

# PHYSIOLOGIC VARIATIONS OF GASTRIN IN SERUM OF GERMAN SHEPHERD DOG.

Cavallone E.\*\*, Gualtieri M.\*, Corti M.\*, Ciorba A.\*\*\*, Rimoldi E.M.\*, Leonardi L.\*

\* *Institute of Veterinary Surgery and Radiology - University of Milan (Italy)*

\*\* *Radioisotopes Laboratory of Veterinary Medicine - University of Milan (Italy)*

\*\*\* *Department of Veterinary Biopathological Sciences - University of Perugia (Italy)*

## INTRODUCTION

Gastrin is the pool of various polypeptides at different molecular weight, 90% of which is secreted by G cells of antral and pyloric mucosa and the remaining 10% by duodenal mucosa. Various types of gastrin (e.g. G-34 and G-17) are named on the number of aminoacids forming their polypeptide chain (*Shulkes A. and Baldwin G., 1997*). The synthesis and release of gastrin into the bloodstream are related to the ingestion of proteic foods and also to the mere gastric filling (*Gee J.M. and others, 1996; Ghatei M.A. and others, 1997; Leib M.S. and others, 1985*) and result from a “cephalic control”, being able the simple vision or even the thought of food to activate a parasympathetic stimulation of G cells’ activity (*Johnson C.D. and others, 1996; Karnik P.S. and others, 1990; Lenz H.J. and others, 1988; Riquet W., 1989*). The complex interactions with hormonal axis of calcium metabolism are still under study. Recent investigations in rats seem to confirm the action of vitamin D alone on gastrin’s secretion (*Gagnemo-Persson R. and others, 1999; Hakanson R., 1996; Inoue K. and others, 1985; Rumenapf and others, 1998; Surve V.V. and others, 1998*).

Gastrin is synthesized as a pre-propeptide of 101 aminoacids, which is converted in the endoplasmic reticulum and in the Golgi apparatus to G-34, which is then resolved to G-17 by the pro-hormone-convertase enzyme. The terminal sequence of 5 aminoacids (pentagastrin or G-5) is identical to the terminal sequence of cholecystokinin (CCK), an important regulator of pancreatic secretion (*Shulkes A. and others, 1997*).

When artificially administered, pentagastrin stimulates HCl secretion and seems to be able to induce crisis of panic by binding a specific CNS receptor (*Agugini G. and others, 1996; Gomez G.*

*and others, 1996; Leeuw A.S. and others, 1996; Megen H.J. and others, 1994; Nustede R. and others, 1993*). The regulation of gastrin secretion is summarized in Scheme 1. It is well-known that all the types of gastrin stimulate gastric and pancreatic secretion (*Agugini G.- Beghelli V. and others, 1996; Gomez G. and others, 1996; Nustede R. and others, 1993; Strombeck D.R.- Guilford W.G., 1991*).

Gastrinemia raises in the course of acute and chronic gastritis, of peptic and duodenal ulcers, stress and tumors even if not located in stomach but also in other anatomic zones, as in Zollinger-Ellison syndrome (gastrinoma) or in Type-1 Multiple Endocrine syndrome (MEN-1) observed also in dogs, and being excreted by kidney raises also in the course of nephrosis (*Diaz- Sanchez C.L. and others, 1998; Faglia G., 1997; Fox L.E. and others, 1990; Feldman E.C. and others, 1992; Hayden D.W., 1997; Perkins S.E. and others, 1996; Simpson K.W. and others, 1999; Strombeck D.R. and others, 1991; Sukhodolia A.I., 1999*). On the contrary, gastrin's hematic concentration is reduced in the presence of mastocytoma or mastocytosarcoma (*Hayden D.W. and others, 1997*).

### **Scheme 1 Regulation of Gastrin secretion**

Aim of present investigation has been to evaluate the validity of a procedure to determine the gastrin in dogs, to establish the reference range of our laboratory for the German shepherd dog and to establish if variations related to sex and age exist.

### **MATERIALS AND METODS**

Blood samples have been drawn from 40 German shepherd dogs, 20 female and 20 male, of the Perugia Revenue Guard Corps breeding. The drawings have been taken in the course of routine controls. The dogs were grouped according to the age in groups of 8 subjects, 4 female and 4 male, each.

The age ranges were:

1<sup>st</sup> group: 3-3.5 months

2<sup>nd</sup> group: 7-15 months

3<sup>rd</sup> group: 2-3 years

4<sup>th</sup> group: 4-6 years

5<sup>th</sup> group: 7-10 years.

All the subjects, fasting for 18 hours, have been submitted to clinical examination and routine laboratory tests (complete blood count, creatininemia and uraemia)

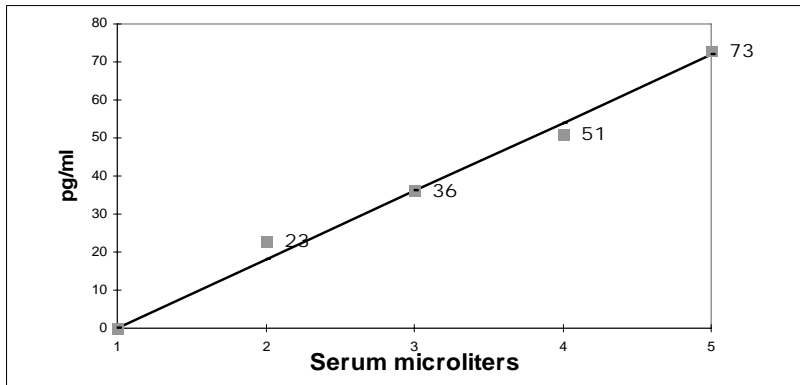
The blood samples, taken at 10 a.m., were immediately centrifuged and serum was subdivided in aliquots and stored at  $-20^{\circ}\text{C}$  until determination performed 48 hours after the blood drawing.

For the determination of gastrin a polyclonal double-antibody RIA kit, labelled with  $^{125}\text{I}$  has been used (DPC, Diagnostic Products Corporation, Los Angeles CA, USA, distributed in Italy by Medical Systems, Genoa). This procedure was declared to be able to recognize the main molecular fragments of gastrin, G-34 and G-17 (*Rehfeld J.F. and others, 1983*). Being this procedure non specific for canine species, and being a dog-specific commercial kit not available on the market, it has been necessary to perform a dose-response curve to ascertain the validity of the procedure applied to canine species. To this aim, increasing serum doses have been tested (100, 200, 300 and 400  $\mu\text{L}$ ). In all the samples the hormone concentration has been determined in the bound fraction, obtained by centrifugation at  $+4^{\circ}\text{C}$  and after supernatant aspiration. All the tests have been performed in duplicate. The samples have been read in a computerized gamma counter with NaI (T1) detector (Canberra Packard, Meriden, CT, USA).

## **RESULTS**

The results obtained with the dose-response curve, shown in Figure 1, show an optimal linearity between increasing serum doses and gastrin concentrations obtained.

**Figure 1**  
**Gastrin kit in the dog. Dose–response curve.**



1=0 2=100 3=200 4=300 5=400  $\mu$ L serum

In table 1 coefficient of variation % of each point of curve, corresponding to the means of duplicates, are reported.

**Table 1**

DATA POINT	MEAN VALUE	% CV
2	23.2	1.509
3	36.4	1.928
4	50.9	2.029
5	73.1	1.958

In present study sera from 40 subjects homogeneous for race and diet have been tested. They resulted to be healthy at clinical examination and laboratory tests resulted within normal values. These factors rendered possible the evaluation of a reference range and restricted the variability to the parameters under study, i.e. age and sex. The calculated reference range is shown in Table 2.

**Table 2**

**Gastrin values (pg/ml) in German shepherd dog sera**

Subjects number	Mean value	SD (standard deviation)	Reference range
40	<b>38.2</b>	<b>11.8</b>	<b>14.6 - 61.8</b>

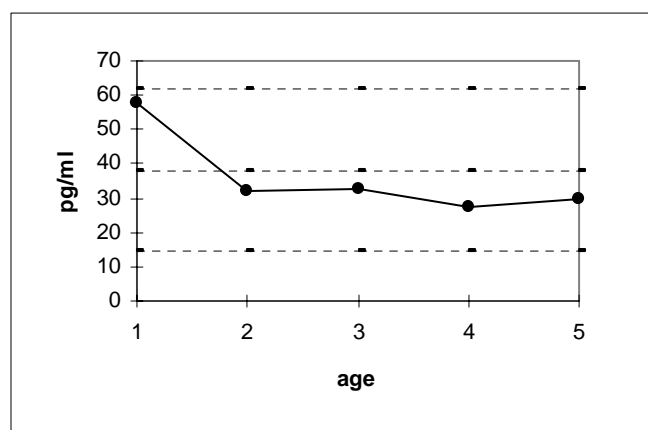
As regards the sex, we did not observe any difference, except for a lightly higher variability in males, considered fortuitous. The observed values are shown in Table 3

**Table 3: Gastrin values (pg/ml) in both sexes**

males (mean value)	SD (standard dev.)	females (mean value)	SD (standard dev.)
38.2	<b>12.7</b>	<b>38.2</b>	<b>10.4</b>

As regards variations in different groups of ages, the results are shown in Figure 2. A steep decrease of serum gastrin concentration was observed from the first (3-3.5 months) to the second (7-15 months) group. The values remain rather constant in the following age groups fig.2 and tab.4)

**Gastrin values in serum (pg/ml) according to age**  
**Fig.2**



group 1: 3-3,5 months - group 2 = 7-15 months - group 3: 2-3 years – group 4: 4-6 years - group 5: 7-10 years

**Table 4**

Group	Mean value	SD (Standard deviation)
-------	------------	----------------------------

<b>1° (3-3,5 months)</b>	<b>57.7</b>	<b>3.7</b>
<b>2° (7-15 months)</b>	<b>32.1</b>	<b>1.5</b>
<b>3° (2-3 years)</b>	<b>32.8</b>	<b>2.3</b>
<b>4° (4-6 years)</b>	<b>27.3</b>	<b>2.6</b>
<b>5° (7-10 years)</b>	<b>29.8</b>	<b>2.1</b>

## **DISCUSSION**

The main aims of present study have been achieved. In fact the procedure adopted to determine gastrin in dog has been validated, as results from the dose-response curve (Figure 1) showing that kit's reagents were specific also for this species. Then we have been able to determine in our laboratory the reference ranges for German shepherd dog.

The population submitted to the study, even though not much large, was considered statistically significant. In fact the minimum number of subjects required to evaluate the variations of a biological parameter in a homogeneous population should be at least 31, corresponding to 30 degrees of freedom (31-1). Probably it is possible to extend our reference range to the whole canine species, being the values here observed corresponding to those reported in literature based on studies mongrel dogs (15.0-78.6) (*Della Longa A. and others, 1986; Romussi S. Gualtieri M., 1991*). It is important to underline that the assessment of reference range in healthy subjects is an essential prerequisite for the study of pathological variations of any biochemical or hormonal parameter, which can be influenced by instruments and assay procedure (*Hesser and others, 1983*), by animal species, race, sex, age, life style, climate and geographical area of population under study. Besides, variations of hormones and in particular of gastrin have been observed in various physiological condition, as after exercise (*Furr M. and others, 1994*). Variations observed in present study for gastrin in relation to age confirmed previous data for parathormone and calcium ions (*Cavallone E. and others, 1998*), and demonstrate that growth is a phase of turbulent metabolic processes, requiring a thorough attention of Veterinary and a careful evaluation of laboratory

parameters, especially of hormones. Present data have an interest in clinical evaluation of laboratory results, and have also a relevant physiological significance. In fact the high values observed in the younger subjects could be related to the need to absorb quickly proteic substances, valuable in the intense growth of puppies. This interpretation is supported by recent investigations performed in humans. In fact it has been observed that in children aged 1-15 years the higher concentration of gastrin was observed in periods of more intense growth, related not only to the high protein needs but also to the higher vagal tone and hypothalamic activity (*Alfiven G. and others, 1995; Ohning G.V. and others, 1996*). Other Authors observed that in pigs the haematic concentration of gastrin was much higher in breast-fed than in weaned subjects (*Mao X.R. and others, 1996*). Being the animals object of present study already weaned, we could expect to find much higher concentrations in dog during suckling. The subsequent stabilization at the various ages of serum gastrin values can be related to the needs to maintain unchanged the protein asset of the body. As regards sex, we did not observe any significant variation in healthy subjects. It is however timely to remind that tumors of uterus, breast and prostate can influence its secretion (*Feldman E.C. and others, 1992*).

## **CONCLUSIONS**

In conclusion, although we consider preliminary the present study, we believe it represents the basis for further widening of investigation. Lastly, we have to underline that, in this first experimental approach, the blood drawings have been taken at 10 a.m. after 18 hours fasting, therefore in conditions corresponding to the common laboratory practice. Nevertheless, considering the circadian cycles of hormonal secretion (*Agugini G and others, 1996; Faglia G., 1997; Feldman E.C. and others, 1992*), it will be of great interest, in different animal species, a further study in depth with serial determination of gastrin and other hormones.

## **ACKNOWLEDGEMENT**

We wish to record our tanks to Dr. Amodio and to the “Guardia di Finanza” of Perugia (Italy)

## **SUMMARY**

Gastrin is a pool of peptide hormones at different molecular weight secreted by gastric and duodenal mucosa during feeding and involved in the intake of proteins. Aim of present study was to establish the gastrin concentration in serum samples of 40 healthy German shepherd dogs belonging to 5 different age groups. A RIA commercial kit has been used, which has been validated for the animal species under study with preliminary dose-response experiments. Results obtained showed specificity of the procedure used for canine gastrin. The obtained reference range (14.6-61.8 pg/ml) resulted to be superimposable to the range reported in literature for italian mongrel dogs (15- 78.6 pg/ml). No differences related to sex have been observed (females  $38.2 \pm 10.4$  pg/ml males  $38.2 \pm 12.7$  pg/ml). The gastrin concentration resulted to be elevated in puppies aged 3-3.5 months (58 pg/ml) and stabilized in dogs from 7 months to 10 years on values ranging 27-33 pg/ml. Physiological and pathological role of this hormone is discussed. This study supports the critical evaluation of pathological variations of gastrin in serum of the dogs..

**Key words: gastrin; dog; reference range; aging.**



## REFERENCES

- Agugini G., Beghelli V., Giulio L.F. (1996) Apparecchio digerente in Fisiologia degli Animali Domestici con Elementi di Etologia, 2th edn. Ed. *UTET*, Torino pp 527-572
- Alfiven G., Gustavsson P. and others (1995) Age-related decrease in plasma levels of gastrin, cholecystokinin and somatostatin. *Acta Paediatrica*; 84:12, 1344-1346
- Cavallone E., Di Giancamillo M., Guazzoni M., Leonardi L., Rimoldi E.M., Note preliminari sulle variazioni fisiologiche del Paratormone (PTH) nel cane (1998) *Archivio Veterinario Italiano*, vol. 50 (5/6), 145- 152
- Della Longa A., Martelli F. and others (1986) Studio dei valori della gastrina plasmatica nel cane in condizioni normali e patologiche. *Atti della Società Italiana delle Scienze Veterinarie*; 39:2, 135-137
- Diaz-Sanchez CL and others (1998). Gastric neuroendocrine tumor. *Review Gastroenterology of Mexico*. Apr-Jun; 63(2), 97-100
- Faglia G. (1997). Malattie del Sistema Endocrino e del Metabolismo. *Mc Graw-Hill*, 2° ed.; Cap. 16.
- Fox L.E., Rosenthal R.C. and others (1990). Plasma histamine and gastrin concentrations in 17 dogs with mast cell tumors. *Journal of Veterinary Internal Medicine*; 4:5, 242-246
- Furr M., Taylor L. and others (1994).The effect of exercise training on serum gastrin response in the horse. *Cornell Veterinarian*; 84:1, 41-45
- Feldman E.C., Nelson R.W. (1992) Gastrinoma, Glucanoma e altri APUDomi in Endocrinologia e riproduzione del Cane e del Gatto. 2th edn. Ed. *UTET*, Torino pp 489-497.
- Gagnemo-Persson R and others (1999) Rat stomach ECL-cell histidine decarboxylase activity is suppressed by ergocalciferol but unaffected by parathyroid hormone and calcitonin. *Regulatory Peptides*; 5; 79 (2-3): 131-9.
- Gee J.M., Lee-Finglas and others (1996). Fermentable carbohydrate modulates postprandial enteroglucagon and gastrin release in rats. *British Journal of nutrition*; 75:5, 757-766
- Ghatei M.A., Ratcliffe B. and others (1997). Fermentable dietary fibre, intestinal microflora and plasma hormones in the rat. *Clinical Science*; 93:2, 109 112
- Gomez G., Udipi V. and others (1996). Growth hormone upregulates gastrin and peptide YY gene expression. *American Journal of Physiology*; 271:3, 1, e582-e586
- Hakanson R. and others (1996).Perturbations in blood Ca(2)+ do not affect the activity of rat stomach enterochromaffin-like cells. *Scandinavian Journal of Gastroenterology*; 31(3): 217-27
- Hayden D.W., Henson M.S. and others (1997). Gastrin-secreting pancreatic endocrine tumor in a dog (putative Zollinger-Ellison syndrome). *Journal of Veterinary Diagnostic Investigation*; 9: 100-103

- Hesser, Kao (1983). Comparison of two assay for serum gastrin. *Clinical Chemistry*; 29: 1161-2
- Inoue K., Fried G.M. and others (1985). Effect of divalent cations on gastrointestinal Hormone release and exocrine pancreatic secretion in dogs. *American Journal of Physiology*; 248,1,I, G28-G34
- Jin H.O., Lee K.Y. and others (1994). Physiological role of cholecystokinin on gastric emptying and acid output in dogs. *Digestive Disease and Sciences*; vol.39 (11), 2306-2314
- Johnson C.D., Chayvialle J.A. and others (1986). Neural pathways for the release of gastrin, cholecystokinin and pancreatic polipeptide after a meal in dogs. *Digestive Diseases and Sciences*; vol. 31 (12), 1361-1369
- Karnik P.S., Wolfe M.M. (1990). Somatostatin stimulates gastrin mRNA turnover in dog antral mucosa. *The Journal of Biological Chemistry*; vol.265 (5), 2550-2555
- Leeuw A.S. et al (1996). Pentagastrin has panic-inducing properties in obsessive convulsive disorder. *Psychopharmacology*; 126(4): 339-44
- Leib M.S., Wingfield W.E. and others (1985). Gastric distention and gastrin in the dog. *American Journal of Veterinaary Research*; vol.46(9), 2011-2015.
- Lenz H.J., Hester S.E. and others (1988). CNS actions of calcitonin gene-related peptide on gastric acid secretion in conscius dogs. *American Journal of Physiology*; 255: 3, 1, G298-G303
- Mao X.R., Tso Y.L. (1996). Stability of gastrin in the gastrointestinal lumen of suckling, weanling and adult pigs. *Biology of the neonate*; 70: 1, 60-68
- Megen H.J. et al (1994). Pentagastrin induced panic attacks: enhanced sensitivity in panic disorder patients. *Psychopharmacology*; 114(3): 449-55
- Nustede R., Schmidt W.E. and others (1993). Gastrin-releasing Peptide and cholecystokinin in The regulation of exocrine pancreatic secretion in dogs. *Digestion*; 54: 79-83
- Ohning G.V., Wong H.C. and others (1996). Gastrin mediates the gastric mucosal proliferative response to feeding. *American Journal of Phisiology*; 271:3,1, G470-G476
- Perkins S. E., Fox J.G. and others (1996). Helicobacter mustelae-associated Hypergastrinemia in Ferrets (Mustela putorius furo). *American Journal of Veterinary Research*; 57:2, 147-150
- Rehfeld J.F. and others (1983). Misure of gastrin radioimmunoassay kits. *Lancet*; II: 338
- Riquet W., Duet M. and others (1989). Initiation psycho-sensorielle de la sécrétion gastrique du chien. *Gastroenterologie Clinnique et Biologique*; 13: 364-361
- Romussi S., Gualtieri M. (1991) Gastrin determination as aid in the treatment of gastric out-flow obstruction in 14 dogs. *Proc. B.S.A.V.A. Congress, Birmingham, 114.*

- Ronning K., Sandvik K. and others (1996). The fade of gastrin-stimulated gastric acid secretion in the rat is due to depletion of releasable mucosal histamine. *Acta Physiologica Scandinava*; 157:4, 487-491
- Rumenapf and others (1998). Gastric fundectomy in the rats: effects on mineral and bone metabolism, with emphasis on the gastrin- calcitonin-parathyroid hormone-vitamin D axis. *Calcified Tissue International* ; 63(5): 433-41
- Shulkes A., Baldwin G. (1997). Biology of cholecystokinin and gastrin receptors. *Clinical and Experimental Pharmacolgy and Physiology*; 24: 209-216
- Simpson K.W. and others (1999). Helicobacter felis infection in dogs: effect on gastric structure and function. *Veterinary Pathology*; May 36(3): 237-48
- Strombeck D.R., Guilford W.G. (1991). Smal Animal Gastroenterology. *Wolfe*, 2°ed.
- Sukhodolia A.I. (1999). The use of extragastric vagotomy in the surgical treatment of duodenal ulcer. *Klinichna Khirurgiia*; (2): 14-5
- Surve V.V., Hakanson R. (1998). Evidence that peroral calcium does not activate the gastrin-ECL-cell axis in the rat. *Regulatory Peptides*; 27; 73(3): 177-82

### Scheme 1

### Regulation of Gastrin secretion

