kidney, VIP immunoreactive nerve fibers were detected. All controls were negative. The relative amounts of nerves containing VIP varied; they were especially numerous in the vagina, uterus, and ureters (Fig. 1). The bulk of VIP-containing nerves occurred in the smooth muscle wall of the genitourinary organs. However, in the uterus, in particular, numerous VIP-containing nerves were found also in the endometrium. In this location the nerves were intimately associated with vessels in a pattern suggesting innervation. Since they showed a tortuous course in the endometrium some of these vessels may be coiled arteries. In the cervical portion of the uterus and in the vagina, large bundles, having up to 50 to 100 VIP-containing nerves each, were found (Fig. 1). Such bundles invariably occurred in the smooth muscle coat or in the adventitia of these organs. At the zone of transition between uterus and vagina small collections of specifically immunoreactive nerve cell bodies were detected in the adventitial connective tissue. Nerves containing VIP were few in the Fallopian tubes and only occasionally seen in the ovaries. They were, however, numerous in the urinary tract, where they seemed to innervate the smooth muscle coat and also to form a subepithelial nerve plexus of varying intensity. Nerves containing VIP were most numerous in the ureters; few were found in the urinary bladder and urethra (*8*). It is interesting that arteries occurring in the vicinity of the genitourinary organs were heavily endowed with VIP-containing nerves. These nerves always occurred at the zone between the adventitia and media, a fact suggesting vascular innervation. However, neither the kidney nor the vessels supplying it contained nerves with VIP.

Table 1. Distribution of VIP immunoreactivity (expressed as picomoles per gram, wet weight) in the female genitourinary tract.

Location	Species			
	Feline		Porcine	
	Concentration	*N**	Concentration	*N**
Uterus	120	3	82	2
Vagina	171	1	†	
Fallopian tube	9	1	31	2
Ovary	6	2	2	2
Ureter	208	1	†	
Urinary bladder	32	3	37	2
Urethra	169	1	†	

* Number of animals tested. † Not tested.

Although, in essence, the described distribution holds true for all species examined, VIP-containing nerves were much more abundant in the feline than in the murine genitourinary tract.

Radioimmunoanalysis revealed the presence of varying, though sometimes very high, quantities of VIP immunoreactivity in all organs studied (Table 1). Gel chromatography of extracts from feline vagina revealed that the VIP immunoreactivity eluted in one single peak, corresponding to that of highly purified porcine VIP (Fig. 2).

The present results show that VIP-containing nerves are of widespread occurrence in the body. Immunoreactive nerve cell bodies from which these nerves may originate have been detected in the ventromedial hypothalamus (*3*), in the gastrointestinal nervous plexa (*3*), and at the zone of transition between the uterus and vagina. Gel chromatographic and immunologic evidence indicates that we are dealing with VIP rather than with a cross-reacting peptide. In the genitourinary tract the nerves containing this vasodilatory peptide are found in connection with vessels and smooth muscle in an innervation-like pattern. Recently, we have shown by means of electron microscopic immunocytochemistry that VIP occurs within the vesicles of the terminals of a special type (p-type) of nerve (*9*). Thus, evidence is accumulating that VIP represents a peptide neurotransmitter. If so, its effects on smooth muscle contractility and blood flow (*10*) may be of great importance for many bodily functions.

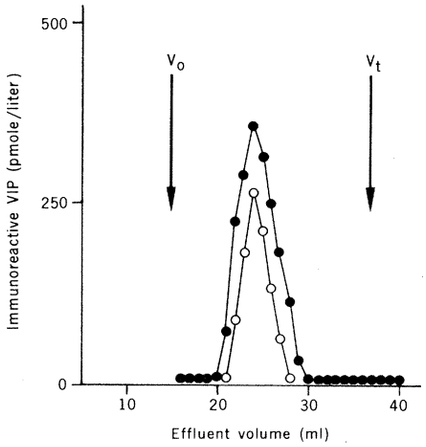

Fig. 2. Elution diagram of VIP immunoreactivity from an extract of feline vagina (●). Gel permeation chromatography on a Sephadex G-50 superfine column (11 by 400 mm) eluted with 0.25*M* ammonium hydrogen carbonate, *p*H 8.0, containing 72.5 μmole of bovine serum albumin per liter at a flow rate of 10.0 ml per hour at 4°C. The column was calibrated with highly purified porcine VIP (○), ^{125}I-labeled albumin, and K ^{125}I (V_o, void volume; V_t, total mobile phase).

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