

Motilin-Immunoreactive Cells in the Duodenum, Pyloric Stomach and Pancreas of Caimans (*Caiman latirostris* and *Caiman crocodilus*, Alligatorinae): A Further Comparison Using Region-Specific Motilin Antisera*

Junzo YAMADA¹, Marco A. M. RODRIGUES², Nobuo KITAMURA¹, Vitalino D. PAI³, Tadayuki YAMASHITA¹, Tohru MOTIZUKI⁴ and Noboru YANAIHARA⁴

Department of Veterinary Anatomy¹, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan; Departamento de Morfohistologia², Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil; Departamento de Morfologia³, Universidade Estadual Paulista Campus de Botucatu, Botucatu, Brazil; and Laboratory of Bioorganic Chemistry⁴, University of Shizuoka School of Pharmaceutical Sciences, Shizuoka, Japan

Received April 15, 1991

Summary. Motilin-immunoreactive cells in the duodenum, pyloric stomach and pancreas of *Caiman latirostris* and *Caiman crocodilus* were investigated using region specific antisera for porcine and canine motilin molecules. Motilin-immunoreactive cells were found in the duodenum, pyloric stomach and pancreas of both caiman species. These cells were primarily open-type endocrine ones in the epithelium of the duodenum and pyloric stomach. Motilin-immunoreactive cells were observed in both the exocrine and endocrine portions of the pancreas, and frequently exhibited one or more cytoplasmic processes of variable length. Since motilin-immunoreactive cells do not cross-react with serotonin or any of the other pancreatic and gut hormones, they are considered to be cell type independent from any of the other known pancreatic or gut endocrine cells. The molecular similarity between caiman motilin and porcine and canine motilins and the heterogeneity of the motilin molecule in the caiman digestive system is discussed.

Motilin, a 22 amino acid polypeptide first isolated and characterized from porcine small intestine (BROWN et al., 1971), is known to stimulate phase III activity of the interdigestive migrating contractions in the stomach and duodenum (ITOH et al., 1976; WINGATE et al., 1976). The cellular source of intestinal motilin has tentatively been established as a population of argyrophil, or non-argentaffin cells in the gut mucosa,

although this definition is still controversial (KISHIMOTO et al., 1981). Motilin-like immunoreactivities also have been reported in porcine and rat tissues distinct from the gut, most notably in the central nervous system (YANAIHARA et al., 1980; NILAVER et al., 1988). In our previous studies (YAMADA et al., 1986a, 1987), we reported the presence of motilin-immunoreactive cells not only in the intestine but also in the pancreas and pyloric stomach of *Caiman latirostris* (Alligatorinae). The aim of the present study is to demonstrate the presence of motilin-immunoreactive cells in the pancreas and pyloric stomach of another caiman species and to conduct immunocytochemical comparisons of the motilin molecule using region-specific antisera.

MATERIALS AND METHODS

Samples of the pancreas, pyloric stomach and duodenum were obtained from both sexes of *Caiman latirostris* (n=5) and *Caiman crocodilus* (n=5), measuring 53-140 cm and 45-119 cm in body length, respectively. All tissues were fixed in Bouin's fluid, embedded in paraffin according to routine methods, and serially cut either at a 1.5 or 4 μ m thickness. To demonstrate motilin-immunoreactive cells, tissue sections

*This work was supported in part by a Research Grant-in-Aid (No. 63560283) from the Ministry of Education, Science and Culture, Japan, and the Brazilian National Research Council (CNPq).

were stained immunocytochemically using the peroxidase-antiperoxidase (PAP) method (STERNBERGER, 1979). Five different motilin antisera were used in this study. Antisera R-1104 (working dilution 1:1,000) and R-1106 (1:1,000) recognize whole molecule of the porcine motilin; antiserum R-1105 (1:1,000) reacts significantly with the C-terminal sequence of the porcine motilin molecule; and antiserum MBR-02 (1:400) is specific for the N-terminal sequence of the porcine motilin molecule. Antiserum R-0220 (1:2,000) recognizes the canine motilin molecule. The methods employed to produce and characterize these antisera have been reported previously in detail (YANAIHARA et al., 1980, 1990). To prevent non-specific staining, the sections were incubated with non-immunized goat serum prior to incubation with specific antisera, and the specific antisera were absorbed with bovine liver powder and diluted in 0.01 M phosphate-buffered 0.1 M saline (pH 7.3). Tissues from the duodenum of a dog and pig fixed with Bouin's fluid served as positive controls.

RESULTS

The pancreas

Antisera R-1104 and R-1106, both of which recognize the whole molecule of porcine motilin, and antiserum R-1105, which cross-reacts significantly with the C-terminal portion of the porcine motilin molecule, demonstrated immunoreactive cells in the exocrine and endocrine portions of the pancreas from both species of caiman. These motilin-immunoreactive cells also were clearly detected by antiserum R-0220 which recognizes the canine motilin molecule. Antiserum MBR-02, which is specific for the N-terminal portion of the porcine motilin molecule, could be demonstrated in most but not all motilin-immunoreactive cells when adjacent thin serial sections were stained with the five types of motilin-antisera used (Fig. 1). A few motilin-immunoreactive cells reacted to antisera R-1104, R-1105, R-1106 and R-0220, but not to antiserum MBR-02 (Fig. 2).

In *Caiman crocodilus*, the motilin-immunoreactive cells never showed any evidence of cross-reactivity with antisera for established pancreatic hormones or serotonin as was shown previously in *Caiman latirostris* (YAMADA et al., 1986b). The polygonal motilin-immunoreactive cells frequently possessed one or more cytoplasmic processes of variable length that ended in synaptic button-like swellings. The processes appeared to establish contact with adjacent endocrine and exocrine cells in the pancreas. There were no appar-

ent differences in the distribution or in the frequency of the occurrence of motilin-immunoreactive cells between *Caiman latirostris* and *Caiman crocodilus*.

The pyloric stomach and duodenum

Motilin-immunoreactive cells in the pyloric stomach and duodenum of both caiman species appeared as elongated triangles or were spindle-shaped with an elongated apical cytoplasmic process. These were recognized as the open-type endocrine cell; most were demonstrated by all the motilin antisera used in this study (Figs. 3, 5, 6). However, a few motilin cells were not stained with the MBR-02 antiserum (Fig. 4); the immunoreactivity being found with R-1104, R-1105, R-1106 and R-0220. Generally the immunoreactivity with MBR-02 was weaker than with the other four kinds of antisera (Fig. 3).

The motilin-immunoreactive cells examined failed to show cross-reactivity with serotonin and other established gut hormones. There were no significant differences in the distribution or frequency of occurrence of motilin-immunoreactive cells in the species of caiman examined in this study.

DISCUSSION

Although motilin cells are now considered one of the major endocrine cell types in the duodenum (SOLCIA et al., 1981), they also have been demonstrated in the pancreas (YAMADA et al., 1986b) and the pyloric stomach (YAMADA et al., 1987) of the alligator (*Caiman latirostris*). The results of the present study suggest that the presence of motilin-immunoreactive cells in the duodenum, pyloric stomach and pancreas is characteristic of the caiman family. Motilin-immunoreactive cells have also been reported in the gizzard (YAMADA et al., 1986a; RICHARDSON et al., 1988) and pyloric region (RICHARDSON et al., 1988; SAITO et al., 1990) of certain avian species and in the pancreas of the echidna (*Tachyglossus aculeatus*, Monotremata) (YAMADA et al., 1990). Because motilin-immunoreactive cells have been identified in the stomach and pancreas as well as in the small intestine, it is possible that they exist in these regions in other species as well.

It has been well established that motilin is an active regulatory peptide which induces phase III activity of the interdigestive migrating contractions in the stomach and duodenum in fasted but healthy, conscious dogs, and that the motilin-induced contractions of the stomach and duodenum move in a caudal direction along the small intestine. The motilin cells detected in the pyloric stomach of caimans may also

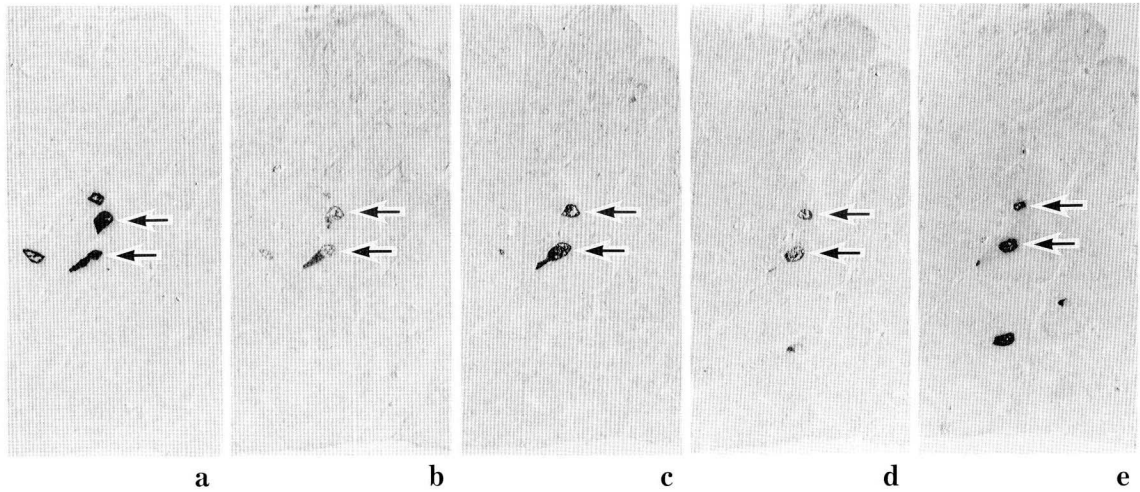


Fig. 1. Five serial sections of the pancreas of *Caiman crocodilus* immunostained with five different antisera: R-1104 (a), MBR-02 (b), R-1105 (c), R-0220 (d) and R-1106 (e). Two cells indicated by *arrows* in each micrograph show immunoreactivities for all of the antisera. $\times 200$

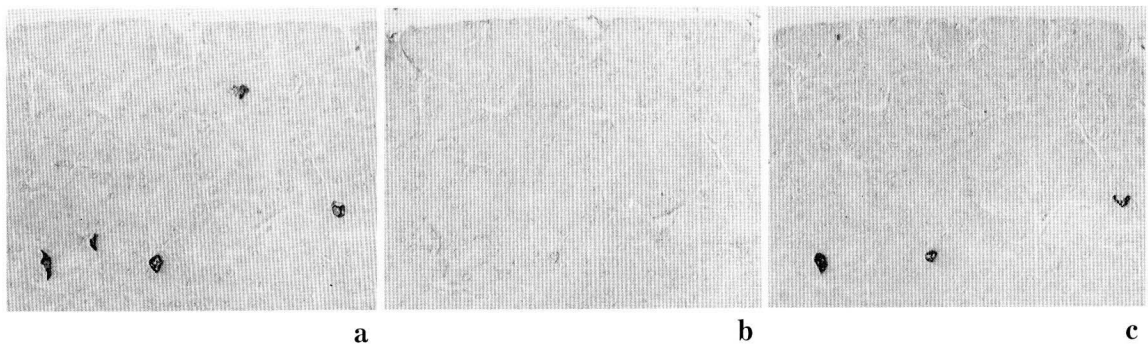


Fig. 2. Motilin-immunoreactive cells from another field of the pancreas but from the same sections as in Figures 1a-c. Motilin-immunoreactive cells in a and c are negative for antiserum MBR-02 as shown in b. $\times 200$

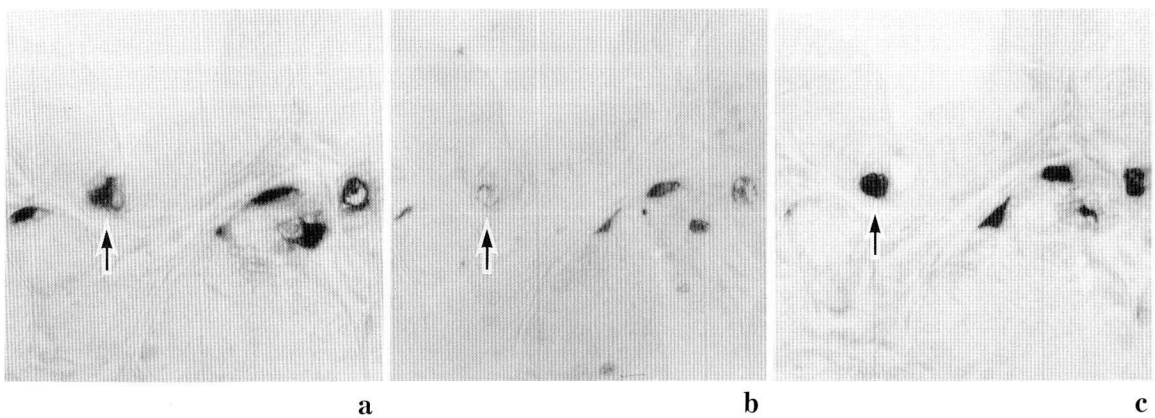


Fig. 3. Three serial sections from the pyloric stomach of *Caiman latirostris* are immunostained with antisera R-1106 (a), MBR-02 (b) and R-1105 (c), respectively. The cells indicated by *arrows* are strongly immunoreactive for antisera R-1106 (a) and R-1105 (c), but weakly stained using antiserum MBR-02 (b). Other immunoreactive cells are clearly stained with all of the antisera. $\times 300$

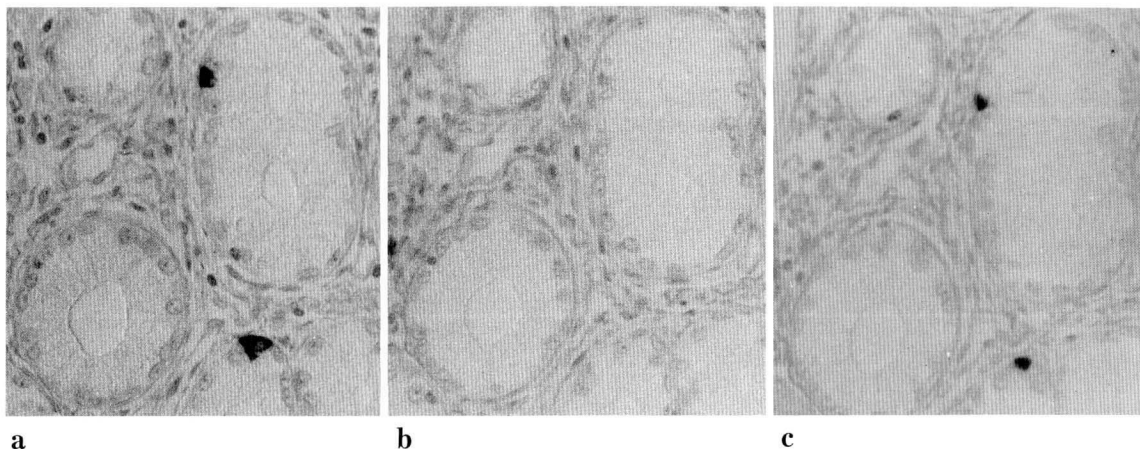


Fig. 4. Three serial sections of the pyloric region of *Caiman crocodilus* are immunostained with antisera R-1104 (a), MBR-02 (b) and R-1106 (c), respectively. Two motilin-immunoreactive cells shown in a and c are negative to antiserum MBR-02 (b). $\times 300$

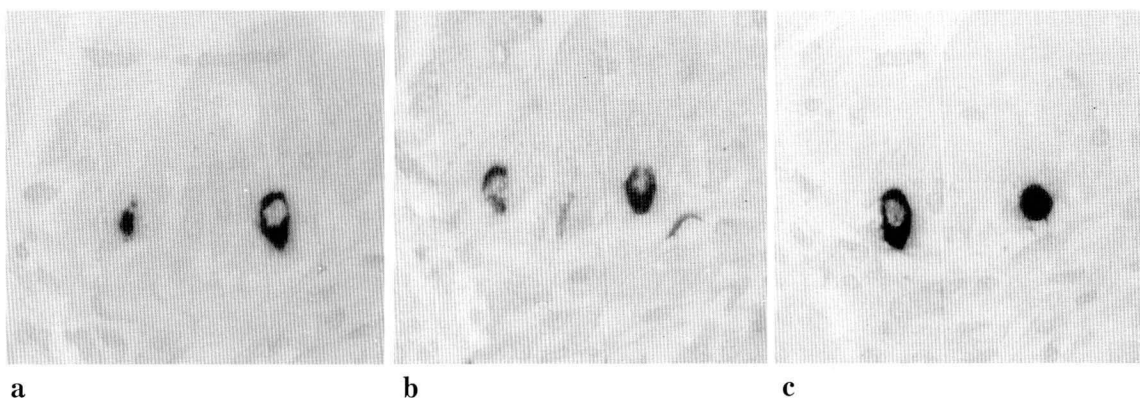


Fig. 5. Three serial sections of the duodenum of *Caiman crocodilus* are immunostained with antisera R-1106 (a), MBR-02 (b) and R-1104 (c), respectively. Motilin-immunoreactive cells are detected with all of the antisera. $\times 300$

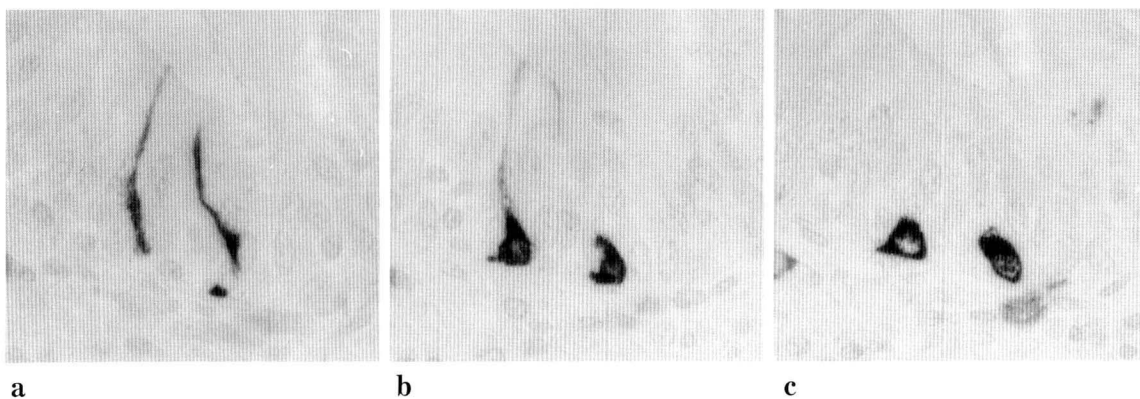


Fig. 6. Three serial sections of the duodenum of *Caiman latirostris* are immunostained with R-1106 (a), R-0220 (b) and R-1105 (c), respectively. Motilin-immunoreactive cells are immunostained with all of the antisera. $\times 300$

play a regulatory role in the gastric motility together with duodenal motilin cells. A stimulatory effect of motilin on pancreatic secretion, first suggested by KONTUREK et al. (1976), has been confirmed by LEE et al. (1986). Based on these views and on the morphological characteristics of the pancreatic motilin-immunoreactive cells described here and previously (YAMADA et al., 1986b) it seems reasonable to support the concept that pancreatic motilin cells of the caiman may be involved in the regulation of pancreatic secretion by a paracrine mechanism.

There were no remarkable differences in the immunoreactivities between the gut and pancreatic motilin cells with regard to the use of specific antisera for either porcine or canine motilin. Although most motilin cells were immunoreactive for all the antisera used, a few motilin-immunoreactive cells were immunoreactive for antisera R-1104 and R-1106 (recognizing the whole molecule of the porcine motilin), R-1105 (cross-reacting significantly with the C-terminal portion of porcine motilin molecule) and R-0220 (recognizing the canine motilin molecule), but not for MBR-02 (specific for the N-terminal portion of the porcine motilin molecule). Such observations suggest that a dominant molecular form of caiman motilin resembles both the porcine and canine forms of motilin and that a second minor molecular form of motilin may be present, causing an N-terminal sequence different from the major molecular forms of caiman motilin. These results suggest that there may be at least two molecular forms of motilin in the caiman.

The heterogeneity of the motilin molecule in mammalian tissues has been suggested by a report utilizing radioimmunoassay (NILAVER et al., 1988). The heterogeneity of the motilin immunoreactivity demonstrated in the present immunocytochemical study also suggests the heterogeneity of the motilin molecule in the caiman digestive system.

Acknowledgements. We wish to thank Professor W. J. KRAUSE, Department of Anatomy, School of Medicine, University of Missouri-Columbia, MO, U.S.A., for critical reviewing the manuscript.

REFERENCES

- BROWN, J. C., V. MUTT and J. R. DRYBURGH:** The further purification of motilin, a gastric motor activity stimulating polypeptide from the mucosa of the small intestine of hog. *Can. J. Physiol. Pharmacol.* 49: 399-405 (1971).
- ITO, Z., R. HONDA, K. HIWATASHI, S. TAKEUCHI, I. AIZAWA, R. TAKAYANAGI and E. E. COUCH:** Motilin-induced mechanical activity in the canine alimentary tract. *Scand. J. Gastroenterol.* 11 (Suppl. 39): 93-110 (1976).
- KISHIMOTO, S., J. M. POLAK, A. M. BUCHAN, A. A. J. VERHOFSTAD, H. W. M. STEINBUSH, N. YANAIHARA, S. R. BLOOM and A. G. E. PEARSE:** Motilin cells investigated by the use of region-specific antisera. *Virchows Arch. [Cell Pathol.]* 36: 207-218 (1981).
- KONTUREK, S. J., A. DEMBINSKI, R. KROL and E. WÜNSCH:** Effects of motilin on gastric and pancreatic secretion in dogs. *Scand. J. Gastroenterol.* 39 (Suppl): 57-61 (1976).
- LEE, K. Y., K. SHIRATORI, T.-M. CHANG and W. Y. CHEY:** A hormonal mechanism for the interdigestive pancreatic secretion in dogs. *Amer. J. Physiol.* 251: G759-764 (1986).
- NILAVER, G., M. G. BEINFELD, C. T. BOND, D. DAIKH, B. GODFREY and J. P. ADELMAN:** Heterogeneity of motilin immunoreactivity in mammalian tissue. *Synapse* 2: 266-275 (1988).
- RICHARDSON, K. C., J. YAMADA and R. D. WOOLLER:** An immunohistochemical study of the gastrointestinal endocrine cells of the New Holland honey-eater, *Phylidonyris novaehollandiae*. *Aust. J. Zool.* 36: 483-496 (1988).
- SAITO, T., J. YAMADA, N. KITAMURA and T. YAMASHITA:** An immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of domestic pigeon, (*Columba livia* var *domestica*). *Z. Mikrosk.-Anat. Forsch.* 103: 237-246 (1990).
- SOLCIA, E., J. M. POLAK, L.-I. LARSSON, A. M. BUCHAN and C. CAPELLA:** Update on Lausanne classification of endocrine cells. In: (ed. by) S. R. BLOOM and J. M. POLAK: *Gut hormones*. 2nd ed. Churchill Livingstone, Edinburgh, 1981 (p. 96-101).
- STERNBERGER, L. A.:** *Immunocytochemistry*. 2nd ed. John Wiley & Sons, New York, 1979.
- WINGATE, D. L., H. RUPPIN, W. E. R. GREEN, H. H. THOMPSON, W. DOMSCHKE, W. E. WÜNSCH, L. DEMLING and H. D. RITCHIE:** Motilin-induced electrical activity in the canine gastrointestinal tract. *Scand. J. Gastroenterol.* 11 (Suppl. 39): 111-118 (1976).
- YAMADA, J., N. KITAMURA, T. YAMASHITA and N. YANAIHARA:** Immunohistochemical studies on the endocrine cells in avian gizzard. *Biomed. Res.* 7: 39-45 (1986a).
- YAMADA, J., V. J. M. CAMPOS, N. KITAMURA, A. C. PACHECO, T. YAMASHITA and N. YANAIHARA:** An immunohistochemical study of endocrine cells in the pancreas of *Caiman latirostris* (Alligatorinae), with special reference to pancreatic motilin cells. *Biomed. Res.* 7: 199-208 (1986b).
- : An immunohistochemical study of the endocrine cells in the gastrointestinal mucosa of the *Caiman latirostris*. *Arch. Histol. Jap.* 50: 229-241 (1987).
- YAMADA, J., W. J. KRAUSE, N. EDWIN, T. MOCHIZUKI and N. YANAIHARA:** A survey of endocrine cells in the

pancreas of the echidna (*Tachyglossus aculeatus*) with special reference to pancreatic motilin cells. *J. Anat.* 171: 223-231 (1990).

YANAIHARA, N., C. YANAIHARA, K. NAGAI, H. SATO, F. SHIMIZU, K. YAMAGUCHI and K. ABE: Motilin-like immunoreactivity in porcine, human and rat tissue. *Biomed. Res.* 1: 76-83 (1980).

YANAIHARA, N., C. YANAIHARA, T. MOCHIZUKI, K. IGUCHI and M. HOSHINO: Chemical synthesis, radioimmunoassay and distribution of immunoreactivity. In: (ed. by) Z. ITOH: *Motilin*. Academic Press, Sandiego-New York-Berkely-Boston-Sydney-Tokyo-Toronto, 1990 (p. 31-46).

Dr. Junzo YAMADA
Department of Veterinary Anatomy
Obihiro University of Agriculture
and Veterinary Medicine
Nishi-2, Inada-cho, Obihiro
Hokkaido, 080 Japan

山田 純三
080 帯広市稲田町西2
帯広畜産大学畜産学部獣医学科
家畜解剖学教室