VIP-Immunoreactive Nerve Structures of the Gastrointestinal Tract in the Developing and Adult Domestic Duck*

Alfredo VITTORIA, Luciana CASTALDO, Elena LA MURA, Carla LUCINI and Aldo CECIO

Department of Biological Structures, Functions and Technology, University of Naples Federico II, Napoli, Italy

Received December 27, 1991; Revised July 6, 1992

Summary. The first appearance and distribution of VIP-containing nerve structures were studied in the gut of embryonal, newly hatched and adult domestic ducks.

Immunoreactive structures appeared in the mesenchyme of the gizzard-duodenum junction (antrum) at 5 days of incubation, and soon after diffused both cranially and caudally. Early in development, VIP-positive cell bodies were more numerous than positive fibres, appearing 3 days before the latter. Later on, the positive cell bodies decreased in number while the fibres increased. In the foregut, VIP-positive structures firstly appeared in the myenteric plexus while in the mid- and hindgut they appeared simultaneously in the myenteric and submucous plexuses. The gastrointestinal VIP-innervation in newly hatched ducks differed from that of the adult because of the incomplete development of its mucosal component.

The findings obtained indicated following: The time interval from 13 to 17 days of incubation seems critical for the growth of the gastrointestinal VIP-containing neurons. The molecular factors regulating the appearance of neuronal phenotypes are probably synthesized early in the avian gizzard development. VIP is widely diffused in the rectum where it may play important roles in regulating the onset and synchronism of peristaltic and anti-peristaltic movements.

Vasoactive Intestinal Polypeptide (VIP) is a neuropeptide widely distributed within the nerve structures of the vertebrate gut, both in embryos (SAFFREY et al., 1982; SAFFREY and BURNSTOCK, 1988) and adult subjects (FAHRENKRUG, 1979; VAILLANT et al., 1980). Despite such distribution, several important problems

concerning the digestive VIP-ergic innervation remain poorly understood. The aim of this research is to study immunohistochemically the first appearance and distribution of VIP nerves in the gut of embryonal, newly hatched and adult domestic ducks. Studies on this topic are rare for birds, and lacking in the duck.

Avian VIP-containing nerves have been described immunohistochemically in the gastrointestinal tract of embryonal, young and adult chickens (LARSSON et al., 1976; SUNDLER et al., 1979; REINECKE et al., 1981; FONTAINE-PERUS et al., 1981; SAFFREY et al., 1982; EPSTEIN et al., 1983; FONTAINE-PERUS, 1984) and quails (FONTAINE-PERUS et al., 1981; REINECKE et al., 1981; FONTAINE-PERUS, 1984), within the pancreas of embryonal (SUNDLER et al., 1978) and adult chickens (LARSSON et al., 1976) and, finally, in the gastroenteropancreatic system of turkey stags (VAILLANT et al., 1980).

The presence of VIP-immunoreactive structures in the digestive tract of avian embryos has been described early in development but the time of their appearance and distribution in the gut are controversial (SUNDLER et al., 1979; FONTAINE-PERUS et al., 1981; EPSTEIN et al., 1983; FONTAINE-PERUS, 1984). The initial expression of a neuronal phenotype in the guinea pig embryonal gut and its successive development seem to be induced by the presence of both intrinsic and extrinsic factors (SAFFREY and BURNSTOCK, 1988). The former are probably produced by several gastrointestinal structures while the latter are represented by both the extrinsic innervation and the contacts between the developing neurons and

*This work was supported by a Grant-in-Aid from the Ministry of University and Research, Italy.

their "target(s)".

Immunohistochemical studies on the VIP-containing nerve structures of the gut in adult subjects are of particular interest in their comparative and morpho-functional profiles. Such structures have been demonstrated in many animal species both of the protostomian (e.g., cockroach) and deuterostomian (including several mammals) series (LANGER et al., 1979; FAHRENKRUG, 1979; FUJITA et al., 1981; REINECKE et al., 1981). In contrast, the avian species hitherto investigated have been limited to the chicken, quail and turkey, as reported by the cited bibliography.

Recent immunohistochemical studies carried out on both tissue sections and whole mount preparations have allowed the classification of the myenteric VIP-containing neurons of the guinea pig small intestine into four types on the basis of the different neuropeptides co-localized with VIP (LLEWELLYN-SMITH, 1987). Whether such neuropeptides may influence the well known VIP-induced relaxation of the digestive musculature is uncertain. At present, a double cholinergic interneuronal mechanism seems to regulate this relaxation (CHIJIWA et al., 1986).

MATERIALS AND METHODS

Embryonal, newly hatched and adult subjects of both sexes of the domestic duck *Anas platyrhynchos platyrhynchos* were examined. Samples from the oesophagus, glandular stomach (proventriculus), muscular stomach (gizzard), muscular stomach-duodenum junction (antrum), duodenum, jejunum-ileum, caecum and rectum were collected from anaesthetized birds. The embryos were taken from fertilized eggs and incubated at $38.5 \pm 0.2^\circ\text{C}$. Specimens were gathered every two days beginning from the third day of incubation up to birth. Newly hatched ducks were one day old; adult subjects were aged nearly five months. The embryos at early stages of development (up to the sixth day) were fixed and embedded undissected. Later on, the embryonal guts were subdivided as above.

The material was fixed in Bouin's fluid, dehydrated, embedded in Paraplast and cut in $4\text{--}5\ \mu\text{m}$ sections.

Immunohistochemistry was performed by means of the Peroxidase-antiperoxidase (PAP) technique of STERNBERGER (1979) and immunofluorescence following the indirect method of COONS (1956). A specific anti-VIP antibody (Incstar, 20077), raised in rabbits against the natural porcine antigen conjugated with keyhole limpet hemocyanine, was used at a working dilution of 1:300. The sections were incubated for 2 h at 37°C or overnight at 4°C in moistened chambers.

The site of the antigen-antibody reaction was revealed by means of diaminobenzidine hydrochloride (DAB) or aminoethylcarbazole (AEC) or goat anti-rabbit IgG labeled to fluorescein isothiocyanate (GAR-FITC). Control sections were incubated with the specific antiserum preabsorbed with an excess of antigen ($100\ \mu\text{g}/\text{ml}$). The immunoreactivities in these sections were eliminated.

RESULTS

Substantial differences among FITC-, DAB- and AEC-stained sections were not found.

Embryonal ducks

The first appearance of VIP-containing nerve structures in embryonal tissues was observed at 5–6 days of incubation and corresponded to cellular bodies scattered in the mesenchyme of the antrum (Fig. 1) and in portions of the adjacent gizzard and duodenum. Two days later, small clusters of these cells were found in the same area (Fig. 2), while dimly stained pyrenophora appeared in the mesenchyme of the proventriculus. No positive fibres could be found. At 9 days of incubation, the VIP-reactive cell bodies contained in the segments cited above became more numerous and were localized between the smooth muscle precursor layer and the serosa. Rare positive fibres appeared in the corresponding area of the proventriculus, and clearly stained cell bodies were found in the mesenchyme of the jejunum-ileum.

In all the developmental stages, the myenteric plexus of the gizzard was localized between the circular muscle and the serosa as the external musculature was lacking. At 11 days, such a plexus showed numerous immunoreactive cell bodies grouped in ganglion-like structures (Fig. 3). Small ganglia containing positive pyrenophora were present also in the myenteric plexus of the glandular stomach and oesophagus. Moreover, VIP-positive cell bodies appeared in the subepithelial mesenchyme of the duodenal mucosa where the submucous plexus would develop (Fig. 4). They were more numerous than the myenteric ones. At this stage, positive cell bodies appeared in those areas corresponding to the main plexuses of the caeca (Fig. 5) and rectum. VIP-containing nerve fibres were seen infrequently along the whole intestine.

From 17 days onwards the gut of duck embryos assumed a more definitive morphology so that the nerve structures became more evident. In particular, positive fibres increased rapidly in number while the

Fig. 1. Antrum, 5 days of incubation. Dimly stained neuronal cell bodies scattered in the organic mesenchyme. PAP, DAB. $\times 1,240$

Fig. 2. Antrum, 7 days of incubation. VIP-positive cell bodies grouped in small clusters two days after their appearance. PAP, DAB. $\times 1,240$

Fig. 3. Gizzard, 11 days of incubation. Numerous positive nerve cells lining up at the periphery of a myenteric ganglionic structure. PAP, DAB. $\times 1,240$

Fig. 4. Duodenum, 11 days of incubation. VIP-containing cell bodies localized in the mesenchymal areas where the myenteric and submucous plexuses will develop. PAP, DAB. $\times 450$

Fig. 5. Caecum, 11 days of incubation. First appearance of positive pyrenophora within the organic mesenchyme. PAP, DAB. $\times 1,240$

Fig. 6. Proventriculus, 17 days of incubation. Immunoreactive fibres both in the longitudinal (*arrow*) and circular muscle layers. PAP, DAB. $\times 1,240$

Fig. 7. Gizzard, 17 days of incubation. A small cluster of VIP-immunoreactive fibres and cell bodies, contained in the circular musculature. PAP, DAB. $\times 1,240$

Fig. 8. Gizzard, 21 days of incubation. Positive fibres and cell bodies scattered in a chain-like ganglionic structure of the myenteric plexus. GAR-FITC. $\times 450$

Fig. 9. Rectum, 21 days of incubation. Brightly fluorescent nerve fibres grouped in small bundles contained in the circular muscle layers. GAR-FITC. $\times 450$

Fig. 10. Gizzard, 25 days of incubation. Dimly stained cell bodies at their appearance in the basal portion of the gastric mucosa. PAP, DAB. $\times 1,240$

Fig. 11. Caecum, 25 days of incubation. A positive cell body and dimly stained nerve fibres (*arrow*) in the basal portion of the mucosa. PAP, DAB. $\times 1,240$

Fig. 12. Oesophagus at hatching. A mucosal ganglionic structure showing numerous VIP-immunoreactive cell bodies. PAP, DAB. $\times 1,240$

Fig. 13. Jejunum-ileum at hatching. A still immature "deep muscular plexus" is constituted of a few fluorescent nerve fibres mainly running in isolation. GAR-FITC. $\times 450$

Fig. 14. Jejunum-ileum at hatching. Numerous VIP-containing fibres surrounding a periorganic blood vessel. PAP, DAB. $\times 450$

VIP-positive cell bodies decreased. Immunoreactive fibres appeared in both the muscle layers of the proventriculus (Fig. 6), duodenum and jejunum-ileum and in the circular musculature of the gizzard and oesophagus. Similar fibres were recognizable, for the first time, in the mucosa of the foregut, with the exception of the oesophagus. Positive cell bodies appeared within the circular muscle layer of the gizzard (Fig. 7).

At 21 days the myenteric plexus of the gizzard showed a chain of ganglionic structures containing many VIP-immunoreactive cell bodies (Fig. 8), while the same plexus of the proventriculus was found to contain positive fibres often grouped in bundles. Both positive fibres and cell bodies appeared in the submucous connective tissue of the proventriculus and oesophagus. The longitudinal muscle layer of the mid- and hindgut showed the presence of small ganglia containing VIP-immunoreactive fibres and cell bodies. Such a finding was frequent also in the myenteric plexus of the intestine. Positive fibres were described for the first time in the muscularis mucosae of the duodenum and in the circular muscle layer of the caeca and rectum (Fig. 9).

Finally, at 25 days of incubation, positive cell bodies appeared in the mucosa of the gizzard (Fig. 10) and proventriculus. They were rare and scattered among the secretory portions of the glands. VIP-containing fibres were present in the stroma of the duodenal villi and in the muscularis mucosae of the jejunum-ileum, caeca (Fig. 11) and rectum.

Hatching ducks

At hatching, VIP-immunoreactive fibres and cell bodies were observed in the oesophageal mucosa (Fig. 12). The pattern of the distribution of VIP nerves in the gastrointestinal tract differed from that in the adult in the incomplete formation of the "deep muscular plexus" (see below) of the small intestine (Fig. 13) and the scarcity of positive nerve terminals in its mucosal component. VIP-containing fibres were often observed around periorganic blood vessels (Fig. 14).

The results obtained from the study of embryonal and newly hatched subjects are summarized in Table 1.

Adult ducks

In the oesophagus of the adult duck, the myenteric plexus was localized between the circular muscle layer and the serosa because of the absence of the outer musculature. It contained numerous positive fibres distributed within nerve trunks (Fig. 15) or

around blood vessels. Immunoreactive fibres, isolated or grouped in small bundles, were also present in the circular muscle layer, submucous plexus, muscularis mucosae and lamina propria of the mucosa. In the latter they surrounded the secretory portions of the oesophageal glands and sometimes penetrated the connective septa dividing the secretory units (Fig. 16).

In the proventriculus, the outer longitudinal muscle layer, the myenteric plexus (Fig. 17), the internal circular musculature, the submucous plexus and the muscularis mucosae showed the presence of VIP-positive fibres, isolated or grouped in small bundles. They were also found around the secretory portions of the composite glands and in their intralobular connective septa. VIP-immunoreactive terminals frequently contacted single secretory cells. In the upper portion of the lamina propria, positive fibres gave rise to a subepithelial network from which terminals arose, penetrated the stroma of the digitiform projections of the mucosa and ran towards their top (Fig. 18). Rare positive cell bodies were seen as well.

In the muscular stomach, the myenteric plexus contained VIP-positive fibres and cell bodies grouped in large ganglia (Fig. 19) often linked to each other in long chain-like structures. The circular muscle layer and the muscularis mucosae showed the presence of positive fibres isolated or grouped in bundles. Subepithelial nervous networks, similar to those described in the proventriculus, were seen in the upper portion of the mucosa. Rare positive neurons were scattered in their meshes.

In the antrum VIP-containing fibres were present within the external muscle layer, despite its discontinuity and thinness. Such structures, often grouped in bundles, were found also in the circular musculature and the myenteric plexus. The positive nerve components of the mucosal plexus were represented by longitudinal fibres contained in the muscularis mucosae, rare fibres and neurons scattered in the lamina propria (Fig. 20) and interglandular fibres running towards the stroma of the mucosal projections.

The very thin submucous layer of the gizzard and antrum contained no nerve structures.

In the duodenum the diffusion and distribution of the VIP-immunoreactive nerve structures were closely similar to those found in the jejunum-ileum, so that the description of the relative patterns can be unified as follows.

In the small intestine, the external longitudinal muscle layer and the myenteric plexus contained numerous positive fibres (Fig. 21) and cell bodies, isolated or grouped in small ganglia. The internal muscle layer showed the presence of bundles of

Table 1. Time of appearance and distribution of VIP-immunoreactive nerve structures in the gastrointestinal tract of the developing duck embryo.

		5-6	7	9	11	13	17	21	25	Hatching
Oesophagus	Longitudinal muscle				*	*	*	*	*	*
	Myenteric plexus				+	+	+++	+++	+++	+++
	Circular muscle	-	-	-	-	-	+	+	++	+++
	Submucous plexus						-	+	+	++
	Muscularis mucosae						-	+	+	++
	Mucosa						-	-	-	+
Proventriculus	Longitudinal muscle				*	*	+	+	++	++
	Myenteric plexus				++	++	+++	+++	+++	+++
	Circular muscle	-	+	+	-	-	+	++	++	+++
	Submucous plexus						+	++	++	++
	Muscularis mucosae						+	+	+	++
	Mucosa						+	++	++	++
Gizzard	Longitudinal muscle				*	*	*	*	*	*
	Myenteric plexus				++	++	+++	+++	+++	+++
	Circular muscle	+	++	++	-	-	++	+++	+++	+++
	Submucous plexus						*	*	*	*
	Muscularis mucosae						+	++	+++	+++
	Mucosa						-	+	+	+
Antrum	Longitudinal muscle				*	*	*	*	*	*
	Myenteric plexus				++	++	++	+++	+++	+++
	Circular muscle	+	++	++	-	-	+	++	+++	+++
	Submucous plexus						*	*	*	*
	Muscularis mucosae						+	++	++	+++
	Mucosa						+	+	+	+
Duodenum	Longitudinal muscle				*	*	+	++	++	++
	Myenteric plexus				++	++	++	++	+++	+++
	Circular muscle	+	+	+	-	-	++	++	++	++
	Submucous plexus				++	++	++	++	++	+++
	Muscularis mucosae						-	+	++	++
	Mucosa						-	-	+	+
Jejunum-ileum	Longitudinal muscle				*	*	+	+	++	++
	Myenteric plexus				+	+	++	++	++	+++
	Circular muscle	-	-	+	-	-	+	+	++	++
	Submucous plexus				+	+	++	++	++	++
	Muscularis mucosae						-	-	+	+
	Mucosa						-	-	-	+
Caecum	Longitudinal muscle				*	*	-	+	++	++
	Myenteric plexus				+	+	++	++	++	+++
	Circular muscle	-	-	-	-	-	-	+	++	++
	Submucous plexus				+	+	++	++	++	+++
	Muscularis mucosae						-	-	+	+
	Mucosa						-	-	-	+
Rectum	Longitudinal muscle				*	*	-	++	+++	++++
	Myenteric plexus				+	+	++	++	+++	++++
	Circular muscle	-	-	-	-	-	-	+	++	+++
	Submucous plexus				+	+	+	++	++	++++
	Muscularis mucosae						-	-	+	++
	Mucosa						-	-	-	+

*: Structure not present, -: no immunoreactivity, +: very low immunoreactivity, ++: low immunoreactivity
 +++: moderate immunoreactivity, ++++: high immunoreactivity

Fig. 15. Oesophagus in an adult duck. Cross section of a myenteric nerve trunk showing brightly fluorescent fibres. GAR-FITC. $\times 450$

Fig. 16. Oesophagus in an adult duck. A small bundle of fluorescent fibres contained within the connective septa of a mucosal gland. GAR-FITC. $\times 450$

Fig. 17. Proventriculus in an adult duck. VIP-immunoreactive fibres and cell bodies in a myenteric ganglion. PAP, AEC. $\times 450$

Fig. 18. Proventriculus in an adult duck. Fluorescent fibres running in the stroma of mucosal digitiform projections. GAR-FITC. $\times 280$

Fig. 19. Gizzard in an adult duck. VIP-containing fibres and cell bodies present in a chain-like ganglion of the myenteric plexus. PAP, AEC. $\times 450$

Fig. 20. Antrum in an adult duck. A positive neuron isolated in the upper portion of the mucosa. PAP, DAB. $\times 1,240$

Fig. 21. Duodenum in an adult duck. Small bundles of positive fibres contained in the longitudinal muscle layer and myenteric plexus. PAP, DAB. $\times 450$

Fig. 22. Jejunum-ileum in an adult duck. A bundle of VIP-immunoreactive fibres called the "deep muscular plexus" within the inner portion of the circular muscle layer. GAR-FITC. $\times 450$

Fig. 23. Rectum in an adult duck. Circularly oriented bundles of fluorescent fibres running in the internal muscle layer. GAR-FITC. $\times 450$

Fig. 24. Rectum in an adult duck. A small ganglion localized in the submucous plexus. PAP, DAB. $\times 280$

Fig. 25. Various immunoreactive and immunonegative ganglion cells seen at higher magnification. PAP, DAB. $\times 1,240$

VIP-reactive fibres. In the inner portion of the layer these fibres constituted a circular, roughly continuous plexiform network (Fig. 22) that corresponded to the "deep muscular plexus" described in the small intestine of mammals. Positive fibres were also present in the submucous plexus, in the muscularis mucosae, among the secretory portions of the glands and in the stroma of the intestinal villi. Rare immunoreactive cell bodies were observed within the submucous plexus.

In the caeca the quantity and distribution of the positive structures contained in the myenteric, muscular and mucosal plexuses were similar to those described in the small intestine. VIP-containing fibres and cell bodies were numerous in the submucous plexus, and the fibres were frequently seen to contact negative pyrenophora. In the rectum the external longitudinal muscle layer and the myenteric plexus contained immunoreactive fibres grouped in bundles or scattered in small nervous trunks sometimes also showing positive cell bodies. Bundles of positive fibres were observed in the internal circular musculature (Fig. 23). The submucous plexus was constituted mainly of ganglia containing positive cell bodies and fibres, the latter often in contact with negative pyrenophora (Figs. 24, 25). The VIP innerva-

tion of the rectal mucosa was composed, as frequently observed along the intestine, by fibres running around the secretory portions of glands and in the stroma of intestinal villi.

The findings obtained led us to deem that the gizzard and the rectum were the gastrointestinal segments most abundantly supplied by positive structures in the adult duck, as summarized in Table 2.

Finally, the quantity of VIP-immunoreactive cell bodies and fibres observed in this study varied according to the stages of development. The cell bodies were relatively more numerous in embryos than in newly hatched or adult subjects: they appeared about three days before the fibres, and in early stages of the embryonal life were more numerous than they. Later on, the fibres increased greatly in number while the cell bodies decreased so that the former became more diffused.

DISCUSSION

Embryonal ducks

As previously reported, there is a lack of agreement over both the segment of the avian gut where VIP-

Table 2. Distribution of VIP-immunoreactive nerve structures in the gastrointestinal tract of the adult duck.

	Long. muscle	Myenteric plexus	Circ. muscle	Submucous plexus	Musc. mucosae	Mucosa
Oesophagus	*	++++	++++	++	+++	++
Proventriculus	++	++++	+++	++	++	+++
Gizzard	*	+++++	+++++	*	+++	++
Antrum	++	++++	++++	*	+++	++
Small intestine	+++	+++	+++	+++	++	++
Caecum	+++	+++	+++	+++	++	++
Rectum	++++	+++++	++++	+++++	+++	+++

* : Structure not present, ++: low immunoreactivity, +++: moderate immunoreactivity, ++++: high immunoreactivity, +++++: very high immunoreactivity

reactive nerves appear during the embryonal development and the time of their appearance. The earliest immunoreactivities were described in the chick oesophagus at 10 days of incubation (FONTAINE-PERUS et al., 1981; FONTAINE-PERUS, 1984), proventriculus at 13 days (SUNDLER et al., 1979) and gastro-duodenal complex at 5 days (EPSTEIN et al., 1983), and in the quail oesophagus at 9 days (FONTAINE-PERUS et al., 1981; FONTAINE-PERUS, 1984). Such different results probably depend on the sensitivity of the anti-VIP antibodies used, while differences in fixatives employed should not be influent (EPSTEIN et al., 1983). Our findings well correspond to those reported in chick embryos (EPSTEIN et al., 1983) in which VIP-containing nerves appear at 5 days of incubation in the gastro-duodenal complex. This time is coincident or slightly successive to that of the appearance in the same complex of ectodermal cells, retained as precursors of gastroenteric neurons. These cells arise from the neural crest, penetrate the gut at 3-4 days of incubation in proximity to the pharynx and colonize it in a rostro-caudal wave of migration (LE DOUARIN and TEILLET, 1973; ALLAN and NEWGREEN, 1980; PAYETTE et al., 1984; TUCKER et al., 1986). They were described in the walls of the proventriculus and gizzard at 4 and 5 days, respectively (TUCKER et al., 1986).

The differentiation of a neuronal phenotype in the embryonal gut probably depends on the presence of molecular factors which could be synthesized by the developing circular muscle, mesenchyme, nervous cells, connective tissue elements or extracellular matrix (SAFFREY and BURNSTOCK, 1988). Many of these factors should be present in the muscular stomach of the chick embryo in early stages of development because various neuroactive substances appear in its walls at this time. Particularly, VIP, substance

P, somatostatin, met-enkephalin (EPSTEIN et al., 1983), acetyl-cholinesterase (BENNETT and COBB, 1969) and serotonin uptake (GERSHON et al., 1980) have been described in the gizzard of chick embryos between 4 and 7 days of incubation.

On the basis of recent immunohistochemical studies, two patterns have been proposed for the diffusion of the VIP-containing nerve structures in the avian embryonal gut. According to the first hypothesis (SUNDLER et al., 1979; FONTAINE-PERUS et al., 1981; FONTAINE-PERUS, 1984), VIP-positive structures arise from the upper portion of the foregut and extend rostro-caudally up to the lower intestinal segments in quail and chick embryos. On the other hand (SAFFREY et al., 1982), positive structures arise from the gizzard and diffuse both cranially and caudally in chick embryos. This pattern is completed by the appearance of VIP-immunoreactive nerves in the rectum and their progressive caudo-cranial diffusion. Our findings in duck embryos are similar to those reported in the second hypothesis (SAFFREY et al., 1982) with the exception that we did not find a source of VIP-positive structures in the lower segments of the gut.

When the neural crest-derived cellular precursors of the gastrointestinal neurons colonize the chick embryonal gut, descending from the oesophagus, they become situated in the peripheral mesenchyme of the foregut and on the both inner and outer sides of the developing circular muscle layer of the post-umbilical gut (TUCKER et al., 1986). Such findings can be confirmed in the duck by the appearance of VIP-containing neurons in the myenteric plexus of the foregut and in both main plexuses of the intestine, duodenum excepted.

The VIP-positivities found in the gut of duck embryos from 5 days up to 13 days of incubation are

mainly represented by cell bodies. Between 13 days and 17 days, a diffuse increase in VIP-containing fibres was noted. They increased also in the foregut of chick embryos at about 13 days of incubation (EPSTEIN et al., 1983). The time interval of 13–17 days could be important for the growth of VIP-ergic neurons in the gastrointestinal tract of the avian embryo. It has been demonstrated that the invasion of the taenia coli muscle of guinea pig embryos by nervous fibres and the appearance of VIP-immunoreactivity in them were roughly coincident (SAFFREY and BURNSTOCK, 1988). Moreover, during these 13–17 days, the circular muscle layer develops in the gut of chick embryos; it could play a role in the neuronal growth, as reported above (SAFFREY and BURNSTOCK, 1988). On the other hand, the appearance of a specific phenotype in embryonal gastrointestinal neurons precedes by a fairly long time the assumption of a definitive functional role, as hypothesized for peptidergic and cholinergic neurons in the caecum and ileum of the guinea pig embryo (GINTZLER et al., 1980; GERSHON et al., 1981; SAFFREY and BURNSTOCK, 1988).

The number of gastrointestinal VIP-immunoreactive nerve cell bodies decreases progressively from embryonal to newly hatched and adult ducks, confirming results already reported in the chicken (SAFFREY et al., 1982). To explain such a finding, several hypotheses were proposed involving the synthesis of precursor molecules, the intracellular storage site of the neuropeptide and the different amounts of VIP produced in various stages of the neuronal development (HÖKFELT et al., 1980; SAFFREY et al., 1982). The last-mentioned hypothesis seems to be related to the report that an increased synaptic activity of sympathetic and sensory ganglionic neurons, both *in vivo* and *in vitro*, is accompanied by a reduction in VIP storage level and *vice versa* (KESSLER et al., 1983).

Adult ducks

The distribution pattern of the VIP-containing nerves in the gut of adult ducks closely resembles that described in turkey stags (VAILLANT et al., 1980), adult chickens (SUNDLER et al., 1979; SAFFREY et al., 1982) and various adult mammals (LARSSON et al., 1979).

The neuropeptide VIP plays three main functions in mammals: It is a potent vasodilator (EKLUND et al., 1979), a myorelaxant of the gastrointestinal smooth musculature (BODANSKY et al., 1973) and a stimulator of the intestinal secretions (KREJS et al., 1978). As myorelaxant, it acts directly on the smooth mus-

culature of the gut, and the VIP-ergic neuron seems to be stimulated by two different types of cholinergic inter-neurons. This mechanism of action probably involves the serotonin (CHIJIWA et al., 1986) and could be valid also for the duck.

Particular consideration has to be given to the rectum which, in birds, is peculiar in structure and physiology and corresponds only partially to the mammalian rectum. Its main functions are the storage and expulsion of feces, the absorption of water and electrolytes reflowing from the cloaca (SKADHAUGE, 1973) and the filling of the caeca (FENNA and BOAG, 1974). Such functions are performed by the onset of peristaltic and antiperistaltic movements which run along the whole rectum from the caeca to the cloaca and viceversa (FENNA and BOAG, 1974). These movements are much more active than those registered in other intestinal segments. It is possible that VIP and serotonin play a determinant role in the onset and synchronism of both rectal peristaltic and antiperistaltic waves. As reported above, VIP-containing nerve structures are particularly numerous in the walls of the avian rectum and, at least in mammals, they induce the myorelaxation of the intestinal musculature. Serotonin-producing neurons are absent from the avian gut, but the amine is contained in endocrine cells diffused along the gastrointestinal mucosa of many species of birds (ADAMSON and CAMPBELL, 1988). Such cells are particularly numerous in the caeca and rectum (RUTSCHKE, 1976; VITTORIA et al., 1981; VITTORIA and CECIO, 1984). The application of serotonin on the mucosal surface of the rabbit ileum causes the onset of peristaltic-like contractions, while the application of the amine on the serosal surface inhibits any movement (GONELLA, 1981).

Acknowledgements. We thank Mr. Antonio CALAMO for his photographic help.

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Prof. Alfredo VITTORIA
Department of Biological Structures,
Functions and Technology
University of Naples Federico II
Via Veterinaria 1
80137 Napoli, Italy