VALIDATION OF THE ^{13}C-SODIUM-ACETATE BREATH TEST FOR THE MEASUREMENT OF GASTRIC EMPTYING IN DOGS IN COMPARISON TO ^{99M} TECHNETIUM RADIOSCINTIGRAPHY

SILKE SCHMITZ

INAUGURAL-DISSERTATION
zur Erlangung des Grades eines
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eingereicht von

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Tierärztin aus Oberhausen

Gießen 2007

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Silke Schmitz
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Abbreviations

ADP	Adenosinediphosphate
AMP	Adenosinemonophosphate
APT	Applied potential tomography
ATP	Adenosinetriphosphate
BER	Basal electrical rhythm
BIPS	Barium impregnated poyethylene spheres
°C	Degree celsius
^{12}C	Naturally occuring isotope of carbon
^{13}C	Non-radioactive isotope of carbon with 13 electrons
^{13}C-OABT	^{13}C-octanoic acid breath test
^{13}C-SABT	^{13}C-sodium acetate breath test
^{14}C	Radioactive isotope of carbon with 14 electrons
CCK	Cholecystokinine
cm	Centimetres
CNS	Central nervous system
CO_2	Carbon dioxide
CPM	Counts per minute
CT	Computed tomography
DPTA	Diethyltriamine triamine pentacetic acid
DOB	Delta over baseline
ECA	Electrical contractile activity
EIM	Electrical impedance measurements
ENS	Enteric neuronal system
ERA	Electrical response activity
g	Gram(s)
GIP	Gastric inhibitory polypeptide
GEC	Gastric emptying coefficient
GLP-1	Glucagone-like peptide-1
GRP	Gastrine releasing peptide
$G_{t1/2}$	Gastric half emptying time
H_2O	Chemical formula of water
^{111}In	Radioactive 111-Indium
IE	Impedance epigastrography
IRMS	Isotope ratio mass spectrometry
kg	Kilogram(s)
LAG	Lag phase of gastric emptying
mBq	Megabequerell
mg	Milligram(s)
ml	Milliliter(s)
MMC	Migrating motor complex
MRI	Magnetic resonance imaging
NANC	Non-adrenergic-non-cholinergic
NDIRS	Non-dispersive isotope-selective infrared spectroscopy
NO	Nitric oxid
PMP	Electrical pacemaker potentials
ppm	Parts per million
RER	Resting energy requirements
ROI	Region of interest
^{99m}Tc	Radioactive 99m-technetium
VIP	Vasoactive intestinal peptide

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I. Introduction

Delayed gastric emptying is a well known phenomenon in human medicine. It occurs in many different diseases. Therefore, determination of gastric emptying has been used routinely for many years. The most common disease affecting gastric motility in humans is diabetes mellitus, but there are other metabolic dysfunctions and disorders of the gastrointestinal tract itself that have an influence on gastric emptying.

Gastric motility disorders are a significant factor in pathogenesis of many diseases in the dog. Diabetes mellitus plays a role, as well as electrolyte imbalances, effects of drugs or toxins, acute stress or an acute intraabdominal inflammation. In addition, disorders of the smooth muscle or the neuromuscular communication can lead to a defect in gastric propulsion. In many infectious or inflammatory processes, peptic ulcers and after surgical interventions, gastroparesis can occur. In addition, different non-inflammatory processes, as for example neoplasms, foreign bodies, stenosis or hyerplasia of the pylorus can also cause delayed gastric emtying.

The diagnosis of such a delay is unsatisfying, although there are different diagnostic tests available. Diagnostic imaging methods like radiographs with and without different contrast mediums have been used. Bariumsulfate alone, Bariumsulfate mixed with canned dog food or barium impregnated polyethylene spheres (BIPS©) have been tried as well as sonographic examinations of the stomach.

The gold standard method for the measurement of gastric emptying rates in dogs and humans is technetium radioscintigraphy. Emptying of the labeled test meal from the stomach can be watched directly. Lately, non-invasive breath test procedures using ^{13}C-octanoate, -sodium-acetate, -glycin and other marker substances have been used successfully in various species but have not been compared to radioscintigraphy in dogs.

The aim of this study was to evaluate the non-invasive ^{13}C-sodium-acetate breath test (^{13}C-SABT) and compare it to radioscintigraphy in dogs to assess gastric emptying.

II. Literature review

1. Historical background

The main role of the stomach concerning the digestion of food has been known for several centuries.
The word "pylorus" dates from the 5th century BC and means "keeper of the gate", an expression that shows that the early physicians were aware of its physiological function.[1]
The rate of gastric emptying was first examined non-invasively 1898 by the Harvard physiologist W.B. Cannon. He used X-rays to assess the passage of a radiopaque meal in the stomach and intestines of a cat.[2] In the following 6 years he used this method to study the significance of fundic and antral contractions and he could show the effects of emotions and food on the rate of gastric emptying.[1] More than 100 years later the radiographic assessment of gastrointestinal motility is still the most commonly performed method in veterinarian medicine.
The dog has frequently been used as an animal model to study physiological and pharmacological aspects of gastric emptying, and thus gastric emptying is now better understood in this species than in any other.

2. Physiology of gastric emptying

Gastric emptying is defined as the process by which food particles are moved into the proximal parts of the small intestine in a way that allows optimal absorption of nutrients. Solid phase gastric emptying is of greater interest to the veterinary clinician because there is some evidence that pathological disorders of gastric emptying are not always detectable in the liquid phase.[1]

2.1. Morphological and functional structure of the canine stomach

The stomach is divided into fundus, corpus and antrum pyloricum (fig. 1). Motor function can be differentiated into the gastric storage consisting of fundus and corpus, and the gastric pump, an area in which peristaltic smooth muscle waves occur. The latter consists of the distal part of the corpus and the antrum. Because of the different properties of these two functional parts, the gastric storage is also called the tonical and the gastric pump the phasic or contractile part of the stomach.[3]
In the proximal stomach the resting potential of the smooth muscle cells is above the threshold potential which results in a permanent tonic contraction. In the distal parts of the stomach the resting potential is lower than the threshold potential and therefore contractions occur only when this potential is rising.[3]

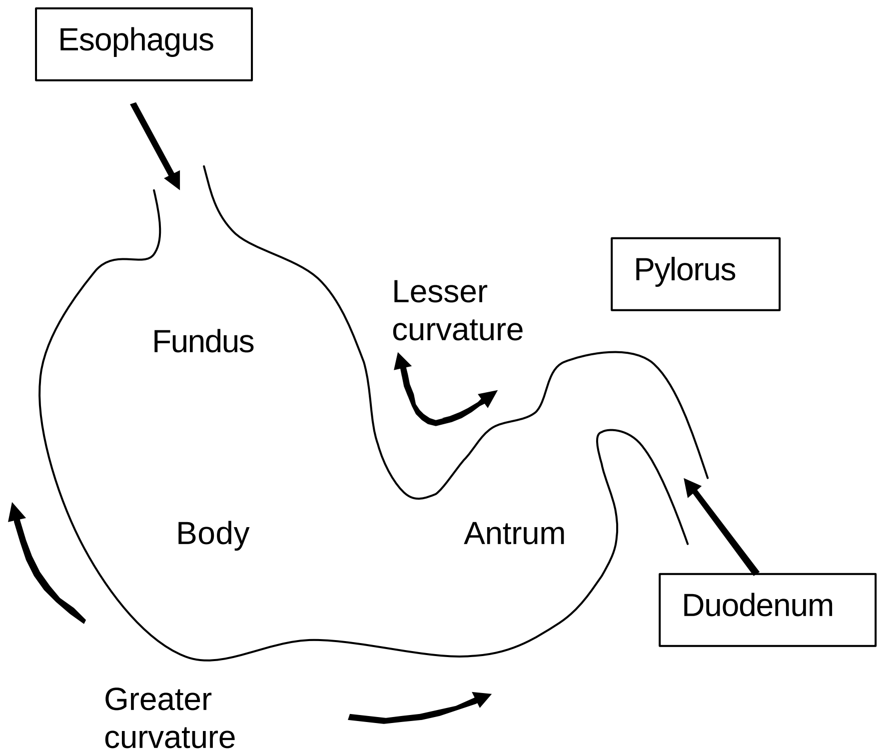


Figure 1: Anatomical regions of the canine stomach.

2.2. The stomach as a store

At the beginning of the 20th century it was revealed that the inner pressure of the stomach increases only minimally during the filling process, i.e. 1.2 cm H_2O per 100 ml content in the dog.[3] This is due to a reflectory dilatation of the stomach and not only a passive stretching.
There are three different forms of gastric relaxation: The *receptive*, the *adaptive* and the *feedback-relaxation*.
The *receptive relaxation* occurs during chewing and swallowing. By stimulation of mechanoreceptors in the mouth and the pharynx vagal reflexes are triggered. They lead to a short-period relaxation of the stomach so it can prepare for receiving a food bolus (fig. 2).
During the filling process of the stomach, stimulation of tension receptors in the stomach wall accelerates gastro-gastric reflexes. An *adaptive relaxation or accommodation* of the smooth muscles occurs (fig. 2). This mechanism ensures that the gastric content is not emptied into the small intestines before it is mixed and “triturated” properly. Then a slow depolarisation of the smooth muscle cells begins, which ends in a contractile wave. A pressure gradient between stomach and duodenum leads to emptying of the liquid and viscous gastric contents.[1]
Finally, a *feedback-mechanism* ensures that gastric emptying is adapted to the type and amount of food which empties into the small intestine.

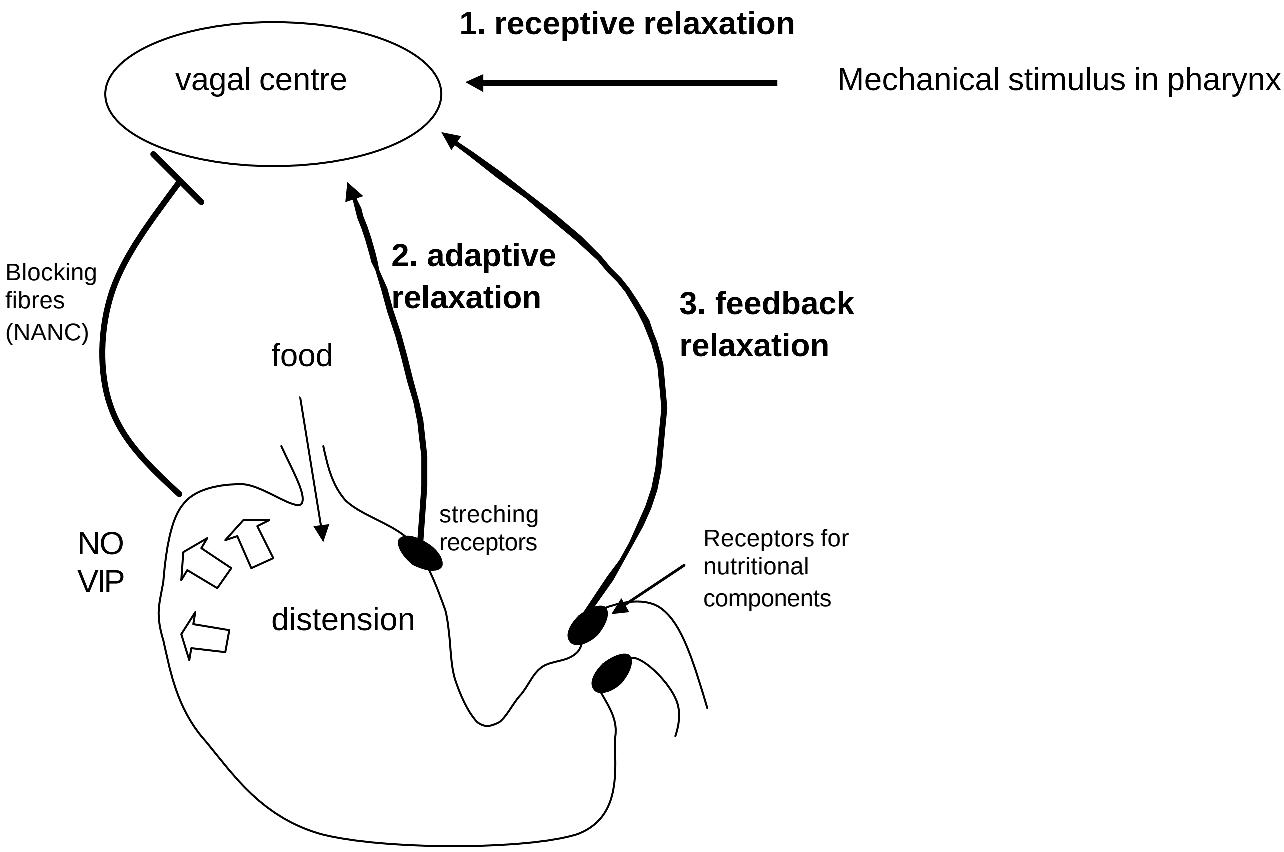


Figure 2: Regulation of gastric motility.
NANC = not-adrenergic-not cholinergic fibres, NO = nitric oxide,
VIP = vasoactive intestinal peptide (modified[3])

2.3. The gastric pump

The antral wall consists of several layers of smooth muscles in circular and longitudinal bands. The muscle cells in this area are able to cause regular cycling basal depolarisations, the so called basal electrical rhythm (BER), which are synonymous for electric pacemaker potentials (PMP), electrical contractile activity (ECA) or slow waves. The pacemaker centre is situated in the area between the proximal and distal parts of the stomach. Its potentials are produced by non-neuronal cells (so called interstitial cells of Cajal) which are located between the circular and longitudinal muscle layers. The frequency of these PMPs varies in different species (for example 3 cycles per minute in humans). Starting at the pacemaker centre these potentials spread towards the pylorus but are not causing a contraction. This only happens with the addition of faster and deeper depolarisations called active potentials, spikes or electrical response activity (ERA), which define the distribution speed of the peristaltic waves. Whilst moving aborally, the pressure and amplitude of the contractions increases.[4] As a result, only the superficial semi-solid, acidic and partly digested portion of the food bolus is transported into the small intestine, while the more alkaline inner portion remains in the stomach for further digestion.

2.4. Physiology of gastric emptying and trituration

There are three consecutive phases of gastric motility that lead to emptying of the stomach: the *phase of propulsion*, the *phase of emptying and mixing* and finally the *phase of retropulsion and "trituration*".[1] These phases occur in cycles due to reoccurring pace maker potentials. While the proximal part of the antrum contracts, the distal part is relaxed, into which the gastric content is then transported (phase of propulsion). When the peristaltic wave has reached the middle of the antrum the pylorus opens partially, the duodenal peristalsis is blocked so that the food can pass into the small intestine. In this phase of emptying and mixing the peristaltic wave is still at a certain distance to the pylorus, so that the food is not pressed into the duodenum by force, but rather "flows" into it. Because liquids flow faster than viscous and solid contents, there is a sieving process at this stage, where small particles, suspended in liquid, pass the pylorus, while the more solid food remains in the stomach.

An important function of solid phase gastric emptying is to act as a mill for the "trituration" of solid food. Trituration refers to the mechanical breakdown and mixing of food to a semi liquid chyme, and this effect is achieved by the repeated to-and-fro movement of ingesta in the antrum. As the wave of contraction approaches the distal antrum, the pylorus and proximal antrum close, the transpyloric flow is stopped and particles too large to pass the pylorus are propelled back into the body of the stomach (fig. 3).[1] This jet-like phenomenon causes a very strong mixture and trituration of food particles. By this, digestible food particles are reduced to a size suitable for gastric emptying (in the range of 0.1-0.63 mm in the dog).[1] Solid meals empty in a slow, linear pattern that often is preceded by a lag phase thought to be representative of the process of trituration.[1]

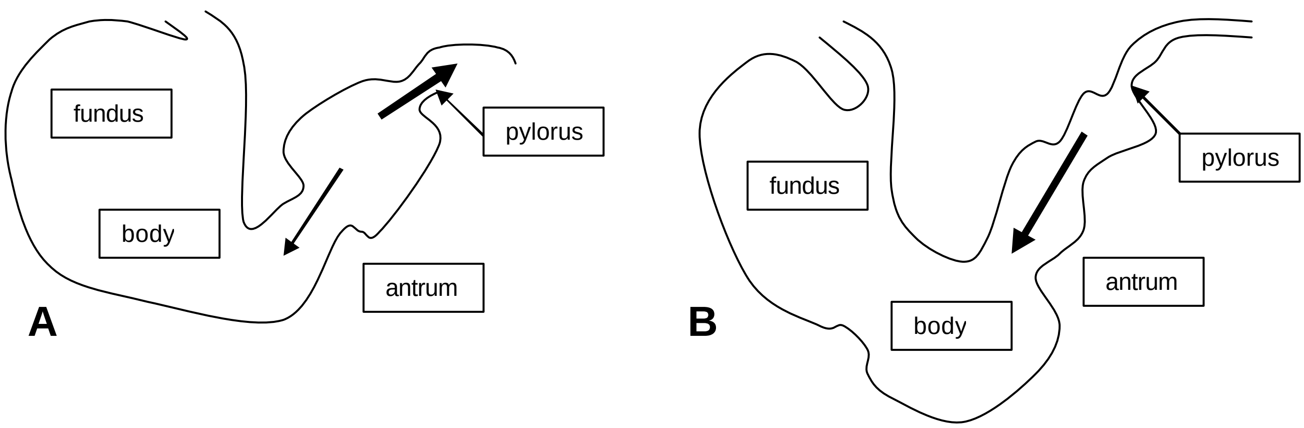


Figure 3: Phases of gastric emptying.
A: phase of emptying: contraction of the central antrum and transpyloric flow (big arrow), greater particles are propelled back into the antrum (small arrow).
B: Trituration of particles, jet-like propulsion back into the antrum (big arrow).

In the dog, small particles (diameter < 1,6 mm) empty promptly from the stomach, whereas large indigestible solids (diameter > 2 mm) that are resistant to trituration, are retained until the digestible solids have emptied and the interdigestive motor pattern (migrating motor complex; MMC) occurs.[1,3]
Emptying of liquid contents starts immediately after the ingestion of the liquid. After a fast beginning, the emptying rate is decreasing and follows an exponential pattern (fig. 4) in both men and dogs.[3]

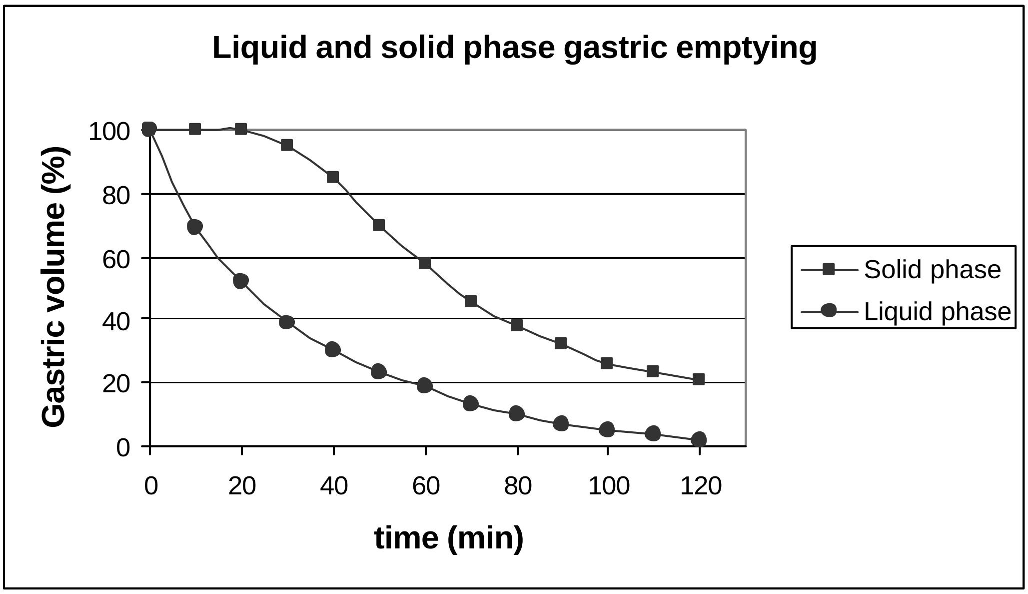


Figure 4: Schematic reduction of gastric contents (liquid and solid phase) plotted against time.[3]

Reference ranges for solid phase gastric emptying in the dog and cat, measured by non-invasive techniques, are shown in table 1. Animals with clinical signs of gastroparesis that have rates of gastric emptying outside these ranges with the same protocol applied, might have delayed gastric emptying. However, many of these studies report gastric emptying rates in normal animals kept under experimental conditions; consequently, the reference ranges reported might not be representative of the gastric emptying rate of a normal animal presented to a veterinary clinic. Few studies have documented delayed gastric emptying associated with a pathological process in the dog, possibly because of the technical difficulties in assessing the rate of solid phase gastric emptying in this species outside the laboratory. The limited information available on the rate of gastric emptying in the diseased animal combined with the wide variability of gastric emptying in the normal animal makes the definition of delayed gastric emptying difficult. Furthermore, studies are often not comparable because different test meals and methods for assessment of gastric emptying were used. The definition of delayed gastric emptying in the dog requires further work, and establishment of standard methods and reference ranges in large groups of healthy animals is necessary. Investigation of the sensitivity of each method in detecting delayed gastric emptying in diseased animals also is required.

Method	**Test meal**	**n**	**Gastric half emptying time**
Radioscintigraphy	Egg, starch + glucose	27	66 min (median), 45 – 227 min (95% CI)[5]
	Beef baby food + kibble	6	4.9 ± 1.96 hours (mean ± SD)[6]
	Liver	4	About 2 hours[7]
	Canned dog food + egg	6 (18 tests)	172 ± 17 min (mean ± SE)[8]
	Canned dog food + egg	7 (14 tests)	285 ± 34 min (mean ± SD); 294 ± 39 min (mean ± SD)[9]
	Canned dog food	6	77 min (mean)[10]
Radiography	Dry dog food + radio-opaque solids	10	3.5 hours (median), 1-6 hours (range)[11]
	Canned dog food + eggs+ BIPS	6 (18 tests)	Small BIPS 416 ± 81 min (mean ± SE)[8]
	Canned dog food + BIPS	20	Small BIPS 6,05 ± 2.99 hours (mean ± SD) Large BIPS 7.11 ± 3,60 hours (mean ± SD)[12]
	Kibble + BIPS	8	Small BIPS 8.29 ± 1.62 hours (70% of dogs ± SE) Large BIPS 29.21 ± 18.31 hours (70% of dogs ± SE)[13]
	Kibble + liquid barium	9 (27 tests)	Total gastric emptying time: 7-15 hours (range)[14]
	Kibble + liquid barium	4	Total gastric emptying time: 7.6 ± 1.98 hours (mean ± SE)[15]
Ultrasonography	Canned dog food + corn oil	12	High energy meal: 127 ± 28 min (mean ± SD) Standard energy meal: 54 ± 21 min (mean ± SD)[16]
	Bread, egg, milk	10	1.89 ± 0.78 hours (mean ± SD)[17]
Breath tests	Bread, egg + margarine	6 (18 tests)	3.43 ± 0.50 hours (mean ± SD)[18]
	Bread, milk + margarine	24	3.38 ± 0.79 hours (mean ± SD)[9]
	Bread, egg, milk	10	3.44 ± 0.48 hours (mean ± SD)[7]

Table 1: Gastric emptying times in the dog measured with different methods, radiopaques substances and test meals.
BIPS© = barium impregnated polyethylene spheres

2.5. Neuronal regulation of gastric motility

There are four neuronal systems influencing gastric motility: The *enteric neuronal system* (ENS) dominating the BER, that is regulating motility, secretion and absorption.[20] The neuronal impulses are produced in the plexus myentericus which is more distinct in the aboral region of the stomach wall. In this plexus, non-adrenergic-non-cholinergic (NANC) afferent vagal fibres have been located.[17] Efferent vagal fibres are producing the gastric tone. These opponent functions of the vagus are coordinated in the *medulla oblongata*. The fibres of the plexus myentericus are not using acetylcholine, but nitric oxid (NO) as neurotransmitter, which relaxes smooth muscle cells by activating guanylatcyclase. Another transmitter is serotonin that has shown to cause contraction of the proximal stomach and simultaneously relaxation of the antrum.[17] Adenosinetriphosphate (ATP), adenosinediphosphate (ADP) and adenosinemonophosphate (AMP) are released by the vagal neurons of the ENS. By stimulation of purinergic receptors they lead to a relaxation of the smooth muscles.[22]

The *autonomic nervous system* is also regulating gastrointestinal motility. Parasympathic effects are stimulating motility and secretion by cholinergic muscarinergic neurotransmission.[22] Sympathic fibres are working in the opposite direction, norepinephrine and epinephrine as postganglionic agonists of a2-receptors block motility and secretion of the stomach. Postsynaptic a1-receptors lead to contraction of the gastrointestinal sphincters, while ß-receptors relax the smooth muscle cells of the gastrointestinal wall.[22]
The highest level of regulation of the gastrointestinal motility is the central nervous system (CNS). By stimulation of certain brain regions special effects on intestinal motility are caused. The coordination of CNS and ENS is regulated by the autonomic nervous system, which also carries viscero-sensible afferences to the CNS and is therefore part of visceral reflex bows.[22]

2.6. Influences on gastric motility

Gastric emptying is linked to exocrine and endocrine functions of the stomach and subject to alteration by many physiological, pharmacological, dietary or pathological conditions, i.e. meal composition[18,23] or dietary viscosity.[24,25]
In humans it has been shown that liquids like water or isotonic saline solution are emptied in an exponential pattern after a short lag phase.[3,26] Solid food and liquids with a high caloric content are emptied more linear and the lag phase is longer (fig. 4).[3,23,26] Fat is staying longer in the stomach than proteins and carbohydrates.[23,27] Particles of a higher density are emptied slower than food with a lower density.[24,25] A high content of fibres[28] or a high osmolality of the food[27] prolong gastric emptying. The temperature of the food has an effect on gastric emptying as well. Very cold (4° C) and very warm (50° C) liquids are emptied slower that fluids with 37° C.[29] Volume also plays a role in the regulation of gastric emptying. The more volume has been taken up, the more is emptied per time unit.[30] Stress blocks gastric motility and can even stop the postprandial motility pattern in humans,[31] there is no information available about the impact of stress on gastric emptying in dogs.
There are many other factors that alter gastric emptying in humans, for example body size,[32,33] nicotine,[34] the female menstruation cycle[35] or blood glucose levels.[36] Hyperglycaemia decreases gastric emptying rate, a fact important in diabetic patients.[36] Gastric emptying is slower whith human patients laying on their left side in

comparison to the right.[37] The influence of exercise and sports on gastric emptying has also been examined and is irregular.[38]

There is a very close relationship between gastric and duodenal motility, called the antro-duodenal coordination. The pylorus is working as an “electrical isolator”, thus electrical activity and contractions of the stomach stop here. The frequency of the pacemaker potentials in the duodenum is higher than in the stomach, its motility is faster and shorter.[3]
Gastric emptying can be inhibited by the small intestine. This feedback-blocking system is activated via several factors, such as acid, increased or decreased osmolality of the food as well as the amount of intraluminal nutrients and is mediated via enterogastric reflexes, the release of intestinal hormones or vagal fibres. By measuring the frequency of afferent vagus impulses gluco-, osmo-, acid-, amino-acid- and fatty-acid- receptors could be detected. They are not specialized morphological structures but the afferent vagal fibres themselves. The most potent inhibition factors on gastric emptying and the small intestines are various portions of digested nutrients such as proteins and fat.[3,22]
Beside the classical neurotransmitters, there are various gastrointestinal peptides produced and released in the distal parts of the intestine with a transmitting function, regulating the feedback-mechanisms under physiological conditions: peptide Y, enteroglucagone, galanine, gastric inhibitory polypeptide (GIP), gastrin releasing peptide (GRP), gastrin, ghrelin, the glucagone-like peptide-1 (GLP-1),[22] vasoactive intestinal peptide (VIP), peptide histidine-methionine, cholecystokinine (CCK), substance P, neurotensine and somatostatine.[20,21] The most important is CCK, which is produced by the I-cells of the intestinal epithelium (duodenum and jejunum) after stimulation by acid, fat and amino-acids. CCK reaches the stomach via the blood stream and causes its relaxation. Simultaneously, it stimulates CCK-receptors on afferent vagal fibres of the intestinal mucosa and activates enterogastric blocking reflexes. In that way, the intestine can detect certain food elements before they have been absorbed.

2.7. Interdigestive motility patterns and the migrating motor complex

Interdigestive patterns of gastrointestinal motility vary greatly between species. The interdigestive state in humans and in the dog is associated with a cyclic recurring complex of motor activity, the migrating motor complex (MMC) that migrates periodically from the stomach to the distal small intestine[1,3,39] and empties the non-digestible food components into the duodenum. The MMC (fig. 5) is characterized by a band of intense contractile activity (Phase III) that is followed by a period of relative quiescence (Phase I) and then a period of irregular activity (Phase II).[3] The interdigestive MMC is interrupted by food intake and is replaced by a pattern of persistent phasic contractile activity that mixes and propels the gut contents.

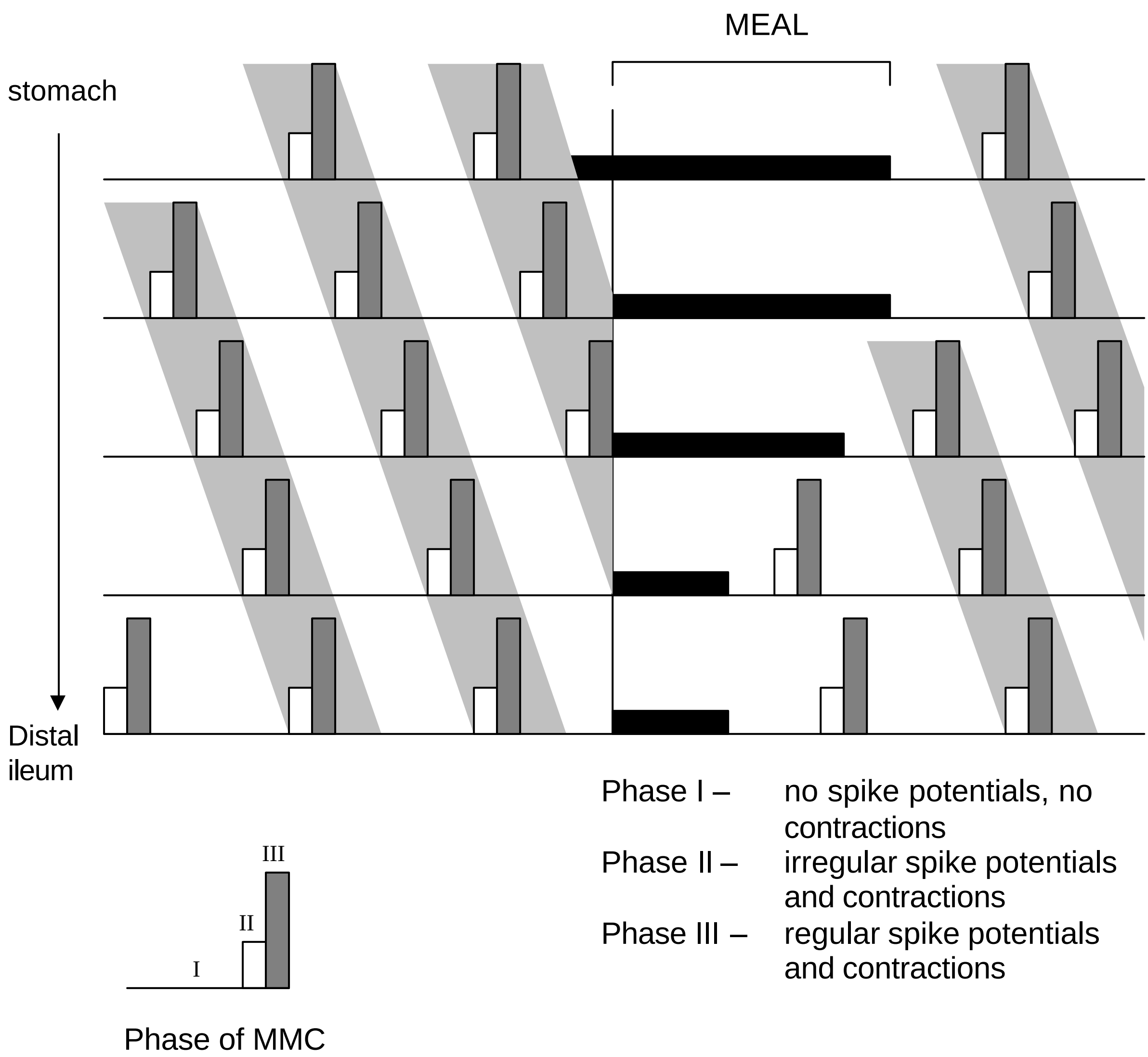


Figure 5: Migrating motor complexes (MMCs). Complexes move down the gastrointestinal tract at a regular rate during fasting, they are completely inhibited by a meal and then they resume after a meal.[39]

2.8. Pathology of gastric emptying

Delayed gastric emptying in humans is associated with type I and type II diabetes mellitus.[40] Examinations in the rat have shown signs of axonal de- and regeneration after hypoglycaemia of peripheral nerves. In contrary, hyperglycaemia leads to non-enzymatic glycolysation of proteins and to other metabolic changes. Morphological changes, such as segmental degeneration of the myelin-cores, axonal degeneration and vacuoles occurring in the cytoplasm of neurons[41] have been found in healthy volunteers as well as in diabetic patients with and without enteropathy and are therefore non-specific concerning neuropathies of the ENS.
These results are not consistent with earlier studies in which a higher number of lymphocytes in the ganglions and irregularities in nervous fibres of the plexus myentericus and extrinsic nerval roots have been found.[42] In another study in 3

patients with long-term diabetes the authors described a degeneration of myelin and an increasing number of nuclei of the Swann-cells.[42] It is not clear if micro- or macroangiopathic lesions lead to the manifestation of gastrointestinal transit disorders in diabetic human patients.[43]
Delayed gastric emptying is also seen in human paediatric patients as a result of gastro-oesophageal reflux[44] and other conditions and a gastric emptying breath test has recently been used to assess intestinal absorption in critically ill human patients.[45]

It has been shown that delayed gastric emptying also occurs in dogs with experimentally induced diabetes mellitus.[46] The prevalence of delayed gastric emptying in diabetic dogs warrants investigation, particularly because erratic delivery of nutrients to the small bowel could contribute to poor glycaemic control. The role of gastric emptying in the aetiology of canine gastric dilatation-volvulus has also been the focus of detailed research, and considerable evidence suggests that delayed gastric emptying might be a primary pathogenic mechanism in this disorder.[11]
Delayed gastric emptying has also been reported in dogs with chronic hypertrophic pyloric gastropathy,[47] endotoxaemia,[48] dysautonomia,[49] neoplasia[50] and radiation-induced vomiting.[51] In addition functional lesions of the smooth muscle or dysfunction of the neuromuscular connection, for example inflammation, infection, peptic ulcers and gastroparesis after surgical interventions can lead to abnormal propulsion.[52]
Further potential causes of a delayed gastric emptying are electrolyte imbalances, metabolic diseases, drugs, acute stress or acute inflammation in the abdominal area.[1]
In comparison with human medicine, delayed gastric emptying has been described rarely in the veterinary literature. This observation is likely due to the limited access for measuring solid phase gastric emptying in animals. The development of simple and inexpensive methods for assessment of solid phase gastric emptying in veterinary medicine might show that clinical disorders of gastric emptying are more prevalent than previously reported.

3. Methods for the assessment of gastric emptying in dogs

Several methods to assess gastric emptying in the dog have been used with various success. Gastroscopy can only be performed to examine the inner surface of the stomach and to exclude mechanical obstruction,[53] but it is not useful to reveal more subtle functional disorders of the stomach caused by other pathomechanisms. In the last decades there has been a great progress concerning the techniques for measuring gastric emptying rates in animals[1,18] and some of these methods can be realistic options for clinical settings (fig. 6).

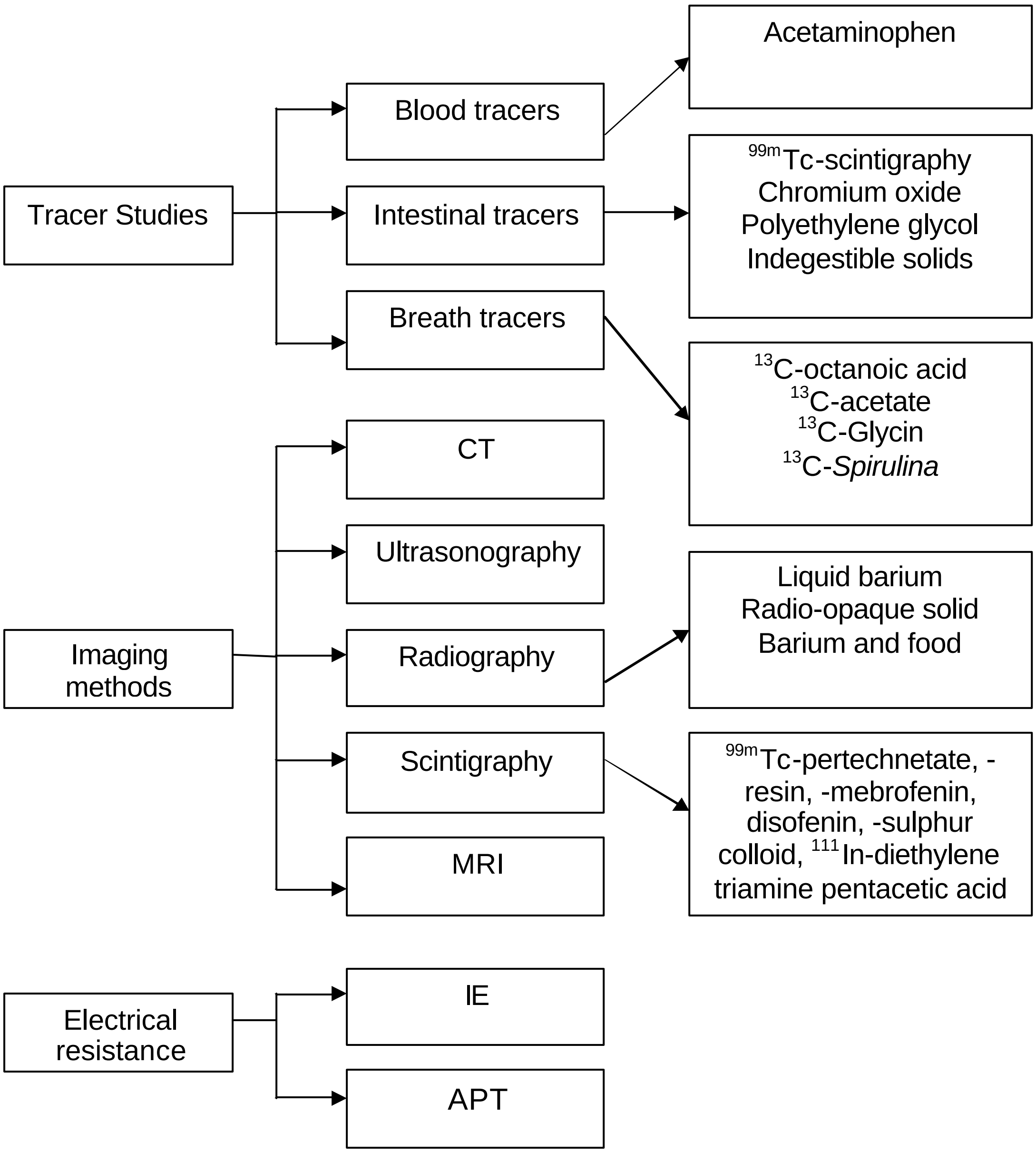


Figure 6: List of methods for measuring gastric emptying.[1]
CT = computed tomography
MRI = magnetic resonance imaging
IE = impedance epigastrography
APT = applied potential tomography

3.1. Diagnostic imaging

Diagnostic imaging methods are frequently used to assess gastric emptying in dogs, despite the potential requirement for restraint or sedation of the animal during the procedure. Manual restraint, sedation, anaesthesia[1] or fixation in a Pavlov-sling[14] have all been used. All these interventions can potentially affect gastric motility. In a routine veterinary setting these methods are difficult to apply, because sequential imaging over a long period of time is necessary.

3.1.1. Radiography

Radiography and fluoroscopy can be used to monitor gastrointestinal transit and these methods can provide qualitative and limited quantitative data on the rate and pattern of gastric emptying. Many radiopaque test meals have been used for radiographic assessment of gastric motility, the contrast medias used have been liquid bariumsulfate alone to measure liquid phase gastric emptying,[1] bariumsulfate mixed with food[1,7,14,15] to assess solid phase gastric emptying, or BIPS©[1,8,12,13,18] mixed with food. Multiple laterolateral and dorsoventral radiographs of the stomach and intestines have to be taken over a 4 to 12 hour period. The oral-aboral movement of the radiopaque meal along the gastrointestinal tract can be assessed qualitatively or by counting the radiopaque particles judged to be in the stomach.
Unfortunately, the contrast media used are neither chemically nor physically similar to normal food; consequently, the results might be of restricted relevance and limited physiological importance. Furthermore, the palatability of radiopaque test meals has presented a difficulty in veterinary studies, often necessitating the use of chemical appetite stimulants, force feeding or tube feeding.[14,54] Radiographic methods are useful tools for identification of gross abnormalities of gastric emptying and for the detection of mucosal defects, gastric obstruction, neoplasia and foreign bodies. These methods are less useful in detecting subtle modulations of the rate of gastric emptying because it is difficult to accurately quantify the rate of passage of radiopaque material from the stomach.

3.1.2. Ultrasonography

Because of the technological advances in the last decades it is now possible to visualise gastric emptying by ultrasound examinations. In human medicine a close relationship between gastric emptying rates of liquid and solid contents measured by scintigraphy and ultrasonography has been determined.[55] The rate of gastric emptying measured by ultrasound is based on the estimated change in antral volume, and this measurement might not accurately represent gastric emptying, especially if antral contents are retropelled into the fundus. It also allows visualization of antral contractions.
Ultrasound examination of the stomach is possible in the dog, but depends on a skilled and experienced examiner and may therefore be subjective and non-reproducible. Other studies have shown that the variations among different operators are not large.[17] Unfortunately, this technique is not able to differentiate between liquid and solid phase gastric emptying and the results are affected by gastric fluid secretion and the presence of gas in the stomach.[56]

The principle advantage of ultrasound examination of the stomach in veterinary medicine is the assessment of the gastric motility in real-time mode and with minimal restraint of the animal.
Latest studies propose that ultrasonography could be potentially useful to determine emptying of liquid[16,56] and solid meals[57] in the dog. Ultrasonographic equipment is widely available in veterinary practice, so that this technique could be a useful non-invasive method to study gastric emptying in the dog and cat. Further research is necessary to validate this method against radioscintigraphy in the dog and to describe reference ranges in healthy and diseased animals.

3.1.3. Magnetic resonance imaging

This technique can provide a three-dimensional image of the stomach. Gastric motility and emptying are assessed simultaneously and non-invasively[1]. Initially, magnetic resonance imaging (MRI) was used to diagnose liquid phase gastric emptying disorders, but a solid phase marker substance has been described in human medicine.[1]
Recently, several studies evaluating gastric emptying in healthy human subjects,[58] the influence of posture[59] and macronutrients (proteins, fat and glucose)[60] was evaluated using MRI.
Although the MRI examination of the stomach seems to be useful in veterinary medicine, its use is limited because specialized equipment and personnel is needed and anaesthesia would be necessary in most cases.

3.1.4. Radioscintigraphy

The examination of gastrointestinal motility by radioscintigraphy after ingestion of a radioactive labelled test meal has first been described 1966[61] and radioscintigraphy is now considered the gold standard, against which all new methods must be compared.[1]
Radioscintigraphy has been applied to assess gastric emptying in a variety of animal species including dogs.[1,9]
The radioisotopes used are ^{99m}Technetium (^{99m}Tc) bound to pertechnetate, sulphur, tin or albumin colloid, disofenin, mebrofenin or resin beads, and ^{111}Indium (^{111}In), bound to diethyltriamine triamine pentacetic acid (DPTA). These radionuclides have short half lives of 6 hours to 2.2 days, respectively. Since they emit gamma radiation of different energies, they can be used to simultaneously label liquid and solid gastric contents. This dual isotope method allows to monitor mechanisms and rates of liquid and solid phase emptying at the same time.
The radiopharmaceutical used to assess emptying of solids must remain bound to the test meal and must not be absorbed or adsorbed within the gastrointestinal tract. ^{99m}Tc-disofenin and ^{99m}Tc-mebrofenin have been recommended for use in the dog on the basis of in vitro and in vivo studies of labelling efficiency.[1] Solid phase labels are generally baked into liver or egg or are mixed with canned animal food or meat.
In most veterinary studies using scintigraphy the tested animals were fasted for a period of 12 - 24 hours before ingestion of the labelled test meal to make sure the stomach is empty. Right and left lateral views as well as ventral images of the stomach are taken at certain time intervals using a gamma camera and the radioactive counts in this region are recorded, usually at regular time intervals, for 6-9 hours. Data are assessed with an integrated nuclear medicine computer system.[1]
The gastric region of interest (ROI) can be defined manually or by computer.[1]

Correction factors are necessary to take into account the radioactive decay of the isotope for the duration of the test. Movement of the marker within the stomach can cause attenuation of the radioactivity and the lag phase of gastric emptying might be overestimated because the marker has moved to lie closer to the camera.[62] Spreading of food in the stomach might cause some of the readings early in the test to be greater than the initial count, and overlap of activity in the small intestine or colon over the ROI in the stomach also might displace a problem.[54] Data are converted to fractional retention of radiation after decay correction. Mathematical curve fitting is used to model the data and to calculate coefficients to describe the rate of gastric emptying. Alternatively, the percent isotope remaining in the stomach at 2 and 4 hours can be calculated. This calculation reduces the number of scans necessary and has been shown to have a high predictive value in detecting alterations in gastric emptying in humans.[53]
The application of scintigraphy in animals is limited by the requirements for a nuclear medicine facility and the radiation hazard involved. Scintigraphy protocols have been described for assessment of gastric emptying in the dog.[1] The test meal used in scintigraphic studies is similar to the food the animal might normally ingest; in small-animal studies, canned pet food or radiolabel liver are often used.

3.2. Other methods to assess the rate of gastric emptying

Because there are several problems trying to assess gastric emptying by diagnostic imaging procedures, other methods have been developed. They had to be simple in performing, non-invasive and should reflect the physiological situation as accurately as possible.

3.2.1. Electrical impedance measurements

The rate of gastric emptying can be measured by assessing the electrical impedance changes during contractile waves. Impedance-epigastrography (IE) and applied potential tomography (APT) have been used experimentally to monitor gastric emptying by measuring the fluctuation of an alternating current applied across the epigastric region after ingestion of a test meal.[1] The ingestion of a meal causes increased electrical resistance across the stomach followed by an exponential decrease in impedance, and these changes are an indirect representative of gastric emptying. APT involves the use of a multi-electrode array to enclose the upper abdomen, whereas impedance epigastrography uses just two pairs of standard electrocardiographic electrodes. Unfortunately, both techniques are very sensitive to artifacts induced by even slight body movements during the test. Nevertheless, impedance epigastrography correlates well with liquid phase gastric emptying rates measured by scintigraphy in humans,[63] but a solid phase marker for use with this methods has not yet been described. The use of either technique in small animals has not been reported.

3.2.2. Gastric tracers

Tracer studies involve serial aspiration of gastric or duodenal contents after administrating a known concentration of a nonabsorbable marker substance with food. Samples of gastrointestinal contents are obtained through an oro- or

nasogastric tube or a fistula or catheter inserted through the gastric or small intestinal wall. The change in concentration of the marker is used to calculate gastric emptying rate. This method has been widely applied to assess gastrointestinal transit times in dogs in experimental settings.[1,31]
Nonabsorbable substrates used as intestinal tracers of solid phase gastric emptying include non-digestible solids,[1] radiolabel food, freeze-dried food and chromium oxide. Liquid phase markers include polyethylene glycol and phenol red.[1] Studies with these agents assume that a homogeneous suspension is formed within the gut. Intestinal tracer studies have provided valuable information on the physiology of gastric emptying, but their invasiveness confines the use to the research laboratory.

3.2.3. Plasma tracers

The paracetamol (acetaminophen) absorption test has been described to assess liquid and solid phase gastric emptying rates.[1] Plasma drug concentrations are measured in serial blood samples after ingestion of paracetamol in solution, and the rate of gastric emptying is related to the appearance of paracetamol in the blood[1]. Paracetamol is poorly absorbed in the stomach but rapidly from the duodenum and does not affect the rate of gastric emptying.[1] The paracetamol absorption test has been correlated with scintigraphic measurement of gastric emptying in humans but not yet in the dog. Paracetamol can be measured easily in serum by chromatography, and the absorption test protocol is relatively simple. The paracetamol absorption test has been applied for assessment of the effect of prokinetic drugs and different test meals on the rate of gastric emptying in the dog.[1,46] A disadvantage of the paracetamol absorption test is that intravenous catheterization is required, and the method has not been validated for assessment of solid phase gastric emptying. The limited absorption of paracetamol in the stomach might result in substantial variation of the results.[1] As with all methods for assessment of gastric emptying that rely on the metabolism of a tracer, the paracetamol absorption test might be affected by abnormal small intestinal or liver functions.

3.2.4. Breath tests/ breath tracers

3.2.4.1. General commentary about breath tests

The last decades have seen the development of various breath tests in human and veterinary medicine as a new method to assess metabolic processes in vivo and to examine special body functions. The use of stable isotopes as marker substances and the spreading of this technique to monitor physiological and pathological processes in the body have added to the armamentarium in gastroenterology.
Twenty-five years ago breath tests were performed with radioactive markers, such as carbon-isotopes, for example ^{14}C, to assess exocrine pancreatic and liver functions or intestinal reabsorption. Because of the increased awareness of problems occurring with radioactivity and the evolution of quick and accurate methods for the detection of stable isotopes, these techniques have gradually been replaced with non-radioactive isotopes, for example ^{13}C.[64]
Isotope ratio mass spectrometry (IRMS) is used to measure the difference between the molecular mass of the naturally occurring ^{12}C and molecular mass of the tracer element ^{13}C. Newer techniques, such as non-dispersive isotope-selective infrared spectroscopy (NDIRS) have been developed recently, which are less expensive and quicker without any loss in precision.[1]

Basics of the ^{13}C-breath test:
Eight isotopes of carbon are known, but only ^{12}C and ^{13}C are stable. ^{13}C is an isotope with 6 protons and 7 neutrons and has a natural abundance of 1.11% of the total carbon so that all ^{13}C breath tests are carried out against a background level of the naturally occurring isotope.[65] The assessment of gastric emptying by stable isotope breath tests involves ingestion of a ^{13}C-labelled substrate that is rapidly absorbed and metabolized to $^{13}CO_2$ after gastric emptying to produce a detectable increase in the exhaled breath. Therefore serial breath test samples have to be collected and analysed. The rate-limiting steps in measuring the marker substance before it occurs in the exhaled breath are metabolism of the substrate in the intestinal tract, abnormal transport mechanisms or other potential metabolic abnormalities. It is postulated, that if gastric emptying is the rate-limiting step in the absorption and excretion of the ^{13}C-labelled substrate, then the pattern of isotope recovery in exhaled breath is a reflection of the rate and pattern of gastric emptying,[34] and all other metabolic processes are either negligibly fast or at least constant. There is a wide variety of ^{13}C labelled substances available.
The principal advantages of the gastric emptying breath test are their non-invasiveness and that they can be carried out easily away from the hospital. Test meals are similar to normally ingested food, and neither the marker substances nor the ^{13}C-substrate pose any health risk.

3.2.4.2. Use of breath tests in human medicine

Breath tests with marker substances have been widely used in human medicine to assess infection with *Helicobacter pylori*,[66] which has as well been done in veterinary medicine[67] as well as to measure liver function,[68] exocrine pancreatic function,[69] to assess the orocoecal transit time,[70] different intestinal enzyme activities[71] and, eventually, to measure gastric emptying rates.
The ^{13}C-octanoic acid breath test (^{13}C-OABT) was described to assess solid phase gastric emptying in humans in 1993.[34] It has been compared to scintigraphy[72] and against ultrasonography.[73] Octanoic acid is a medium chain fatty acid and entry of the carbon label into the bicarbonate pool imposes an inevitable delay before recovery of isotope in the breath. Studies in humans and dogs[18] have proposed suitable correction factors to compensate this delay.
Double-labelling of breath test meals, like in scintigraphic examinations, is only possible by using ^{13}C-substrates and in addition the radioactive ^{14}C-isotope,[74] however, this is not permitted in Germany.
Recent innovations in the use of the ^{13}C gastric emptying breath test include the validation of a novel substrate *^{13}C-spirulina*,[1] an edible alga that is cultured in $^{13}CO_2$ to produce a 99% ^{13}C-enriched substrate that is thought to be more representative of the food matrix than octanoic acid[1].

3.2.4.3. Use of breath tests in veterinary medicine

Performing breath tests in animals follows the same principles as in humans. The breath test protocol is simple and does not require any special equipment or expertise[1]. Breath samples can be collected from animals with a face mask,[75,76,77] with a nasal prong[18,78] or by placing the animal in a breath collection chamber.[79] The breath sampling procedure can be completed rapidly and with minimal restraint.[18,76,77]
Because of the natural abundance of ^{13}C it is necessary to ensure that basal levels of

$^{13}CO_2$ excretion are stable before commencing a breath test. Foods derived from tropical grasses such as cane sugar and maize should be avoided because these have photosynthetic mechanisms that cause natural enrichment of ^{13}C. However, studies in dogs have demonstrated that under conditions of routine animal husbandry in the United Kingdom and abstinence from ^{13}C-rich foods, variation in ^{13}C-excretion in dogs is negligible.[75] The animal must remain at rest during the test to ensure that the rate of CO_2 production remains constant. Breath samples can be stored in sealed tubes for up to 60 days[60], and samples can be shipped to the laboratory for analysis.
Measurement of solid phase gastric emptying via ^{13}C-OABT has been performed in the horse,[76,80] pony,[75] dog,[18] cat[77] and mouse.[79] It has been compared to scintigraphy in horses[76] and against ultrasonography in dogs.[57] Inter- and intrasubject variability in the dog was relatively low[18] and comparable to previous studies with radioscintigraphy.[1] The test was shown to be sensitive to alterations in gastric emptying induced with different meal composition in dogs and horses.[18]

Acetate-Breath Test
Recently, studies have shown that the use of ^{13}C-sodium acetate could be of more benefit in comparison to the use of octanoic acid.[81] Because of its unpleasant taste, octanoic acid is only poorly accepted by patients, especially in the paediatric sector in human medicine or in veterinary medicine. In addition, preparation of the test meal is time-consuming as it has to be prepared freshly for every patient. Most importantly, however, the absorption of octanoic acid as a fatty acid has considerable individual variation. As such the absorption rate of fatty acids in the duodenum may be the time-limiting step.
The ^{13}C-sodium acetate breath test (^{13}C-SABT) seems to be devoid these problems. It was shown that the ^{13}C-acetate breath test accurately reflects gastric emptying in both liquid and semisolid meals in humans.[82]
The ^{13}C-SABT has been used to assess liquid phase gastric emptying in the dog in comparison to scintigraphy.[83]

4. Aim of the study

This study should establish the non-invasive ^{13}C-SABT to assess solid phase gastric emptying in the healthy dog and compare it to gastric radioscintigraphy.

III. Hypotheses

- If compared to scintigraphy, ^{13}C-SABT provides a useful and reliable diagnostic tool for measuring gastric emptying rates in dogs
- Measuring gastric emptying in dogs is easy to perform with ^{13}C-SABT
- ^{13}C-SABT provides repeatable measurements in the same individual tested
- Gastric radioscintigraphy serves as gold standard method to measure gastric emptying in dogs

IV. Material and methods

1. General considerations

1.1. Subjects

All dogs had to be clinically healthy to be included in this study. There were neither breed nor sex or age specifications. Exclusion criteria were - besides any abnormal clinical signs - medication during the last 12 months (with the exception of immunization, flea products or anthelmintics) and special dietary requirements. The dogs were dewormed with fenbendazole (50 mg/kg) over 5 days one to three weeks prior to the first test. Two hour post prandial bile acid serum concentrations were measured enzymatically and colorimetrically (reagents obtained from DiaSys, Holzheim, Germany) in all dogs after ingestion of the breath test meal. This was done on both occasions when breath tests were conducted. Measurements were performed with the clinical chemical analyser ABX Pentra 400 (Axon Lab, Reichenbach, Stuttgart, Germany).
In all animals breath test analysis and scintigraphy were performed after an overnight fast on two consecutive days; this procedure was repeated in an alternating order.

1.2. Test meal composition

The test meal used in this study consisted of commercially available canned dog food („Intestinal"/ Royal Canin©). The amount of dog food was calculated based on body weight according to the following formula:[84]

The <u>resting energy requirement (RER)</u> was calculated and multiplied with a factor indicating normal exercise of a standard dog (multiplication factor: 1.6):

$70 \times \text{kg BM}^{0.75} \times 1.6$	=	requirement in kcal/d (kilocalories per day) for a neutered dog with a normal daily exercise. (kg BM = kg body mass)

Kilocalories (kcal) were converted into megajoule (MJ) :

$$\frac{(\text{kcal} \times 4.184)}{1000} = \text{MJ (Megajoule)}$$

The daily megajoule requirement was calculated for each dog. Because dogs used for this study are normally fed twice daily, half of the calculated daily requirement represented one test meal.

For example:
Dog no. 11 had a weight of 25,6 ~ 26 kg.

$70 \times 26^{0.75} \times 1.6$	= 1289 kcal/d
1289 x 4.184	= 5395 kJ/d
5395/1000	= 5.4 MJ/d
5.4/ 2	= 2.7 MJ per meal

100 g “Intestinal” (Royal Canin©) canned dog food contains 0.1116 kJ, thus dog no. 11 was fed 577 g.

Prior to feeding the diet was thoroughly mixed with a household blender (Ito Electronics©; www.ito-electronics.de; Standmixer; 400 watt; 1300 ml content, 2 speed rates, glass container, stainless steel blades) for 3-5 minutes. After proper homogenization, either 100 mg of ^{13}C-sodium acetate (Sigma-Aldrich Chemie GmbH, Munich, Germany; www.sigmaaldrich.com) for breath test analysis or 150 to 250 MBq of ^{99m}Technetium (from the Institute of Nuclear Medicine, Justus-Liebig-Universität Giessen, Prof. Dr. R. Bauer, amount depending on availability of the technetium on the day of testing) already added to an albumin colloid solution (Solco-Nanokoll©; Sorin-Biomedica, Munich, Germany) for scintigraphy was added and the test meal was mixed again for 3 minutes on the highest level (level 2).

1.3. Study design

Following an overnight fast (at least 12 hours) to ensure an empty stomach, the test meal containing either ^{99m}Technetium colloid or ^{13}C-sodium acetate labeled food was given to the dogs at 8 am. The dogs were kept separated in a quite room during the entire time of the study and were leash walked for 30 minutes 4 hours into the study. They had free access to water.

2. ^{13}C-sodium acetate breath test

2.1. Test procedure

The first breath sample was collected immediately prior to the ingestion of the test meal (baseline sample). Afterwards breath samples were collected every 15 minutes for 4 hours and then every 30 minutes for additional 2 hours.

Breath test collection technique
The breath samples were collected by a commercially available anesthetic mask for small animals (Heiland Vet© GmbH und CoKG, Hamburg; Germany; www.heiland-vet.de; article no. 730-246). This mask is made of plexiglass and has a rubber ending that fits tightly around the dog’s muzzle. At the other end it has a standardized metal port for the connection of the sampling system. The mask was connected to a self-constructed one-way valve (fig. 7), attached to a commercially available breath reservoir bag (Fischer Analysen Instrumente GmbH, Leipzig, www.fan-gmbh.de; Germany; one-way breathing bag; 0,3 l content, article no. F201-VP-05a). The mask was fitted snuggly around each dog`s muzzle, and the dogs were allowed to breath normally until the reservoir bag was filled with exhaled air. The soft part of the reservoir bag tube was then compressed manually and the bag was separated from

the mask without loss or dilution of the breath sample. Then, the breathing bag was immediately closed with a fitting cap. Within half an hour the $^{12}CO_2/^{13}CO_2$ ratio of the breath sample was measured. The reservoir bags were stored at room temperature until analyzed.

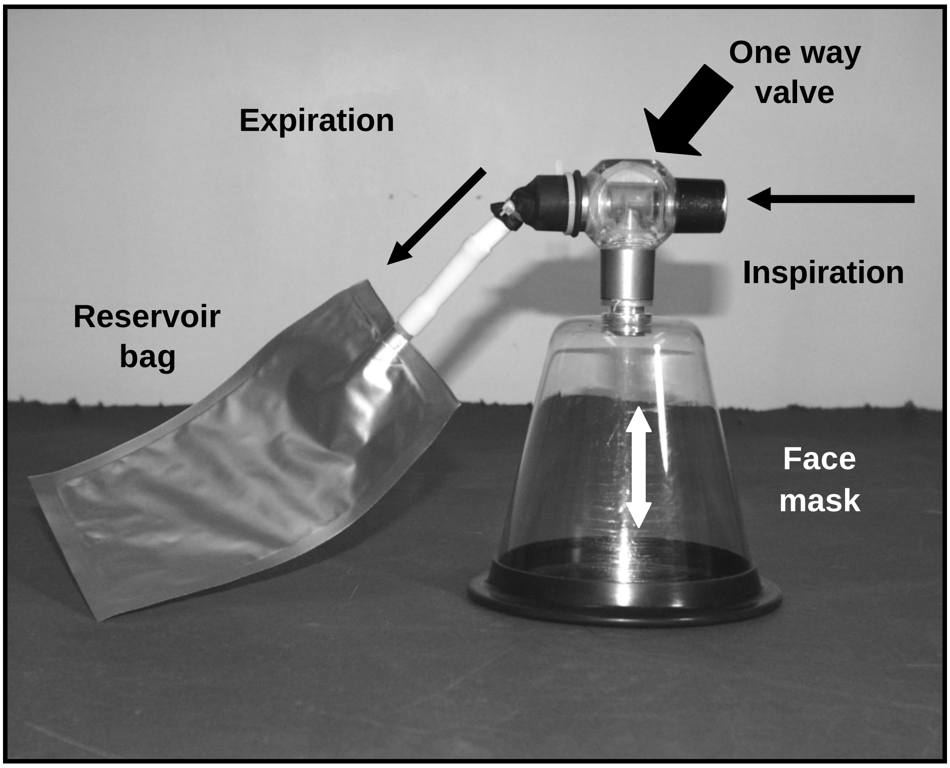


Figure 7: Breath sampling system with the self constructed one way valve. The one-way valve shifts from side to side depending on the respiration phase, allowing air to flow in only one direction. Small arrows are indicating breath direction.

2. 2. Automatic data analysis and calculation of gastric emptying values:

Exhaled $^{13}CO_2$ was measured with the HeliFAN plus© (Fischer Analysen GmbH, Leipzig; Germany), an analyser determining $^{12}CO_2/^{13}CO_2$ ratios by non-dispersive infrared spectrometry (NDIRS) (fig. 8a). The reservoir bags were connected to special ports and the content of $^{13}CO_2$ in the exhaled air was subsequently measured via broad band infrared light. This light is sending rays in direction of two acusto-optical collectors that respond exclusively to specific wave lengths (fig 8b). The NDIRS measurement relies on the principal of physics, which separates the absorption spectrum of both molecules ($^{12}CO_2$ or $^{13}CO_2$, respectively) by their asymmetric oscillation. Therefore, interferences that could potentially affect measured concentrations are minuscule. Initially, the concentration of total CO_2 is measured, then the concentrations of $^{12}CO_2$ in % and $^{13}CO_2$ in parts per million (ppm) are analysed and finally the $^{12}CO_2/^{13}CO_2$ ratio is calculated and displayed as delta. The baseline delta-value is defined as zero-value, against which all the following measurements are compared and calculated as delta-over-baseline (DOB).

Figure 8a: $^{13}CO_2$ measuring device HeliFANplus© (Fischer Analysen GmbH, Leipzig; Germany) based on non-dispersive infrared spectroscopy.

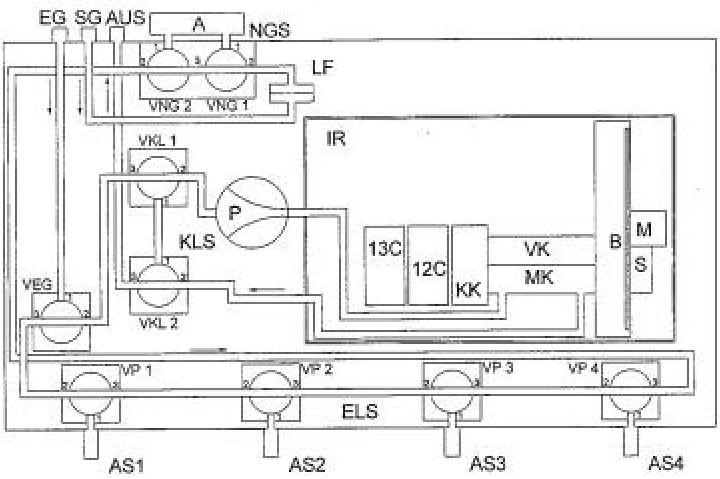


Figure 8b: Principle setup of the HeliFANplus© (courtesy Fischer Analysen GmbH)
Arrows are indicating direction of air flow

SG	= insertion of flushing gas	p	= pump
LF	= air filter	IR	= infrared module
NGS	= zero gas system	MK	= measuring chamber
A	= absorber	VK	= comparison chamber
VNG 1 and VNG 2	= ventile of zero gas	S	= infrad red light source
ELS	= inlet system	M	= motor
AS1 ... AS 4	= port 1... 4	B	= lens aperture
Vp 1... VP4	= port ventile 1... 4	KK	= calibration unit
12 C	= $^{12}CO_2$- collector	AUS	= withdrawal of gas
13 C	= $^{13}CO_2$- collector		

2. 2. 1. Formulas used in breath test analysis with HeliFANplus©[85]

? (delta) value

This is defined as the isotope ratio $^{12}CO_2/^{13}CO_2$ in a breath sample (R_s) referring to the reference isotope ratio in a standard (CO_2-free) gas (R_{STD} = 0.0112372).

? = (R_s/R_{STD} – 1) x 1000 ‰

Delta-over-baseline (DOB)

During breath test analysis only the change of the delta value in comparison to a sample that has been taken before ingestion of the ^{13}C-labeled substrate is of interest. This change is expressed in per mil above the basic level.

DOB = $?_{after}$ - $?_{before}$

Isotope relation (R)

$$R = \frac{^{13}C}{^{12}C}$$

Molecular fraction (MF)

$$MF = \frac{^{13}CO_2}{^{13}CO_2 + {}^{12}CO_2} \qquad MF = \frac{1}{1/R + 1}$$

$$R = \frac{1}{1/MF + 1}$$

Procentual recovery rate (PRR) [%/h]

$$PRR = \frac{\text{mmol } ^{13}C \text{ in breath (a)}}{\text{mmol } ^{13}C \text{ given (b)}} * 100$$

a = (MF_t – MF_{t0}) * CO_2 – production

with CO_2 production = 300 mmol/m^2 BSA h
and BSA = 0.024265 x $W^{0.5378}$ * $H^{0.3964}$

W = weight (kg)
H = hight (cm)
BSA = body surface area

Hight was guesstimated in each dog and was either 80 cm, 100 cm or 120 for small, middle sized and large breed dogs, respectively.

$$b = (MF_{substr} - MF_{t0}) * \frac{m}{M} * n$$

With m = amount of given substrate
M = molar mass of given substrate
n = number of ^{13}C-labeled atoms

Cumulative procentual recovery rate (cPRR) [%]

$$cPRR_{ti+1} = cPRR_{ti} + \left(\frac{PRR_{ti} + PRR_{ti-1}}{2} \right) * \frac{?t}{60}$$

2. 2. 2. Mathematical analysis of ^{13}C gastric emptying

a) the cumulative ^{13}C excretion graph correlates well with the inverse retention graph in gastric scintigraphy[1]

$$cPRR = m\,(1-e^{-kt})^{ß}$$

with

t = time
m = recovered percentage of given dosage
e, k, ß = constants

The parameters m, k and ß are calculated by adaptation of the measured data in the model over the method of the smallest sum of square error.

Gastric half emptying time ($Gt_{1/2b}$ in minutes) is calculated with cPRR equal to m/2:

$$Gt_{1/2b} = \frac{-\frac{1}{k}\ \ln(1 - 2^{-1/ß}) * 60 - 66}{1.12}$$

The lag phase (LAG; in minutes) is defined as:

$$Lag = \frac{\frac{\ln ß}{k} * 60 - 66}{0.94}$$

b) To describe ^{13}C-excretion curves, the following mathematical model from the ?^{2}- distribution was created:

$$PRR = a\, t^{b}\, e^{-ct}$$

with t = time

The parameter a, b and c are calculated according to the method of the smallest square error after adaptation of the measured data with the model equation.
The gastric emptying coefficient (GEC) is defined as follows:

$$GEC = \ln a$$

Dog no. 10:
In one dog (dog no. 10) the automatic measurement of these parameters was not possible because the dog exhaled only small amounts of CO_2. As a consequence several breath test samples had to be collected before a representative sample with sufficient amounts of CO_2 was available.
In this dog Delta-values were recorded and DOB-values were plotted against time using Microsoft Excel©. With nonlinear regression analysis from these data points $Gt_{1/2b}$, Lag and GEC were calculated utilizing the same equations as used by the HeliFANplus© software.

2.3. Software for breath test analysis

The software used for automatic measurements is integrated into the HeliFAN plus© device (FANci (PRG), version 2003; using windows XP©). Before measuring the first breath sample, a scheme to use ^{13}C-sodium acetate as labeling substance had to be created based on an already existing octanoate breath test master pattern. For calculating purposes (see above), the molar mass of sodium acetate (83.03 g/mol), the number of labeled C-atomes (1.00), the purity of the substance (99%) and its dosage (100 mg) were entered according to manufacturers datasheet.

3. ^{99m}Technetium colloid radioscintigraphy

After ingestion of the labeled test meal, the dogs were placed on a stretcher above the parallel hole collimator of a large field-of-view gamma camera (Philips Gamma Diagnost Tomo©; Philips Medical Systems GmbH, Hamburg, Germany) in a sternal position with minimal restraint (fig. 9).
Images were taken immediately after the intake of the meal and then every 15 minutes for 4 hours and every 30 minutes for the following 2-4 hours. In some cases a final image was taken after a total of 8 hours, if the stomach still contained radioactivity. If the stomach appeared completely empty at an earlier time point, image acquisition was stopped.

The scintigramms were aquired in 256 x 256 pixel matrices. Pixel size was 2.27 mm, resulting in a field of view of 540 mm. Acquisition time was 60 seconds for each image during which the animal was not allowed to change position. If the dog moved during the imaging sequence, another acquisition was obtained immediately afterwards.
Between aquisition times the dogs remained close-by.

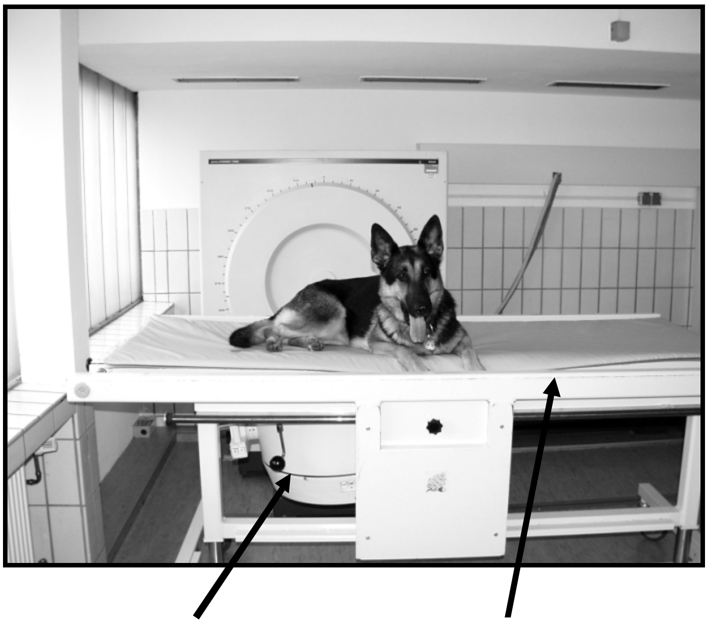


Figure 9: Positioning of a dog above the gamma camera for scintigraphic image acquisition of the gastric region

3.1. Evaluation of the radioscintigraphic image sequences

Scintigraphic images were stored as Dicom© data with the integrated software of the gamma camera (Pegasys© Inc.; Tokyo; Japan). Analysis was performed with the program ImageJ© (http://rsb.info.nih.gov/ij).

A small macro-programme was created in ImageJ© which allocated each picture to a certain time point of the study. Subsequently, images that could not be evaluated were excluded, for example if the animal had moved during the imaging acquisition or if the ROI could not be defined properly.

Brightness and contrast were adjusted for each picture in a standard manner (pressing “auto” button twice). Then, pictures were zoomed (magnifying glass-tool) to get the optimal view of the stomach so that a ROI could be drawn with maximal accuracy. The ROI was manually drawn for each picture (free hand selection-tool) and radioactive counts were measured within this area. ImageJ© displayed the total amount of pixels in the ROI as well as the mean, minimal and maximal size of the ROI (table 3):

No.	Time (min)	Pixels in ROI	Mean	Min	Max
1	0	8967.979	174.428	0	571
2	15	9177.604	144.095	0	583
3	30	7091.189	159.936	1	517
4	45	6527.824	155.567	1	488
5	60	5725.357	164.042	3	473
6	75	5853.097	140.059	1	429
7	90	5663.125	116.020	1	367
8	105	4595.353	115.041	3	316
9	120	5715.531	78.930	3	220
10	135	5073.558	86.379	2	252
11	150	4768.948	68.384	1	230
12	165	4847.557	64.222	1	217
13	180	3832.190	60.418	1	195
14	195	7281.161	30.124	0	114
15	210	4985.123	26.654	0	89
16	225	3085.404	26.764	0	81
17	240	1356.006	18.174	1	46
18	270	1395.310	5.915	0	20

Table 3: Example of scintigraphic data (dog no. 3).
no. = number of image
pixels in ROI = total number of pixels in the region of interest
mean = mean number of pixels in the ROI
min, max= minimal and maximal number of pixels in the ROI

The number of pixels times the mean size of the ROI gives the absolute number of radioactive counts per image. Next, the radioactive decay of ^{99m}Technetium over time using the law of radioactive decay was calc ulated:

$$A = A_0 * e^{-l * \frac{t}{T}}$$

Law of radioactive decay:

A: radioactivity
A_0: radioactivity at time point 0
T: physical half-life time of ^{99m}Technetium (= 6 hours)
t: time lapsed
e: Euler`s constant
?: -ln(2)

3.2. Mathematical curve fitting of scintigraphic images

Gastric half emptying time

The decay-corrected radioactive counts were plotted against time and gastric half-emptying time was calculated. Briefly, a linear regression of the suspected gastric half-emptying time estimated based on the initial counts was calculated. If a gastric emptying lag phase was observed, all radioactivity measurements until the first decline was seen were averaged and this value was subsequently used as initial radioactive count (defined as 100% activity).

On the plotted graph of decay-corrected radioactive counts against time the gastric half emptying time was guesstimated and five measurements in that area were used to calculate a linear regression. This resulted in a trend line with the formula

$$y = mx + b$$

Gastric half emptying times were subsequently calculated using the formula:

$$y_{½} = m\, x_{½} + b$$

$y_{½}$ = half the number of the initially measured radioactive counts
$x_{½}$ = gastric half emptying time = $Gt_{½s}$

Thus,

$Gt_{½s} = (y_{½} - b)/m$

For example:

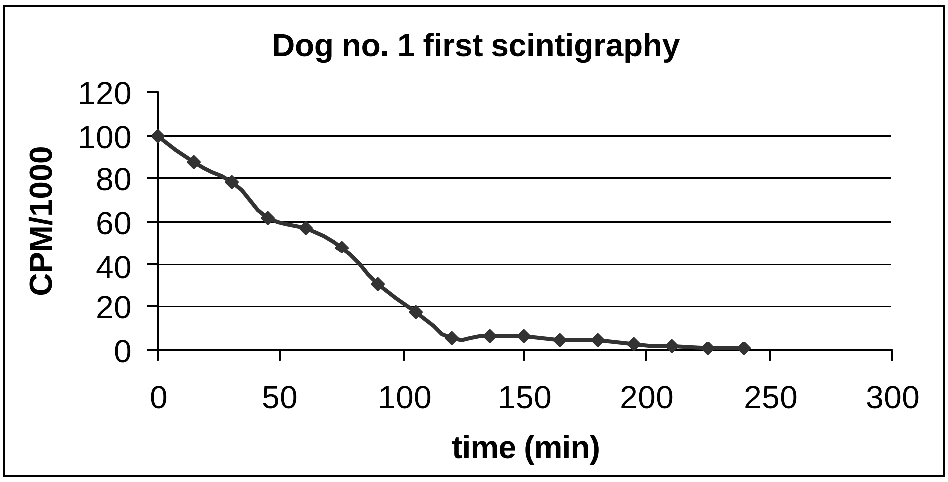


Figure 10: Curve of Scintigraphic gastric emptying of dog no. 1 – there is no lag phase visible
CPM = counts per minute.

In the example above, the initial counts of radioactivity were 1’081’356.251 counts per minutes (CPM; see fig. 10). Presuming that the gastric half emptying time point is at half of this radioactivity (540’678,126 CPM) the five measurements around this value were chosen for further evaluation. A trend line was calculated with Microsoft Excel© (fig. 11) and the formula was obtained.

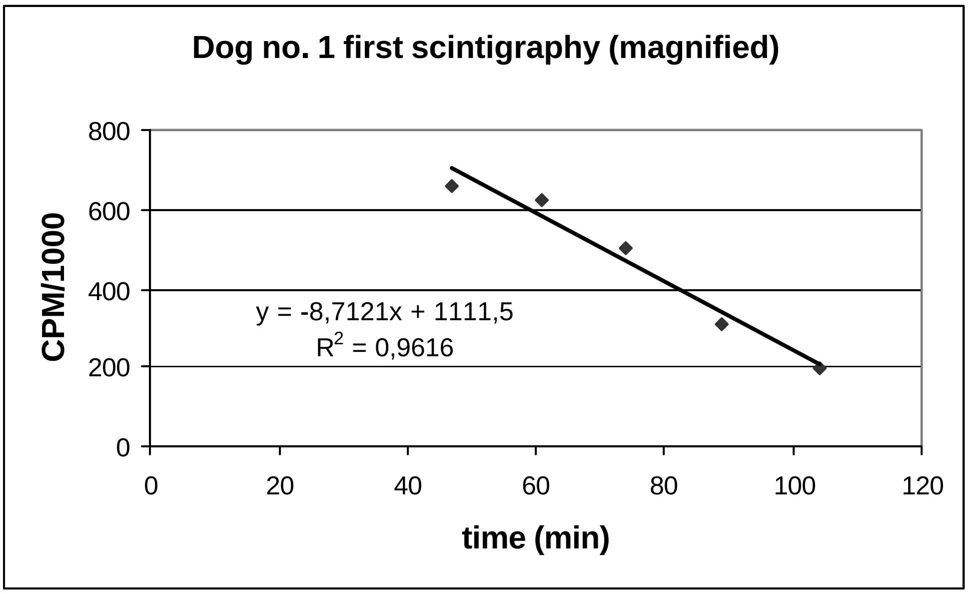


Figure 11: Calculated trend line of five measurements close to the guesstimated gastric half emptying time (dog no.1).
CPM = counts per minute
R^2 = correlation coefficient
y = linear equation of the trend line

In this case (dog no. 1) the values for the formula:

$Gt_{½s}$ = ($y_{½}$ -b) / m

are

$y_{½}$ = 540.5
m = -8.7121
b = 1111.5

→ $Gt_{½s}$ = (540.5 – 1111.5)/ -8.7121
= 65.54

Thus, gastric half emptying time is 65.54 minutes for the dog in this example.

Lag phase
The lag phase is defined as the time from ingestion of the test meal to the beginning of gastric emptying and a decline in radioactive counts can be observed. Lag phase was recorded by visual inspection of the graphical depiction of each scintigraphy.

4. Statistical evaluation of results

For statistical analysis the programm SPSS© (SPSS GmbH Software, Munich, Germany, version 14.0 for Microsoft Windows©) was used.
Variables were evaluated for normality by the Kolmogorov-Smirnov test and are reported as median and range.
A Spearman correlation analysis was performed to compare data of the different sets of measurements.

V. Results

1. Baseline results

From May 2005 to September 2005 twelve healthy dogs privately owned by staff of the small animal clinic were included in this study. There were three female intact, five female spayed, three male neutered dogs and one male intact dog. Age ranged from 1 to 12 years (median 6.5 y) and weight ranged from 9.2 to 38.1 kg (median 19.7 kg).
Most dogs (n= 6) were of mixed breed, other breeds represented included Beagle (n=1), Dobermann Pinscher (n=1), Fox Terrier (n=1), Labrador Retriever (n=1), Old German Shepherd (n=1) and West Highland White Terrier (n=1) (table 4).

No.	Weight (kg)	Breed	Age	Gender
1	9.5	Mix	9 y	mn
2	15.1	Beagle	7 y	mn
3	10	West Highland White Terrier	11 y	mn
4	22.1	Mix	7 y	f
5	20	Mix	8 y	fs
6	9.2	Fox Terrier	12 y	fs
7	22	Mix	4 y	fs
8	14.5	Mix	1 y	f
9	27.5	Labrador	4 y	fs
10	38.1	Old German Shepherd	5 y	f
11	25.6	Dobermann Pinscher	7 y	fs
12	22.3	Mix	4 y	m

Table 4: Data of the dogs participating in this study.
fs = female spayed
f = female
mn = male neutered
m = male
y = years

Liver function, assessed by bile acid stimulation test was within normal limits in all dogs (table 5).[85]

Dog no.	BA 1 (µmol/l)	BA 2 (µmol/l)
1	14	19
2	11	11
3	12	23
4	10	17
5	10	15
6	11	11
7	11	15
8	11	17
9	12	26
10	10	13
11	9	8
12	18	17

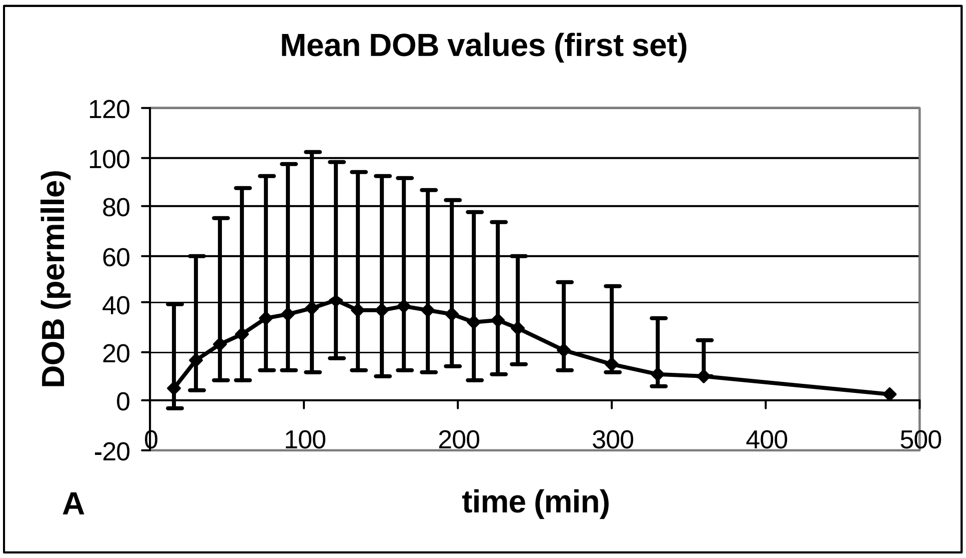
Table 5: BA1 = bile acid concentrations 2 hours after ingestion of the first breath test meal
BA2 = bile acid concentrations 2 hours after ingestion of the second breath test meal
(reference value < 30 µmol/l)

2. ^{13}C-sodium acetate breath test

2.1. Test procedure

Breath tests could be successfully performed in all 12 dogs and in eleven of the twelve CO_2 levels in the collected breath samples were sufficient for automatic measurements with NDIRS.
The test meal was well accepted in 9 dogs, 2 dogs (dog no. 1 and no. 5) had to be force fed for every test, and another dog (dog no. 3) was force fed on his first test but accepted the test meal in the 3 following measurements.

In all dogs a rapid increase of $^{13}CO_2$ in expired air was noticed during the first hour. The peak concentrations were reached between 75 and 360 minutes (median 141 minutes in the first and 162 in the second series of tests) (fig. 12).



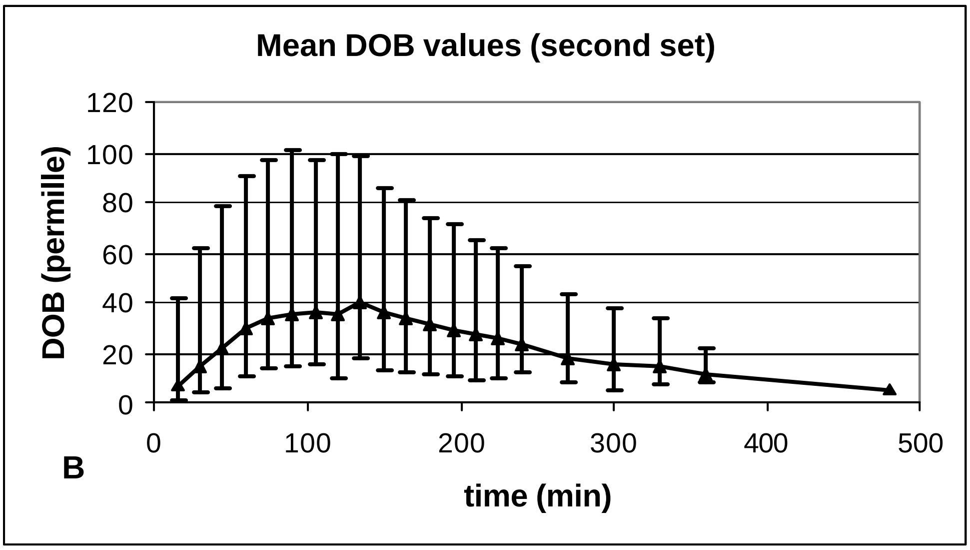
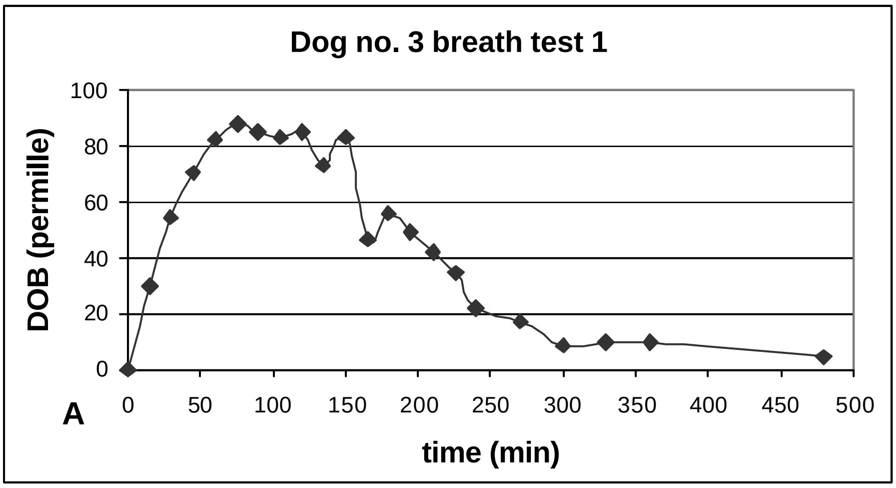


Figure 12: Data over baseline (DOB)-values of all dogs in the first (A) and second (B) set of breath tests over time presented as mean values (data points) and standard deviation (whiskers).

At the end of the collection period (360 minutes in five and 480 minutes in nineteen tests) nearly basal levels of DOB were reached in all but one dog. In this dog (no. 11), after 480 minutes DOB level was still nearly 50% of the peak value. No further breath test sample was obtained in this dog.

Figure 13 shows two typical examples of breath test curves in 2 dogs. The graph of dog no. 3 shows a rapid gastric emptying rate ($G_{t1/2b}$ = 51.9 min) and dog no. 7 has a moderate to fast gastric emptying rate ($G_{t1/2b}$ = 85.0 min) with a slightly different emptying pattern.



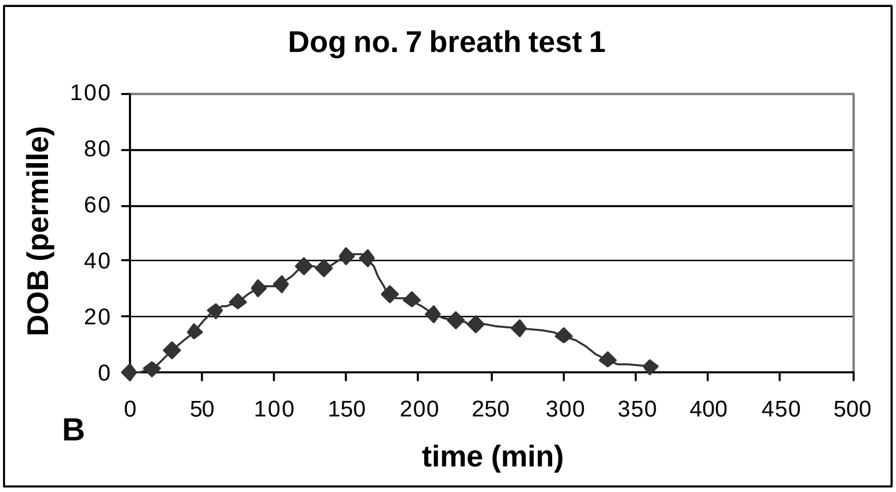


Figure 13: Two examples of gastric emptying patterns in the ^{13}C-SABT. DOB = delta over baseline

2.2. Gastric emptying parameters

2.2.1. Gastric half emptying time measured by sodium acetate breath test

The median gastric half emptying time ($Gt_{1/2b}$) in the first and second set were 121 min (range 52 to 244 min) and 115 min (range 32 to 312 min), respectively; there was no significant difference.
Results of $Gt_{1/2b}$ in both sets of measurements showed no correlation (r = 0.364, p = 0.245).

2.2.2. Lag phase measured by sodium acetate breath test

The median lag phase in the first set and second set were 63 min (range 17 to 137 min) and 66 min (range 0 to 181 min), respectively.
LAG_b values of both breath test analyses correlated fairly well with each other (r= 0.624; p = 0.040).

2.2.3. Gastric emptying coefficient measured by sodium acetate breath test

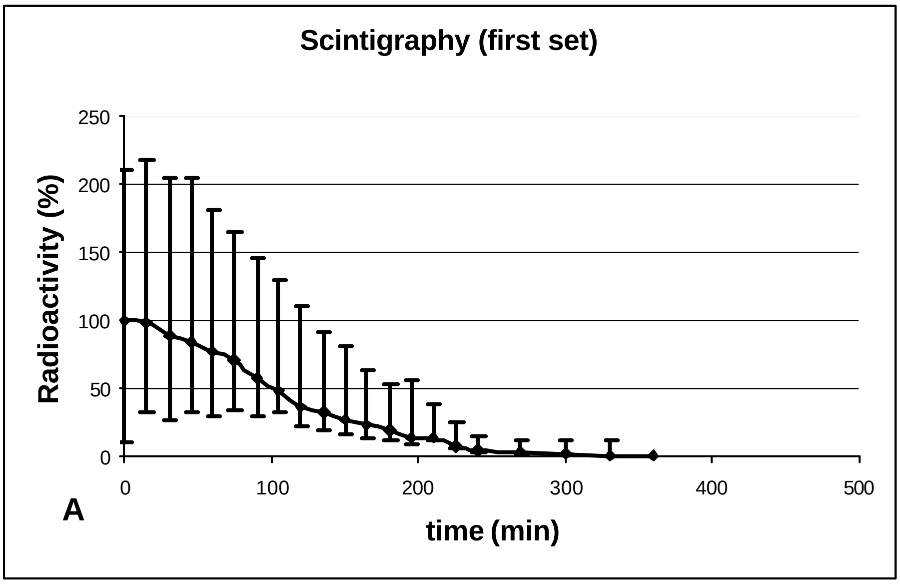
The median gastric emptying coefficient in the first and second set of measurements were 2.5 (range 1.0 to 3.0) and 2.0 (range 0.7 to 3.9), respectively.
Gastric emptying coefficient (GEC) showed no consistency between the two sets of tests (r = 0.183; p = 0.591).

3. ^{99m}Technetium colloid radioscintigraphy

3.1. Test procedure

Gastric radioscintigraphy was successfully performed in all 12 dogs in both sets of measurements.

The decrease of the gastric content of all dogs in percent in the first and second set of measurements can be seen in figure 14:



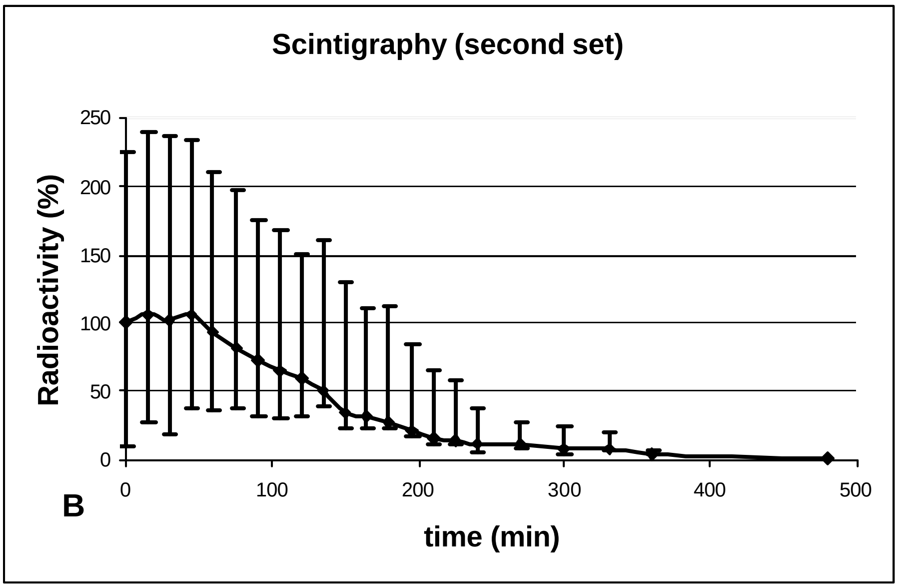


Figure 14: Percentage of decrease in gastric volume, measured by scintigraphy in the first (A) and second (B) set of measurements. Data presented as mean values (data points) and standard deviation (whiskers).

Overall gastric emptying patterns showed some variability. In three of 24 scintigraphies (12.5 %) a linear pattern of gastric emptying could be observed (fig 15A). This was preceeded in 1 case by a lag phase of 15 minutes. In 21 scintigraphies (88 %) a sigmoidal shape of the gastric emptying graph was seen (fig 15B), and 18 of them were associated with a lag phase of 15 to 108 minutes (median 41 minutes). In two scintigraphies the occurrence of a lag phase could not be assessed because the graphs showed an erratic course. The gastric emptying pattern was similar between the two sets of scintigraphy in 8 dogs (66 %).

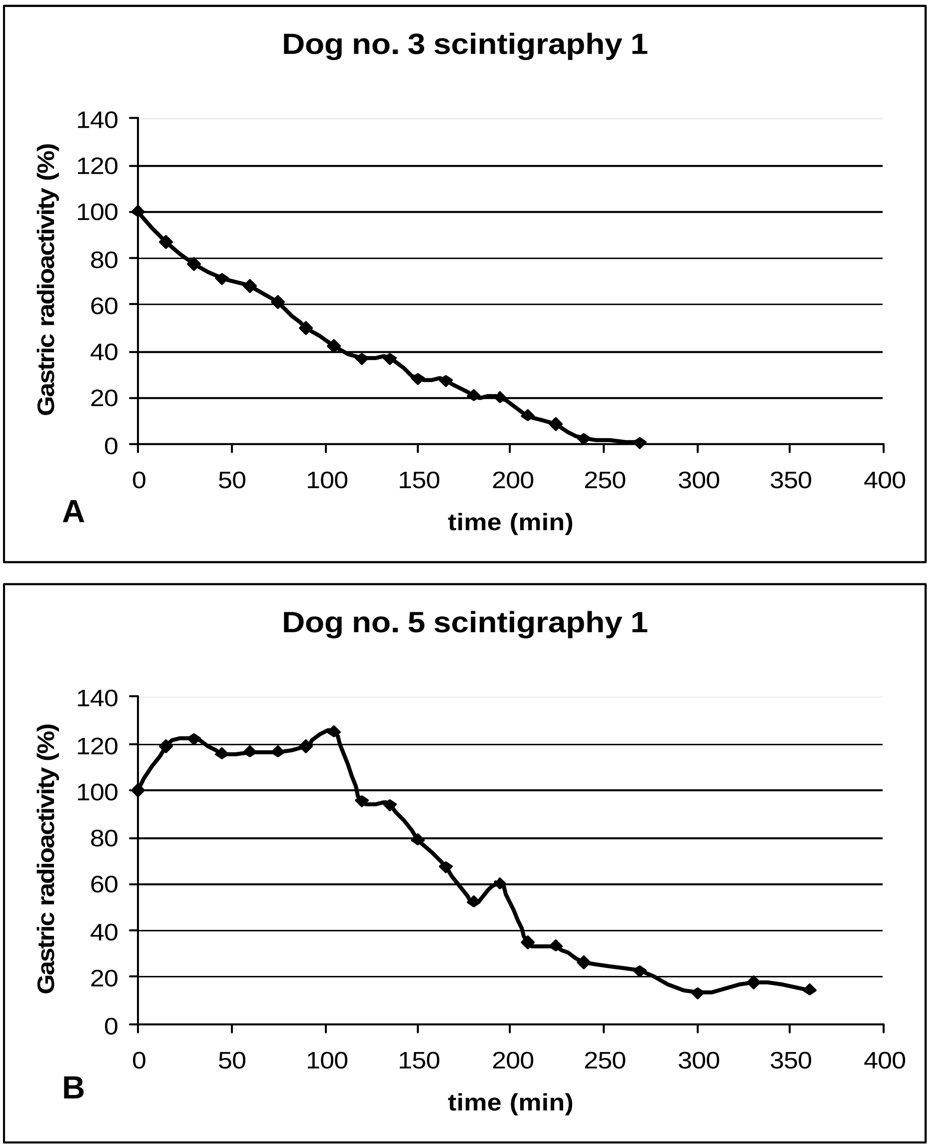


Figure 15: **A**. Dog no. 3 shows a linear scintigraphic gastric emptying pattern without evidence of a lag phase.
B. In contrast, in dog no. 5 a lag phase can be appreciated and the gastric emptying pattern is sigmoidal.

3.2. Gastric emptying parameters

3.2.1. Gastric half emptying time measured by scintigraphy

Median gastric half emptying time in the first set of scintigraphic measurements was 105 min (range 24 to 186 min) and in the second set 149 min (mean, range 70 to 220 min).

There was no significant correlation of the gastric half emptying time between both sets of scintigraphic measurements ($r = 0.207$; $p=0.519$).

3.2.2. Lag phase measured by scintigraphy

A lag phase of gastric emptying was visible in 16 scintigraphic measurements. Only one dog (dog no. 3) had no visible lag phase in both measurements. The median lag phase in the first set of scintigraphies was 54 minutes with a range of 21-108 minutes. In the second set the lag phase median was 35 minutes with a range of 15 to 59 minutes.

LAG_S of both sets of scintigraphic measurements were significantly correlated ($r = 0.609$ and $p= 0.036$).

4. Comparison of ^{13}C-sodium acetate breath test and gastric scintigraphy

4.1. Comparison of gastric half emptying times

Gastric half emptying times measured by scintigraphy and breath test did not correlate in the first set of measurements ($r = 0.155$; $p = 0.650$) but in the second set there was a highly significant correlation ($r = 0.873$; $p < 0.0005$).

4.2. Comparison of lag phases

Lag phases measured by scintigraphy and breath test analysis did neither correlate in the first ($r = 0.252$; $p = 0.455$) nor in the second set of measurements ($r = 0.051$; $p = 0.883$).

VI. Discussion

1. General remarks

In this study, a new breath tracer substance, ^{13}C-sodium acetate, was used to measure gastric emptying rates in dogs and was validated against the gold standard method, gastric ^{99m}Technetium scintigraphy.

Few studies have documented delayed gastric emptying associated with pathological processes in the dog, possibly because of the technical difficulties in assessing the rate of solid phase gastric emptying. The limited information available on the rate of gastric emptying makes the definition of delayed gastric emptying difficult.
The development of a simple and inexpensive method to measure solid phase gastric emptying in veterinary medicine, as for example with the sodium acetate breath test used in the present study, might show that clinical disorders leading to a delay of gastric emptying are more prevalent in dogs than previously thought.

2. ^{13}C- sodium acetate breath test

Breath test protocol and collection of breath test samples
The ^{13}C-octanoic breath test was first described for the examination of solid phase gastric emptying in humans in 1993.[34] It showd a good correlation to scintigraphy and ultrasound.[1,57]

Simplification of the test protocol has established the ^{13}C gastric emptying breath test as an investigative method that can be applied as “point of care” technique in human medicine. It is used in human medicine to detect delays in gastric emptying that occur concurrently with many clinical disorders,[1,4] including cystic fibrosis,[1] diabetes mellitus[1,36] and motor neuron diseases.[1]
In the dog, breath tests have been validated to assess gastric emptying non-invasively. The breath test has been compared to ultrasonography in the dog,[57] but no comparison between gastric emptying breath tests and scintigraphy has been attempted so far.

The principal advantage of gastric emptying breath tests in veterinary medicine is their non-invasiveness. Analysis of breath test data can be completely automated and provides data describing the rate and pattern of gastric emptying that is objective and quantitative.
The assessment of gastric emptying by stable isotope breath tests involves ingestion of a ^{13}C-labeled substrate that is rapidly absorbed and metabolized to $^{13}CO_2$ after gastric emptying to produce a detectable increase in $^{13}CO_2$ in the exhaled breath. The tracer substance mostly used is octanoic acid.

Unfortunately, octanoic acid has been shown not to be an ideal tracer in small animals because of its unpleasant taste. Therefore, we chose to use sodium acetate, an odour- and tasteless powder, that is commercially available as a ^{13}C-tracer substance. Its main advantage in comparison to octanoic acid besides its tastelessness is the ready-to-use formulation and that it can be added to any test meal without special preparations. It is not necessary to cook a fresh, suitable test meal for every feeding since the ^{13}C labelled substance can easily be added to

commercially available canned dog food. The test meal is therefore similar to food normally ingested by the animal and neither sodium acetate nor the ^{13}C label poses any risk to health.
The breath test protocol itself is simple and does not require special expertise. Equipment to measure $^{13}CO_2$ is widely available in human medicine, but not in veterinary medicine. Breath samples can be easily collected with a face mask,[1,18] as it was also done here. With a short period of training, the breath sampling procedure could be completed easily and rapidly in almost all dogs; in this study sampling of breath took at maximum 2 minutes. No special restraint procedures have been necessary.

Collection of breath samples was done up to 6 hours, because this has shown to produce a better accuracy than collecting for shorter periods of time. Sample intervals were relatively short (every 15 minutes for 4 hours, than every 30 minutes for the following 2 hours), however, these might be prolonged in the future to make the test more easily performable in private practice.

$^{13}CO_2$ measurement

The NDIRS-method for measuring of $^{12}C/^{13}C$ in canine breath samples with the HeliFANplus© is simple. Unfortunately, high levels of CO_2 are necessary in the exhaled air in order for the machine to read any samples. A German Shepherd dog (no. 10) did not produce enough CO_2 to be measured automatically with the HeliFANplus©. Thus, alternative measuring techniques, for example gas chromatography would be necessary in those dogs or in other animals that produce only small amounts of exhaled CO_2.

Gastric half emptying time and lag phase

The median gastric half emptying time ($G_{t1/2b}$) measured with this new method was 123 minutes, a value similar to the data determined by other researchers[18,19] but there was a wide range from 32 to 312 minutes, which was not found in other studies. This might be partially due to the number of animals used in other studies, for example Wyse et al. 2001[18] used only 6 dogs and the weight range was very narrow (27-33 kg), which might influence the range of gastric emptying times. It is not entirely sure, if weight or age might influence gastric emptying, controversial results have been published.[18,32]
In the present study, a wide range of different breeds, weights and ages was included to represent a heterogeneous population of dogs. This might explain the wide range of gastric emptying times. To correlate gastric emptying rates with age, gender, breed or weight of the dogs, the examined population in the present study was too small.
In two other studies with a wide weight range (3.5 to 59.1 kg[19] and 6 to 39 kg[32]) a different tracer substance (octanoic acid) was used, hence, comparison with the present study is difficult. Octanoic acid might influence gastric emptying times because it is a fatty acid and its absorption might have considerable individual variation. The influence of sodium acetate on gastric emptying is not known, but it could be shown that it accurately reflects gastric emptying in both liquid and semisolid meals in humans.[82] It has also successfully been used to assess liquid phase gastric emptying in the dog.[1]. However, an influence on gastric emptying times cannot be fully excluded.

There was no correlation between the $G_{t1/2b}$ in both sets of breath test measurements. An influence of the previous scintigraphic test or the abrupt change of food for the tests as well as intraindividual variations might be an explanation.

The lag phase of gastric emptying is a well known phenomenon in humans and it has been assumed that it is also present in the gastric emptying pattern of solid food in dogs. It is most likely due to trituration of the gastric contents during empyting. A lag phase could be seen in 10 dogs during breath test analysis. In one dog the lag phase was absent in one of the two sets of measurements and in another dog the lag phase could not be calculated due to a lack of produced CO_2 for automatic measurement by the breath test device.
Although in the present study the LAG_b values correlated well in both sets of breath test measurement, its significance is controversial[1,87] and no reference ranges for this parameter in dogs are available. Most studies in human medicine do not present this parameter.[87] The LAG_b and LAG_s values did not correlate, the significance of this finding remains unclear.

3. ^{99m}Technetium colloid radioscintigraphy

Radioscintigraphic procedures
Gastric radioscintigraphy is – since its first use in 1966[61] – considered the gold standard method to assess gastric emptying in human medicine, against which all new methods must be compared.[88] In human medicine, the Society of Nuclear medicine defined a standard protocol for radioscintigraphic evaluation of gastric emptying and reference ranges based on large study groups are available.[89] This is not the case for veterinary medicine and no standard protocols are available. Scintigraphy has been used for the assessment of gastric emptying in animals, including dogs, cats, horses, rats, monkeys and pigs.[1]

Although ^{99m}Tc-mebrofenin and disofenin are commonly used in dogs,[1] this study shows that ^{99m}Technetium albumin colloid can also be used. It disperses homogeneously in the test meal as was shown in every single meal before its ingestion by placing the homogenized food on the gamma camera (data not presented). ^{99m}Technetium albumin colloid seems not to separate from the test meal, although determination of the separation of technetium and food is difficult. Still it is possible that radioactive tracer substances might not empty concurrently with the ingested meal, the dispersion of a marker requires further studies.

Before scintigraphy animals are normally fasted for 12-24 hours to ensure an empty stomach before ingestion of the test meal.[1] This was easily performed in all our dogs, and only 3 of 12 dogs had to be forcefed (one of them only once), because they denied the test meal despite sufficient fasting.

Evaluation of scintigraphic images
Correction factors in which the radioactive decay of the isotope over the duration of the test are taken into consideration need to be employed, therefore the law of decay was used. However, there might also be difficulties in measuring the ROI correctly. Movement of the marker in the stomach could decrease the measured activity of the radionuclide. Thus, the lag phase of gastric emptying might be overestimated because the marker has moved to lie closer to the camera. Spreading of food in the

stomach might cause some of the readings early in the test to be greater than the initial count, an effect that could be observed in the majority of the animals in this study. Only 2 dogs had no rise of activity in the initial counts, five dogs showed this phenomenon in one of the two sets of scintigraphies and in the remaining 5 dogs this could be observed in both sets of scintigraphic measurements. Overlap of activity in the small intestine or colon over the ROI in the stomach also is a problem, leading to overestimation of gastric emptying times.

In our study, analysis of the scintigraphic images was performed with the program ImageJ© where the gastric ROI are defined manually. Automatic definition of the ROI seemed inappropriate because in most of the images a subjective estimation of the gastric area was necessary. Obtaining the correct ROI was difficult, because at later time points overlapping between radioactivity in the nearly empty stomach and the large intestines occurred. To test for inaccuracies when obtaining the ROI some scintigraphic pictures where evaluated multiple times after aquisition by the same examiner. This resulted in almost identical values (data not shown) making inaccurate ROI drawings unlikely.
Nearly the same values of radioactive counts within the stomach could again be achieved when the same examiner was asked to define ROIs of the same scintigramms again after several weeks, so that we assume the definition of the ROIs was correct.

Gastric half emptying time and lag phase
$G_{t1/2s}$ values between both sets of gastric scintigraphies showed poor correlation. Because of the wide variability of gastric half emptying times in the examined dogs, the definition of normal gastric half emptying time values seems impossible.
This variability seen in this study is most likely not only due to technical problems that have already been discussed, but also to individual variability, different breeds and weights.
Because of this wide variability of scintigraphic results, we think that scintigraphy itself, although it provides a quite useful standard against which new methods for assessment of gastric emptying can be compared, has its limitations and disadvantages. In addition, the clinical application of scintigraphy in dogs will always be limited by the radiation hazard and the availability and expense of this method. But, because of the limited numbers of dogs used in this study, no final statement about the usefulness of scintigraphy is possible and further studies are necessary to approve or confute these results.

4. Comparison of ^{13}C-sodium acetate breath test and gastric radioscintigraphy

Gastric half emptying times of scintigraphy and breath tests were measured on separate days, whereas in previous studies, especially in human medicine, both measurements were performed simultaneously. Previous studies indicate that both scintigraphy and breath test measurements are reproducible,[87] thus measurement on different days should not be the reason for the poor correlation seen between both methods in this study. A poor correlation has also been reported recently in human medicine.[87] An influence of a preceding test procedure cannot be fully excluded. In addition, in the present study, on the first day of testing there was an abrupt change from the regular dog food to the new diet used for creating the test meals. This could also have had an effect on gastric emptying rate. It should also be considered that in some dogs, intervals between the two sets of measurements were quite long (up to 3 months), so that external variables such as weather or temperature might also have an influence. It is unlikely that the diet used for assessing gastric emptying had any influence since the same diet was used throughout the study.

Mathematical curve fitting was done to model the data acquired by breath test and scintigraphy and to calculate coefficients to describe the rate of gastric emptying, as described in previous studies.[8,9,18,34]
In human medicine, the use of correction factors and the right way of calculating comparable values is discussed vividly at the moment. For example discussion arose if the "corrected" half emptying time used by the first study describing gastric emptying breath tests[34] is a useful parameter at all.[90] Recently, two new mathematical models to achieve a better correlation or comparability between scintigraphy and gastric emptying breath test were presented,[91,92] and therefore it is possible that a better correlation between these both methods could also be accomplished by a different mathematical analysis of gastric emptying times in animals.

Gastric half emptying times of scintigraphic and breath test measurements did not correlate in the fist set of measurements, but correlated in the second set. The first procedure performed in the first set was the scintigraphy, thus an influence from scintigraphy itself or the ^{99m}Technetium on the breath test cannot be fully excluded, although this seems to be very unlikely.
It is possible that gastric emptying in an individual dog varies significantly from one day to another. Environmental factors for such changes have never been proposed for the dog, but cannot be excluded. It would therefore be a proposition for further studies to perform both measurements simultaneously on the same day with one test meal containing ^{99m}Technetium and ^{13}C-sodium acetate.
The reason why one set of measurements correlated while the other did not, may be due to the incoherent results of the scintigraphic measurements. Radioscintigraphy has its drawbacks as a gold standard method for measuring gastric emptying in the dog.
Finally, it has to be discussed whether the results of the two different methods are comparable at all. Scintigraphy is a direct method to measure gastric emptying of a radioactive substance, whereas breath test analysis relies on an indirect detection of metabolites in the expired air. Complicated calculations are necessary to transform these data into the gastric emptying time values. It is therefore questionable whether a comparison between these two different methods is advisable or possible.

5. Conclusion

In conclusion, the correlation between scintigraphy and ^{13}C-SABT in this study was poor. This might be due to difficulties in the correct evaluation of ROIs in scintigraphic measurements. Methodical weakness of the ^{13}C-SABT itself or improper mathematical analysis cannot be excluded as a cause of the poor correlation. The higher correlation in breath test results may indicate that the problem is rather due to the scintigraphy than the breath test measurements.

The gastric emptying breath test still has considerable potential for development as a point of care method for assessment of gastric emptying in small animals.
However, ^{13}C-SABT for measurement of gastric emptying requires further validation before it can be used as a routine diagnostic tool in veterinary medicine. The role of radioscintigraphy as a gold standard method should also be reconsidered, and different mathematical analyses might be necessary.

Whereas the gastric emptying breath test has been validated for application in healthy subjects, relatively few studies have investigated the effect of disease on gastric emptying times and tracer absorption and excretion, so that further research is necessary to determine the metabolic fate of the tracers and to validate the tests for ill animals.

VII. Summary

The gold standard to assess gastric emptying has been suggested to be ^{99m}Tc scintigraphy. Lately, non-invasive ^{13}C-breath tests have been used successfully as an alternative in human medicine, but have not been compared to scintigraphy in the dog. The aim of this study was to evaluate the ^{13}C-SABT and compare it to gastric scintigraphy for a solid test meal in the dog.

12 privately owned healthy dogs were included. Age and weight ranged from 1.5 to 12 years and 9.2 to 38.1 kg, respectively. Normal liver function was ascertained in all dogs via bile acid stimulation test. Test meal consisted of canned dog food; the caloric intake was calculated for each dog based on body mass. The meal, which was labelled with either 100 mg ^{13}C-sodium acetate or 150-250 MBq ^{99m}Tc colloid, was fed after an overnight fast and ^{13}C-SABT and scintigraphy were performed on two consecutive days; this procedure was repeated in an alternating order. Breath samples and scintigrams were obtained before and every 15 minutes after the ingestion of the labelled food for 4 hours, then every 30 minutes for another 2 hours. $^{12}CO_2/^{13}CO_2$ ratio in the breath was measured by non-dispersive infrared spectroscopy.

Gastric half emptying times for scintigraphy ($Gt_{½s}$) and for breath tests ($Gt_{½b}$) were calculated for both sets of measurements. Non-parametric statistical tests were used to compare both scintigraphic as well as both breath test values and to compare scintigraphy to breath test gastric emptying.

The median $Gt_{½b}$ was 121 and 115 minutes (range 32 to 312 min) and median $Gt_{½s}$ was 105 and 149 minutes (range 24 to 220 min). $Gt_{½b}$ and $Gt_{½s}$ did not correlate in the first set of measurements, but showed significant correlation in the second one ($p < 0.0005$). $Gt_{½b}$ did not correlate between both sets of measurements. The same lack of correlation could be found for $Gt_{½s}$.

In conclusion, the ^{13}C-SABT is well tolerated in dogs and the test is easily performed. Since there was no correlation between both sets of scintgigraphic measurements, it is questionable if this type of gastric emptying assessment can indeed be considered a gold standard. Unfortunately, there was also no correlation between both sets of breath test analysis, thus further studies are needed to assess the usefulness of this technique, such as in human medicine.

VIII. Zusammenfassung

Die ^{99m}Tc Szintigraphie ist bisher als Goldstandard zur Bestimmung der Magenentleerungszeit angesehen worden. ^{13}C-Atemtests sind in der Humanmedizin erfolgreich als alternative Methode eingesetzt worden, beim Hund wurden sie allerdings nicht mit der Szintigraphie verglichen. Das Ziel dieser Studie war es, die Magenentleerung einer festen Testmahlzeit beim Hund mittels dem ^{13}C-Natriumazetat Atemtest (NAAT) zu untersuchen und mit der Szintigraphie zu vergleichen.

12 gesunde Hunde aus Privatbesitz gingen in die Studie ein. Alter und Körpergewicht lagen zwischen 1,5 und 12 Jahren bzw. 9,2 bis 38,1 kg. Mittels eines Gallensäurestimulationstests wurde bei allen Hunden eine Leberfunktionsstörung ausgeschlossen. Konventionell erhältliches Feuchtfutter wurde entweder mit 100 mg ^{13}C-Natriumazetat oder 150 – 250 MBq ^{99m}Tc-Albuminkolloid versetzt und nach einer Fastenzeit von mindestens 12 Stunden verabreicht. ^{13}C-NAAT und Szintigraphie wurden an zwei aufeinander folgenden Tagen durchgeführt und in umgekehrter Reihenfolge bei allen Hunden wiederholt. Messung der Atemproben und Szintigramme wurden jeweils direkt vor und anschließend in 15-minütigen Intervallen nach der Aufnahme der Testmahlzeit über 4 Stunden, sowie alle 30 Minuten für weitere 2 Stunden durchgeführt. Das $^{12}CO_2/^{13}CO_2$ Verhältnis in der Atemluft wurde mittels nicht-dispensiver Infrarotspektroskopie ermittelt.

Sowohl für den Atemtest ($Gt_{½b}$) als auch für die Scintigraphie ($G_{t½s}$) sind die Magenhalbentleerungszeiten in beiden Durchgängen bestimmt worden. Nichtparametrische statistische Tests sind zum Vergleich der Daten des Atemtests sowie der Szintigraphie, sowie zum Vergleich beider Methoden miteinander herangezogen worden.

Die mediane $Gt_{½b}$ war 121 bzw. 115 Minuten in beiden Durchläufen (Bereich: 32 – 312 Minuten), die mediane $G_{t½s}$ lag bei 105 und 149 Minuten für beide Durchläufe (Bereich: 24 - 220 Minuten). $Gt_{½b}$ und $Gt_{½s}$ korrelierten im ersten Durchlauf nicht, im zweiten allerdings gut miteinander ($p< 0{,}0005$). Die $Gt_{½b}$ Werte beider Durchläufe korrelierten nicht miteinander, ebenso wenig wie die $Gt_{½s}$ -Werte.

Zusammenfassend wurde der ^{13}C-NAAT von Hunden gut toleriert und ist einfach durchzuführen. Da zwischen beiden szintigraphischen Messungen keine Korrelation bestand, ist es fraglich, ob diese Methode tatsächlich als der Goldstandard zur Bestimmung der Magenentleerung beim Hund bezeichnet werden kann. Leider fand sich auch zwischen den Magenhalbentleerungszeiten im Atemtest keine Korrelation, also sind weitere Studien notwendig, um die Bedeutung dieses Tests zu untersuchen, ähnlich wie in der Humanmedizin.

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