The Incidence of Urolithiasis in Cats and Dogs and the Influence of Diet in Formation and Prevention of Recurrence.

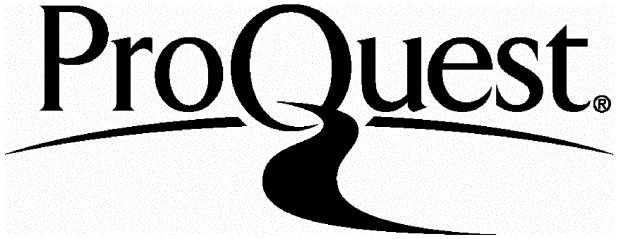
Abigail E. Stevenson

Thesis submitted for the degree of Doctor of Philosophy

Institute of Urology and Nephrology

University College London

2002



ABSTRACT

The two most common minerals found in uroliths from cats and dogs were calcium oxalate and magnesium ammonium phosphate, identified using accurate quantitative analytical techniques. Other mineral types included calcium phosphate and uric acid/ammonium urate. Cystine uroliths were found only in dogs. Trends towards age, breed and sex for each mineral type are discussed.

The major urinary risk factors for calcium oxalate formation in dogs were found to be calcium and oxalate, although uric acid concentration was also increased in some dogs. Although individually these factors differentiated between calcium oxalate stone-forming dogs and clinically normal dogs, the relative supersaturation of urine with respect to calcium oxalate more clearly discriminated between the two groups.

Differences between the urine composition of breeds susceptible to calcium oxalate formation (Miniature schnauzer, Cairn terrier and Cocker spaniel) were identified, when compared to a breed with a low risk of calcium oxalate formation (Labrador retriever). These included a lower urine volume (per kg bodyweight), higher urine specific gravity and increased calcium concentration. Increased dietary moisture and sodium were shown to reduce the severity of these differences, thereby reducing the risk of calcium oxalate crystallisation in susceptible breeds. This thesis also demonstrated that both dietary calcium and oxalate have to be controlled in order to minimise the risk of calcium oxalate formation in susceptible breeds.

It was shown that in cats a moderately acidic urine pH within the range of 6-6.5 could minimise the risk of both calcium oxalate and MAP crystallisation compared to either a more acidic (5.8) or more alkaline urine pH (6.8). Finally, dietary potassium citrate supplementation, which is often recommended for the management of calcium oxalate formation in dogs had limited effects on urine analytes in the majority of dogs.

DECLARATION

This thesis represents my own unaided work, except where acknowledged below.

1. All urolith analyses were carried out by the staff at the Department of Chemical Pathology, University College London Hospitals, Windeyer Building, Cleveland Street, London, W1P 6DB.

2. All urine and diet analyses were carried out by the staff at the Central Nutritional Laboratory, Pedigree Masterfoods, Mill Street, Melton Mowbray, Leics., LE13 1BB.

3. In Chapter 4, the typical nutrient analysis program for human foods, and the risk factor program were supplied by Dr. W.G. Robertson, Institute of Urology and Nephrology, University College London.

Abigail Stevenson

2002

ACKNOWLEDGEMENTS

I would like to thank the many people, colleagues, friends and family, who have assisted me in any way over the past few years, both in collection and analysis of data and in the preparation of this thesis. Without their help and encouragement much of this work would have been difficult if not impossible.

Firstly, I would like to thank my two supervisors, Dr W.G. Robertson, from University College London, and Mr Peter Markwell from the WALTHAM™ Centre for Pet Nutrition, for their continued advice and support. I could not have wished for two more motivating and inspiring mentors!

Particular thanks must also go to Carrick, my three-year-old son and my husband Phil, firstly, for providing both the motivation to complete this thesis and for providing a good reason to go home in the evenings, and secondly, for coping so well without me over the last few months. Along the same lines, huge thanks must also go to my Mum and Dad for all the help and support they have shown in looking after Carrick.

I am also very grateful to the WALTHAM™ Centre for Pet Nutrition who provided all the funding for this thesis, and special thanks must go to Mr. Bill Fry for having the belief in my ability to deliver.

Thanks are also due to the many dogs and cats that participated in generating data for these studies, both at the WALTHAM™ Centre for Pet Nutrition and in the home environment. Close relationships were formed with the owners of many of the clinical cases that were monitored for up to a year, and their co-operation is very much appreciated.

Thanks must also go to the animal technicians at the WALTHAM™ Centre for Pet Nutrition who assisted in the collection of urine samples and the feeding of the dogs and cats, with a special thank you to Virginia Frere and Amelia Wagstaff. I am also grateful to Joy Stevenson for her comments on the statistical methods used to analyse data in this thesis. I must also thank the staff at the Department of Chemical Pathology, UCL Hospitals for the urolith analysis work, with particular thanks going to Dr. Ron Kasidas for his continued support.

Finally, the clinical cases included in this work were predominantly acquired from the Peoples Dispensary for Sick Animals in Nottingham and Leicester, and several other veterinary practices in England. I am indebted to the clinicians at these practices for providing the case material required for this study.

CONTENTS

FIGURES

TABLES

CHAPTER 1. GENERAL INTRODUCTION

"No stretch of chemical or physical imagination will permit so heterogeneous a group of compounds (as renal stones) to be ascribed to a common origin, or their disposition in kidney, ureter or bladder to be so uniformly charged to an identical cause" Howard Kelly

1.1 Definition of urolithiasis

Urolithiasis may be defined as the formation of sediment anywhere within the urinary tract and consisting of one or more of the poorly soluble crystalloids of urine. The formation of uroliths involves multiple physiological and pathological processes (Osborne *et al.*, 1996a). The term *urolith* is derived from the Greek *ouron* meaning urine, and *lithos* meaning stone (Osborne *et al.*, 1999a).

1.2 Historical perspective

The oldest urolith of human origin was found in the Egyptian tomb of a 16-year-old boy and dates from around 5000 years B.C. (Ellis, 1969). This urolith was 6.5 cm in diameter and composed of calcium phosphate and uric acid (Cummings, 1977). Although the removal of such uroliths was not performed in Egypt during this era, it was certainly practised in India and Pakistan from very early times, and is probably the oldest purely surgical procedure in human medicine (Michell, 1989). The symptoms of this disease are colourfully reported by many famous historical figures, for this condition affected all groups of society. Famous people who suffered from urolithiasis include Francis Bacon, Isaac Newton, Horace Walpole, Peter the

Great, Louis XIV, George IV, Oliver Cromwell, William Harvey, Samuel Pepys and Benjamin Franklin (Cummings, 1977).

Even as early as the mid 17th century there were many concepts concerning the aetiology of uroliths including overfeeding, familial predisposition and the composition of blood (Michell, 1989). In 1663, Rofink first classified uroliths according to their size, shape, surface and colour (Schneider, 1985), and by the 18th century common mineral components were recognised as uric acid, oxalic acid, cystine and organic material (Michell, 1989).

Although not so well documented, the oldest urolith found in an animal dates back, before the evolution of mankind, to the Upper Cretaceous age (Hesse *et al.*, 1998). The skeleton of a sea reptile, living around 80 million years ago, was found to contain a urolith composed of calcium carbonate and calcium phosphate. Later findings include uroliths found in the bladder of a cave bear and also in the kidneys and bladders of sacrificial animals (Hesse *et al.*, 1998). More recently stones have been found within the urinary tract of many mammals including humans, horses, donkeys, cows, sheep, rabbits, guinea pigs, chinchillas, cats, dogs (Osborne *et al.*, 1989b) and otters (Weber *et al.*, 2000). Invertebrates such as snails and insects are also known to form concrements in the faecal bladder and the malphigan tubes, which tend to be fatal (Hesse *et al.*, 1998).

The formation of uroliths specifically in cats and dogs is not a new phenomenon. In 1891 Ashmont said of bladder uroliths in dogs, "a cure was out of the question" (Ashmont, 1891). In 1925, Kirk described "retention of the urine" as a very common condition in cats (Kirk, 1925), and Blount also noted that seven different types of uroliths occur in cats, and that magnesium ammonium phosphate

was present in the majority of such deposits in alkaline urine (Blount, 1931). Urinary stones in vertebrates are of special importance; they constitute an economic burden, in both humans and farm animals, and are significant in companion animals such as dogs and cats, where duration and quality of life, rather than maximum growth or production, are of prime concern of the owner.

1.3 Anatomy of the canine and feline urinary tract

The urinary tract of all mammals is designed to dispose of certain body wastes in aqueous form, as urine, in contrast to the gastrointestinal system which eliminates waste from the body in semi-solid to solid form (Osborne and Clinton, 1986). Some of the components of urine may be less soluble than others, and under certain conditions crystal precipitation may occur. Crystallisation, involving the processes of crystal growth and aggregation, may lead to urolithiasis.

1.3.1 Kidney

Cats and dogs have two kidneys of approximately equal size with a smooth surface contour (Osborne and Fletcher, 1995). The kidneys of the dog are bean-shaped while those of the cat tend to be more spherical (Osborne and Fletcher, 1995). An average cat kidney is 4x3x2.5cm, weighing 10-15g (Nash, 1994). When viewed using ventrodorsal radiographs, the feline kidney ranges in size from 2.4 to 3.0 times the length of the second lumbar vertebra (Lee and Leowijuk, 1982). Dimensions of the canine kidney vary with body size; an average 10kg dog will have two kidneys each of approximately 5.5x3.5x2.5cm, weighing about 15g (Nash, 1990). When viewed using radiographs, the kidney length ranges between 2.5 and 3.2 the length of the second lumbar vertebra (Lee and Leowijuk, 1982). In both species the kidneys are

completely enveloped by the peritoneum and are loosely attached to the body wall, allowing movement during respiration, or changes in body position (Grandage, 1975). A hilus is located on the concave surface through which pass vessels, nerves and the renal pelvis (Osborne and Fletcher, 1995). The right kidney is usually more cranial than the left kidney, so that the cranial extremity of the left kidney lies opposite the hilus of the right (Miller, 1962). Urine, formed continuously, though at a variable rate, passes through collecting ducts within the kidneys into the pelvis.

The cut surface of a bisected kidney can be visually divided into the dark coloured cortex, which completely surrounds the lighter coloured medulla (Miller, 1962). The medulla can also be divided into an outer and inner section (Osborne and Fletcher, 1995). Both the cortex and medulla contain renal tubules, vessels and interstitial tissue. In humans, the medulla is divided into 8 to 18 striated conical masses, the renal pyramids (Tisher and Madsen, 2000). The base of each pyramid is positioned on the corticomedullary boundary and the apex extends towards the renal pelvis to form a papilla (Tisher and Madsen, 2000). In contrast, the cat and dog kidney has only a single pyramid and is termed unipapillate (Osborne and Fletcher, 1995; Tisher and Madsen, 2000).

1.3.2 Renal pelvis and ureter

The renal pelvis, located at the hilus of the kidney, is a thin walled distensible funnel-shaped structure that surrounds the innermost portion of the medulla. In humans two or three protrusions extend from the upper dilated end of the renal pelvis, called the major calyces (Tisher and Madsen, 2000). From each major calyx, minor calyces extend towards the papillae to drain the urine produced by each pyramidal unit (Tisher and Madsen, 2000). Unlike the situation in humans, in cats and dogs, the

renal pelvis does not contain calyces, the papilla is directly surrounded by the renal pelvis (Osborne and Fletcher, 1995; Tisher and Madsen, 2000). The renal pelvis collects urine from the papillary ducts and channels it into the ureters (Osborne and Fletcher, 1995). In dogs the renal pelvis has been estimated to hold less than 8ml of urine (Fischer and Sonda, 1979).

The ureter is a thick-walled fibromuscular duct that connects the renal pelvis to the bladder. From a functional perspective the renal pelvis and the ureter comprise a single unit which transports urine from the kidney to the bladder for storage (Osborne and Fletcher, 1995). There is no precise junction between the pelvis and the ureter. They do not significantly alter the composition of the urine produced by the kidneys, although they may produce substances that prevent microbial adherence to, and colonisation of their endothelial lining (Osborne and Fletcher, 1995). Collectively, the kidneys, renal pelvices and ureters form the upper urinary tract.

1.3.3 Urinary bladder and urethra

Micturition is a two-phase process of urine storage and intermittent appropriate voiding during which the urinary bladder and urethra serve as an interdependent unit (Osborne and Fletcher, 1995). During the storage phase of micturition the urinary bladder becomes a low-pressure expandable reservoir while the bladder neck acts as a high resistance outflow valve. During voiding, the urinary bladder becomes a high-pressure pump, normally activated by the distension of the bladder wall, while the urethra provides low resistance for channelling urine out of the body (Osborne and Fletcher, 1995). In normal healthy animals, voiding is very efficient and only 0.2-0.4ml of urine per kilogram bodyweight remains in the bladder (Moreau, 1982).

The urethras of the female dog and cat are similar while those of the male dog and cat are different. In both sexes, the terminal portion of the urogential system is dual purpose. In the female, the urethra opens on to the floor of the vagina, so urine passes through the terminal part of the female reproductive tract. In males, the terminal portion of the urethra, from the level of the prostate, carries both semen and urine (Briggs, 1994). In the male cat, the penis points backwards, and thus the urethra is comparatively short when compared to that of the male dog, which to point anteriorly, has to curve around the bony pelvis (Briggs, 1994). The difference in urethral and penile structure between male cats and dogs may indicate differences in function, such as facilitation of urine spraying as a territorial marker in male cats (Osborne and Fletcher, 1995), and increased chances of reproductive success since male cats only produce a small amount of semen (<1ml) (Sojka, 1980). In both cats and dogs it is likely that the male retains urine in the bladder periodically voiding small amounts to facilitate territory marking. The urethra of the female dog and cat has a larger, more uniform diameter than that of the male (Finco, 1995). Together, the bladder and urethra make up the lower urinary tract.

1.4. The physiology of urine formation

An understanding of the processes involved in the formation and elimination of urine is an essential prerequisite for investigations into the role of diet in urolithiasis. The kidneys have two main functions within the body. The primary function is to eliminate waste materials either ingested or produced by metabolism. The second function is to control the volume and composition of the body fluids (Guyton and Hall, 1996). For water and virtually all electrolytes in the body, the balance between intake (due to ingestion or metabolic production) and output (due to

excretion or metabolic consumption) is largely maintained by the kidneys. This regulatory function maintains a stable environment for cells allowing them to perform various essential activities (Guyton and Hall, 1996).

The kidneys perform their most important functions by filtering the plasma and removing substances from the filtrate at a variable rate, depending on the requirements of the body. The kidneys of a dog normally receive 10 to 20 % of cardiac output. This translates to a renal plasma flow of around 12ml per minute per kg body weight (Finco, 1995). Ultimately, unwanted substances are removed from the filtrate, and therefore from the blood, by excretion in the urine, while required substances are returned to the blood (Guyton and Hall, 1996).

The functional unit of the kidney is the nephron, an intricate epithelial tube that is closed at the beginning and open at the distal end (Randall *et al.*, 1997). Different species of animal have different numbers of nephrons. Estimates show that there are approximately 30,000 nephrons in a rat kidney, 190,000 per feline kidney, 400,000 per canine kidney, and between 0.4 and 1.2 million in the human kidney (Osborne and Fletcher, 1995; Tisher and Madsen, 2000). The nephron is composed of the glomerulus (a set of capillaries) surrounded by the Bowman's capsule, proximal tubule, loop of Henle, distal tubule, and collecting duct. Collecting ducts can collect urine from multiple nephrons (Figure 1.1).

In each nephron, formation of urine results from three basic processes: glomerular filtration, tubular reabsorption and tubular secretion (Figure 1.2) (Guyton and Hall, 1996). The walls of the glomerular capillaries are porous to water and small molecular weight molecules up to a molecular mass of 15,000 Daltons (Despopoulos and Silbernagl, 1991). About one quarter to one third of plasma water escapes from

the glomerular capillaries into the Bowman's capsule (Finco, 1995). The force for filtration is hydrostatic pressure generated by the contraction of the heart. Substances of > 80,000 Daltons are not normally filterable. Molecules of between 15,000 and 80,000 Daltons are partially filterable. Negatively charged substances, such as albumin, pass thorough less readily than neutral substances, probably because the negative charge on the walls of the glomerular filter exert a repellent effect on the anions (Despopoulos and Silbernagl, 1991). Some of the low-molecular weight substances are bound to larger proteins and cannot be filtered in this form (Despopoulos and Silbernagl, 1991).

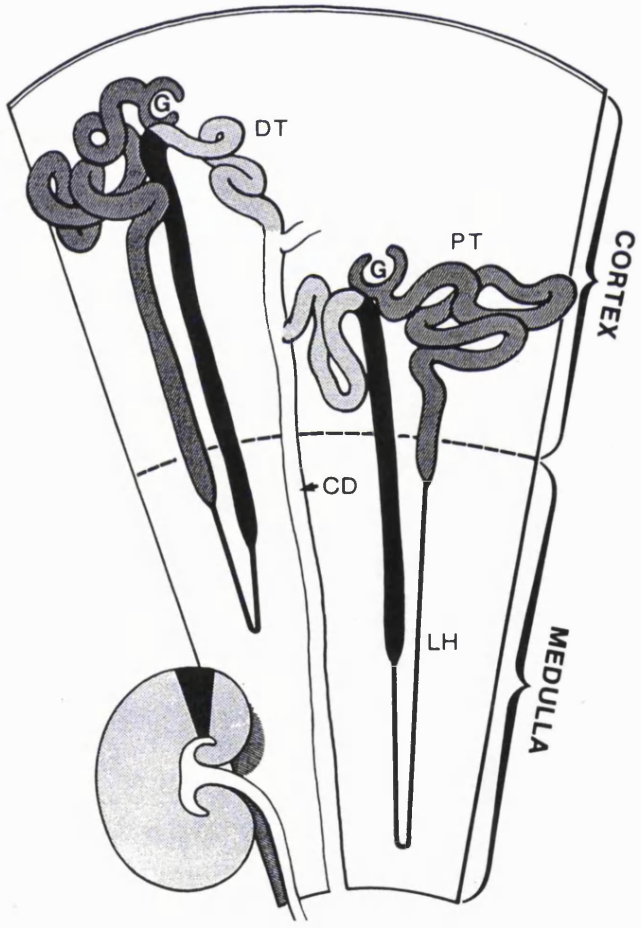


Figure 1.1 Schematic representation of two nephrons.

(G=glomerulus, PT = proximal tubule, DT = distal tubule, CD = collecting duct, LH = loop of Henle), (taken from Finco, 1995)

As fluid leaves the Bowman's capsule and passes through the tubules it is modified by reabsorption of water and specific solutes which are returned to the blood (Guyton and Hall, 1996). Tubular secretion, which refers to the transfer of material from the peritubular area into the lumen, is important in acid-base balance, potassium excretion and secretion of some organic ions (Finco, 1995). For each substance found in plasma a specific combination of filtration, reabsorption and secretion occurs, although secretion is not important for all substances (Figure 1.2). In turn, each process is largely regulated by requirements of the cat or dog.

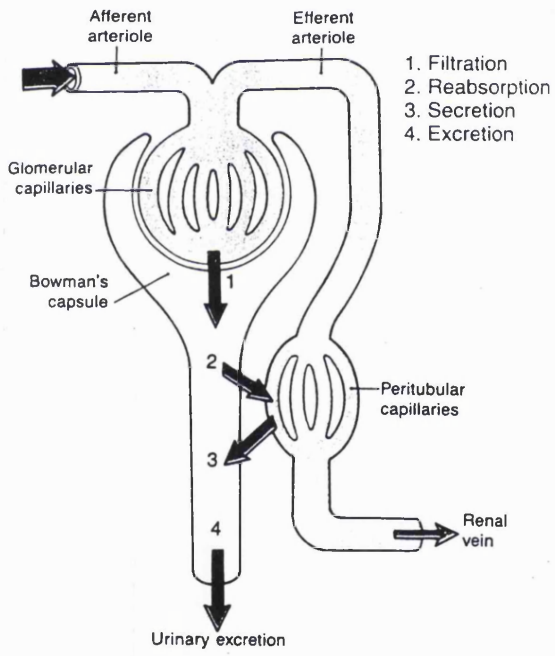


Figure 1.2 Basic kidney processes that determine the composition of urine.
(taken from Guyton and Hall, 1996)

Most substances that must be cleared from the blood, especially the end-products of metabolism, such as urea, creatinine, uric acid and urates, are poorly reabsorbed and are therefore excreted in large amounts (Guyton and Hall, 1996).

Creatinine may be used as a measure of glomerular filtration rate because creatinine appears at the same concentration in the filtrate and the plasma, and is not reabsorbed by the tubules (Finco, 1995). Electrolytes such as sodium ions, chloride ions and bicarbonate ions are highly reabsorbed so that only small proportions of the total amounts filtered appear in the urine. Certain nutritional compounds such as amino acids and glucose are completely reabsorbed in healthy animals and are therefore not usually found in the urine (Guyton and Hall, 1996).

Another function of the kidneys is to produce or convert hormones to their active form. Such hormones include erythropoeitin (to promote the production of red blood cells in bone marrow), calcitriol (25-OH vitamin D_3 is hydroxylated to form the most active form of vitamin D, 1,25-$(OH)_2$ vitamin D_3) and renin (required for generation of angiotensin II) (Finco, 1995). The kidney also responds to hormones including the anti-diuretic hormone (ADH), parathyroid hormone, growth hormone, aldosterone and atrial natriuretic peptide (Finco, 1995). Hormones can also be degraded and eliminated by the kidneys, including PTH, thyroid hormone, insulin and thyrotropic hormone (Finco, 1995).

1.5 Urolithiasis in cats and dogs

1.5.1 Prevalence

Patients with urological diseases account for approximately 2.1% of human hospital admissions in the UK (Robertson, 1993), and 3% of dogs seen at veterinary hospitals (Osborne *et al.*, 1995b). More specifically, the prevalence of urolithiasis in dogs was reported in Sweden and Norway to be between approximately 0.25 and 0.5% (Wallerstrom and Wagberg, 1992). Proportional morbidity rates were reported

as approximately 0.5% in North American veterinary colleges and between 0.5 and 1% in Germany (Hesse, 1990; Lulich and Osborne, 1995).

Urological diseases constitute a more common reason for the presentation of cats at veterinary hospitals, involving over 7% of the feline case load (Osborne *et al.*, 1995a). One study examining obstructed cats found 59% presented with urethral plugs and 12% with uroliths. In the remaining 29%, no specific cause could be ascertained (Osborne *et al.*, 1989a). A second study examining non-obstructed cats found urolithiasis in approximately 13% of cases while the underlying cause of urinary tract signs could not be identified in 64% of cases (Buffington *et al.*, 1997). Other causes of the disease included anatomical abnormalities (9%) and behavioural problems (9%). Typically, when causes cannot be identified, cats are said to have idiopathic lower urinary tract disease. The remainder of this thesis will focus upon urolithiasis and the influence of diet upon the formation and prevention of the most common types of urolith in cats and dogs.

1.5.2 Anatomical occurrence

Upper urinary tract stones are uncommonly reported in cats and dogs and the vast majority of uroliths (>95%) submitted for analysis are removed from the lower urinary tract (Osborne *et al.*, 1995b). The predominant form of stone disease in humans appears to have changed with time, particularly within the past 100 years. During the 19th century one of the major types of urolithiasis was that of bladder stones, particularly in children (Joly, 1929; Ellis, 1969). This form of the disorder has now largely disappeared from the Western Europe, although it still occurs in some developing countries (Andersen, 1973; Thalut *et al.*, 1976). Within the last 30 years, however, there has been a reduction in the number of paediatric bladder stones in

most of the affected countries. In contrast with the decline of bladder stones, the prevalence of upper urinary tract stones (nephroliths) has been increasing in most parts of the world. These changes in humans have been linked to changes in the quality and type of nutrition (Andersen, 1973; Peacock and Robertson, 1979).

The reason for this difference in anatomical distribution between species is speculative. It is possible, but unlikely, that there is a marked underdiagnosis of nephrolithiasis in dogs and cats. It seems more likely that there is a real species difference. It has been hypothesised that this may, at least in part, relate to the positioning of the kidney and bladder relative to gravity in quadrupeds and bipeds (Markwell *et al.*, 2000). Anatomical differences in the structure of the kidney, and in particular the absence of renal pyramids and calyces in the dog and cat kidney, may also contribute to observed anatomical differences in the occurrence of urolithiasis.

1.5.3 Mineral composition of uroliths

Uroliths can range in size from sand-like material to large individual stones that may grow to fill the entire cavity in which they form (White, 1996). Around twenty crystalline substances can occur in uroliths across all species, made up of various mineral combinations of a few chemical substances, including phosphate, calcium, oxalate, urate, cystine, carbonate and silica (White, 1996). Uroliths that form in cats, dogs and humans can be grouped into four main mineral types, namely urate (including ammonium urate, sodium urate and uric acid), cystine, magnesium ammonium phosphate and calcium (calcium oxalate and calcium phosphate) (Figure 1.3). Uroliths composed predominantly of urate or cystine occur infrequently in dogs and cats, each making up less than 10% of uroliths analysed at one centre in the USA,

while struvite (magnesium ammonium phosphate) and calcium-containing uroliths are the two most prevalent mineral types found in cats and dogs (Osborne *et al.*, 2000b).

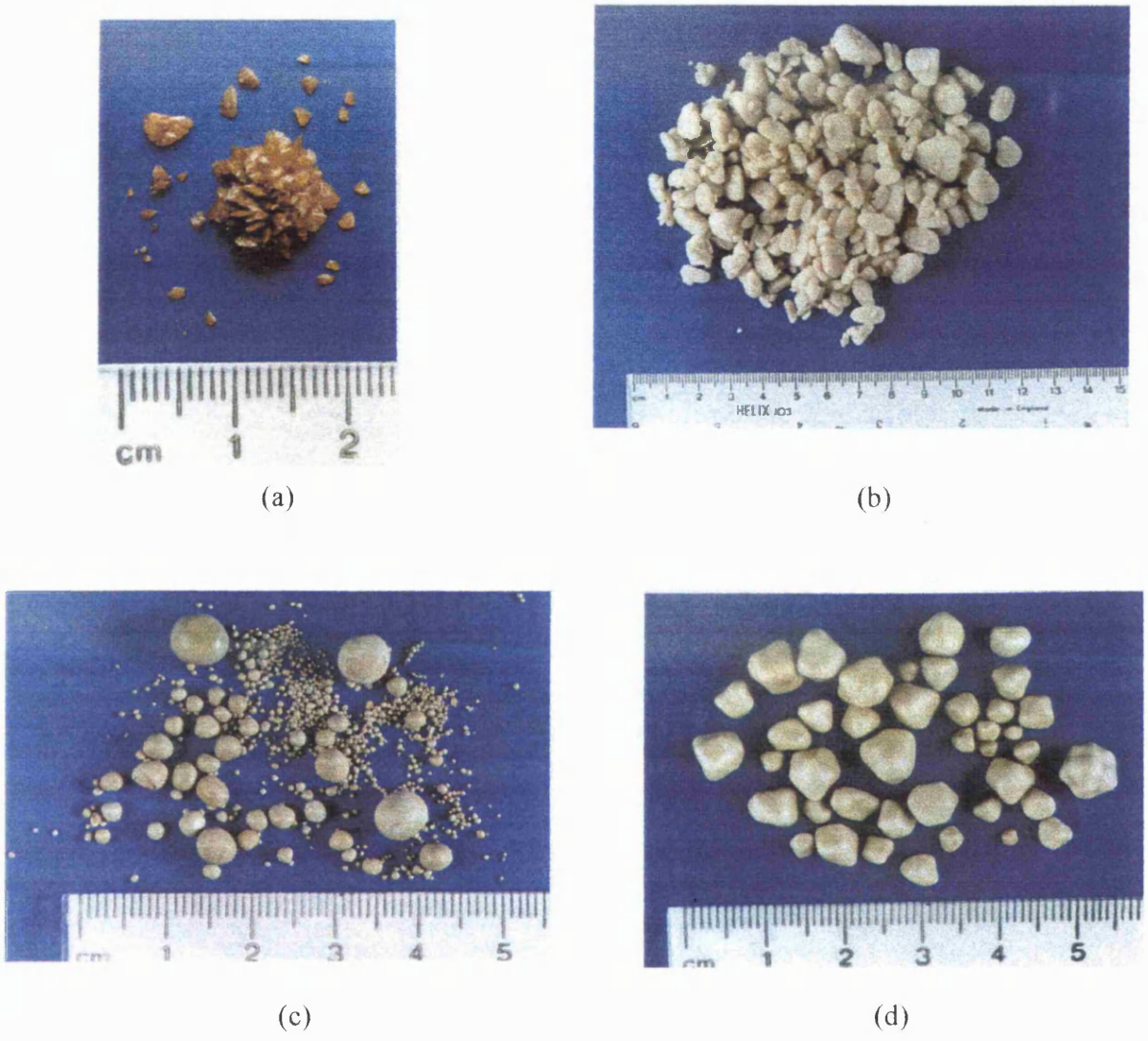


Figure 1.3 The typical appearance of the four most common urolith types found in cats and dogs

(a) calcium oxalate; (b) magnesium ammonium phosphate; (c) ammonium urate; (d) cystine (all measurements in centimetres)

1.5.3.1 Cystine urolith formation

Cystinuria is a congential disorder characterised by the excretion of excessive amounts of cystine, lysine, ornithine and arginine (Treacher, 1962). In normal dogs cystine is freely filtered at the glomerulus, and most is actively reabsorbed in the proximal tubule. Cystinuric dogs reabsorb a much smaller proportion of the amino acid from the glomerular filtrate and some may even have net cystine excretion (Hoppe, 1994). The main manifestation of this abnormality is the formation of cystine uroliths. Dietary intervention plays a relatively minor role in the management of this type of stone disease, and although cystine solubility can be enhanced by inducing an alkaline urine pH, the solubility does not increase significantly until the urine pH is above 7.5 (Dent and Senior, 1955).

Chemical modification of the cystine molecule into a more soluble form which can be excreted safely in the urine, using drugs such as D-penicillamine (DP) or 2-mercaptopropionylglycine (2-MPG), is usually required to effectively prevent recurrence in most individuals (Figure 1.4) (Hoppe, 1994). Although DP is effective in preventing formation, and may also assist with dissolution of cystine uroliths, this treatment is accompanied by frequent complications that may limit its use. In humans around 50% of patients will suffer side-effects including skin rashes, neutropenia, thrombopenia, nephrotic syndrome and fever, while severe proteinuria is experienced in 10-15% of patients (Dahlberg *et al.*, 1977). In dogs, the most common side effect is vomiting (Osborne *et al.*, 1995b). 2-MPG is related to DP, but has a higher oxidation-reduction potential and may, therefore, be more effective in the disulphide exchange reaction (Figure 1.4) (Keene, 1992). In addition, 2-MPG also tends to result in fewer adverse side effects.

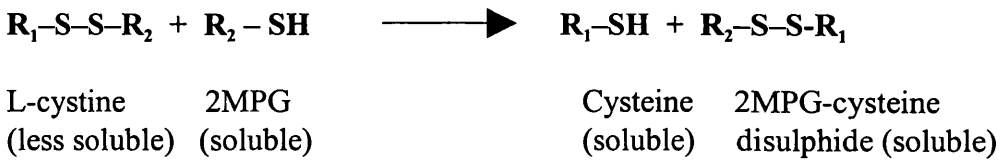


Figure 1.4 The effect of 2- mercaptopropionylglycine (2-MPG) on cystine (Hoppe, 1995)

1.5.3.2 Uric acid / urate urolith formation

In normal healthy humans, dogs and cats, uric acid is one of several products of purine nucleotide metabolism (Figure 1.5) (Bartges *et al.*, 1999). In turn, uric acid is metabolised by hepatic uricase to allantoin, which is excreted by the kidneys and is readily soluble in the urinary environment (Hoppe, 1994). Uroliths containing urate can occur in a number of different forms. In dogs and cats, ammonium urate is the most common form, followed by sodium urate and rarely uric acid. In humans, the form in which urate occurs is largely dependent upon the urine pH during formation. Because it has a dissociation constant pH of 5.46 at 37°C, uric acid tends to form in urine with a pH of <5.5, and within the normal pH range for humans this mineral tends to exist as a mix of the undissociated ion and free urate (Robertson, 1993).

Humans forming uric acid uroliths usually have a very low urine pH either because their ammonium ion excretion is low, the titratable acidity is high or they have hyperuricosuria; the latter form is common in patients with gout (Wyngaarden and Kelly, 1999). Administration of substances such as sodium bicarbonate and potassium citrate, to increase the urine pH to 6.2-6.5 reduce the risk of uric acid formation in humans (Metcalfe-Gibson *et al.*, 1965; Pak, 1987). When human subjects excrete large amounts of uric acid (hyperuricosuria), precipitation can result

in formation of uroliths of mixed mineral composition. In humans uric acid co-precipitates most commonly with calcium oxalate (Coe *et al.*, 1976).

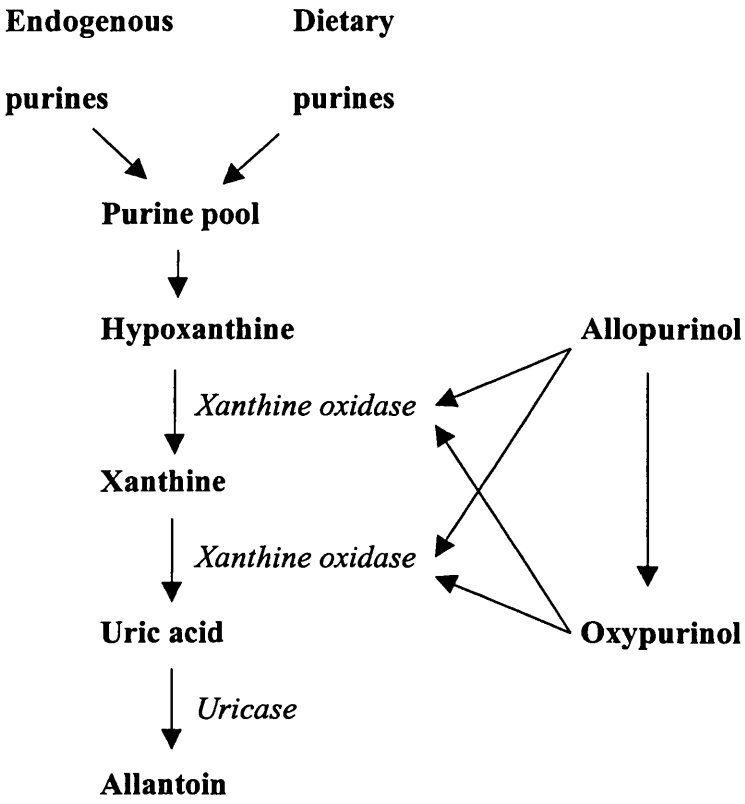


Figure 1.5 Purine metabolism and site of allopurinol action
(taken from Bartges, Osborne, Lulich, Kruger *et al*, 1999)

Two other forms of urate can occur, ammonium urate (the monobasic ammonium salt of uric acid) or sodium urate (the monobasic sodium salt of uric acid). Studies on the solubility of uric acid have shown that under certain conditions there is an upper limit to the total uric acid that can be dissolved with increasing urine pH. In alkaline urine, sodium urate, or ammonium urate will precipitate in preference to uric acid, depending upon the relative urinary concentrations of ammonium and sodium (Teotia and Sutor, 1971; Pak *et al.*, 1977b). This set of conditions lead to ammonium

urate formation within the bladders of boys in developing countries (Thalut *et al.*, 1976). They may also account for renal ammonium urate formation in adults who use excessive amounts of laxatives (Dick *et al.*, 1990), and may be a minor component in stones from patients with urinary tract infections involving a urease-splitting organism (Klohn *et al.*, 1986).

In dogs and cats, the factors determining the form in which urate occurs are not clearly defined, and often stones containing both uric acid and ammonium / sodium urate form. The largest group affected by uric acid / urate urolithiasis is the Dalmatian dog, which only converts 30-40% of uric acid to allantoin (Porter, 1963). This genetically-inherited defect in Dalmatians is thought to be due to impaired transport of urate across the hepatocyte cell membrane, as uricase concentrations tend to be normal (Giesecke and Tiemeyer, 1984). In addition, intestinal uptake of hypoxanthine and urate is delayed (Briggs and Harley, 1986), and renal reabsorption of urate in the proximal tubule is reduced (Roch-Ramel *et al.*, 1976). Dogs and cats with portal-systemic shunts are the second largest group affected (Rothuizen *et al.*, 1982). Ammonia is generated by bacterial action on amino acids and urea in the colon (Rothuizen *et al.*, 1982). After intestinal absorption, normal hepatic function converts most portal blood ammonia to urea so that systemic blood ammonia concentrations remain low. Portal-systemic vascular shunts resulting from congenital malformation or hepatic cirrhosis lead to reduced extraction of ammonia from the portal blood and systemic blood ammonia concentrations become elevated (Hardy and Klausner, 1983). Normal hepatic conversion of urate to allantoin is reduced and plasma urate concentrations are increased in affected dogs (Hardy and Klausner, 1983). Together these factors result in increased renal excretion of ammonia and

urate, with the subsequent increased risk of urate urolith formation (Senior, 1992). In some cases, urate or uric acid uroliths form sporadically in both cats and dogs for reasons that as yet remain unknown (Senior, 1992). The primary goal for the management of urate urolithiasis is to reduce urinary ammonium and urate activity (Senior, 1992). In humans, low purine diets have been found to reduce urate production (Bien *et al.*, 1953). Severe purine restriction has also been found to reduce urinary urate excretion in both healthy dogs and Dalmatians (Senior, 1992). However, allopurinol, a drug which decreases the conversion of xanthine to uric acid (as does the metabolic derivative, oxypurinol), by inhibiting the enzyme xanthine oxidase, may also be necessary to prevent recurrence (Figure 1.5) (Elion *et al.*, 1966; Senior, 1992; Robertson, 1993; Osborne *et al.*, 2000b).

1.5.3.3 Magnesium ammonium phosphate and calcium urolith formation

Calcium salts (phosphate and oxalate) and magnesium ammonium phosphate (struvite), [MAP] are the most common minerals found in uroliths of cats, dogs and humans (Robertson, 1993; Osborne *et al.*, 1995b). In humans, calcium-containing stones predominate with almost one half made up of pure calcium oxalate and the remainder consisting of a mixture of calcium oxalate and calcium phosphate or uric acid (or occasionally ammonium urate) (Robertson, 1993). Historically, MAP has been the most common type of urolith found in both cats and dogs. However, calcium oxalate-containing uroliths appear to have become more common in both species recently, at least in North America. Recent data from the USA suggest that calcium oxalate-containing uroliths may now be more important numerically than MAP in cats, although the latter still predominate in dogs (Buffington *et al.*, 1997; Osborne *et al.*, 1999a; Osborne *et al.*, 2000b). Calcium oxalate uroliths may be pure, but more

commonly present in combination with variable amounts of calcium phosphate, or less commonly MAP or ammonium urate (Osborne *et al.*, 2000b).

The factors that influence the formation of the most common uroliths found in cats and dogs will be discussed in further detail. However, in order to ascertain why certain minerals may precipitate in urine, while others do not, it becomes important to understand some of the physico-chemical aspects of urolithiasis.

1.6 Theories of urolith formation

Examination of the solubilities of the various constituents of uroliths suggests that formation essentially arises from the precipitation of sparingly soluble minerals within the urinary environment (Robertson, 1993). The process of urolith formation should, therefore, be explicable in terms of the chemical laws of nucleation, crystal growth and aggregation, although other aggravating factors such as anatomical abnormalities, or damage to the urinary epithelium may also be involved (Robertson, 1993).

Based upon these processes, many theories have been advanced to account for the formation of uroliths within the urinary tract of humans, focusing particularly on the formation of calcium oxalate within the human kidney, although none completely explains why some individuals form uroliths and others do not. In some individuals, as with cats and dogs, one primary factor such as a urinary tract infection or genetic disorder may be the cause of urolith formation. However, in the majority of individuals the disorder appears to be multifactorial in origin with no one factor being the obvious cause of urolith formation (Robertson, 1993; Ryall, 1993).

The essential feature of a theory of urolith formation is that it should account for both the formation and retention of a critical nucleus within the urinary system, which

then enlarges by growth and agglomeration until it results in clinical signs such as haematuria, dysuria, increased frequency of urination, and pain (Robertson and Nordin, 1976; Robertson, 1993). Additional signs often observed in cats and dogs include changes in urination pattern or behaviour, and incontinence (Allen and Kruger, 2000; Osborne *et al.*, 2000b; Osborne *et al.*, 2000c). Two main groups of theories have been proposed to account for urolith formation, based upon the above principles. Finlayson (1977, 1978) classified these as 'free-particle' and 'fixed-particle' models of urolith formation (Finlayson, 1977; Finlayson, 1978).

1.6.1 'Free-particle' theory of urolith formation

This theory is based upon increased excretion of sparingly soluble minerals, leading to precipitation of minerals within the urinary tract. This process involves a nucleation phase, where crystals are formed by either heterogeneous or homogeneous nucleation, a second phase where the crystals grow and/or aggregate to form larger particles, and a third phase, where the particles become large enough to become trapped in narrow segments within the urinary tract (Vermeulen and Lyon, 1968). The final phase involves growth of the trapped particles to form a urolith.

In humans, this model is supported by the fact that in most forms of urolithiasis, the excretion of at least one mineral component of the urolith is elevated (Robertson *et al.*, 1978; Coe and Favus, 1980; Halabe and Sutton, 1987; Smith, 1991). Since all minerals involved in urolith formation are sparingly soluble under certain conditions, an increase in the concentrations of these minerals will increase the risk of precipitation and crystallisation (Robertson, 1993). It has also been shown that human stone-formers pass more crystals in their urine than healthy subjects (Robertson *et al.*, 1969; Robertson *et al.*, 1971), the crystals within these individuals

tend to be larger and more aggregated (Robertson *et al.*, 1969; Robertson *et al.*, 1971; Kok *et al.*, 1990b); and periods of abnormal crystalluria can trigger off urolith formation in both humans and rats (Vermeulen *et al.*, 1966; Robertson *et al.*, 1969; Werness *et al.*, 1981). In addition, Robertson (1976) observed that the severity of the disease, in terms of the number of urolith episodes, was proportional to the percentage of abnormally large crystals and aggregates within the patient's urine (Robertson and Nordin, 1976). Although crystalluria is commonly observed within the urine of cats and dogs with uroliths, the correlation between the type of urolithiasis, severity of the disease and the observed crystalluria has never been quantified.

The 'free-particle' theory has also been demonstrated theoretically, within the bladder. Finlayson described the bladder as a seeded batch crystalliser, demonstrating empirically that the more frequently urine is voided, the less chance of urolith formation (Finlayson, 1977; Finlayson and Reid, 1978). This observation becomes highly relevant in cats and dogs where bladder stones prevail over uroliths within the kidneys.

1.6.2 Fixed-particle' theory of urolith formation

A number of authors have cast doubt over the 'free-particle' theory of urolith formation, because, through theoretical calculations, they believe the probability of forming a crystal large enough to be trapped within the upper urinary tract is extremely low (Finlayson, 1977; Finlayson, 1978; Finlayson and Reid, 1978). Furthermore, although the supersaturation of urine tends to be greater in individuals that have formed uroliths, healthy individuals can also produce urine that is oversaturated. Supersaturation, measured in isolation, may therefore be an insufficient measure for completely explaining urolith formation in most cases (Ryall,

1993). Finlayson believed that in humans, uroliths were initiated when crystals became attached to the renal epithelium, or that crystal nucleation commenced at a fixed site within renal parenchyma cells or on the tubular cell surface. Lesions within the renal parenchyma, called Randall's plaques were thought to offer an anchoring or nucleating site for crystals (Randall, 1940), although it is now clear that not all humans forming uroliths have these lesions (Low and Stoller, 1997).

However, the 'fixed-particle' model makes a number of assumptions. It assumes that urinary flow is uniformly laminar, implying a lack of turbulence. This would exclude the possibility of crystals colliding and thereby aggregating with each other. A number of studies have demonstrated that there are sites within the kidney where flow is reduced relative to the main stream (Graves, 1982; Schulz *et al.*, 1987; Kok and Khan, 1994). Certainly, once urine reaches the bladder, flow cannot be described as laminar. This theory also ignores possible effects of viscous drag and gravity on urinary flow. In humans, urine has to flow against gravity in upwardly draining tubules within the kidney. This can delay or stop the flow of crystals allowing crystal growth and agglomeration to occur, increasing the probability of a critical particle being formed (Robertson and Peacock, 1985). Gravity will have different, yet still significant, influences over the urinary flow in cats and dogs. This may be one of many factors influencing the differences in anatomical location of uroliths between humans, cats and dogs (Markwell *et al.*, 2000).

1.6.3 Modifiers of crystallisation

For many years it has been considered that a number of components of urine may act as inhibitors or promoters of crystal nucleation, growth or aggregation (Baker *et al.*, 1996). However, it remains unclear which modifiers, of those identified so far,

retain their potency in whole urine and whether or not deficiencies (of inhibitors) or excesses (of promoters) are really important in generating an increased risk of stone-formation (Robertson, 1993). A large number of researchers have carried out extensive work in this area, focusing predominantly on calcium oxalate, and new urinary compounds are still being identified today. It is apparent that very little work has been reported specifically relating to crystallisation modulators in cat or dog urine.

1.6.3.1 The 'Promoter' theory

Promoters are thought to accelerate the rate of nucleation and/or aggregation of calcium oxalate crystals. It has also been suggested that human stone-formers excrete more promoters than normal subjects (Boyce *et al.*, 1962). A typical human calcium oxalate urolith contains significant quantities of macromolecules that may make up 2-3% of the urolith by weight, and are spread throughout the structure (Boyce, 1968). These are primarily a mix of poorly defined mucoproteins (King and Boyce, 1957), glycosaminoglycans (Roberts and Resnick, 1986), albumin and globulins (Boyce, 1968), collectively known as the organic "matrix" (Ryall, 1997). These macromolecules may, if present within the urine, cause the heterogeneous nucleation of crystals at a lower degree of supersaturation than would be required for homogeneous nucleation. Furthermore, their precipitation upon the nucleated crystals is perceived to constitute both a bridge and an adhesive for binding crystals together to form large aggregates, and providing a platform for deposition of more minerals (Ryall, 1993).

Most work has concentrated on the role of the main mucoprotein present in urine, Tamm-Horsfall mucoprotein (THM). However, THM exists in two forms. In

its relatively stable, monomeric form THM behaves as a weak-moderate inhibitor of calcium oxalate and calcium phosphate crystal growth and agglomeration, while in the polymerised form THM acts as an active promoter (Schnierle *et al.*, 1992). One study showed that the effect of THM in urine depends upon the methodology used to analyse it, concluding that under physiological conditions THM would be expected to inhibit calcium oxalate crystal aggregation, and would only function as a promoter under conditions of extreme dehydration (Grover *et al.*, 1990). Some workers have shown differences in the structure of THM in urine isolated from the urine of healthy humans and those that form calcium oxalate uroliths (Schnierle *et al.*, 1992; Knorle *et al.*, 1994). However, other studies have shown no structural differences in THM between healthy and stone-forming humans (Grover and Resnick, 1995). THM has been isolated from cat urine, and was found to occur at higher concentrations in urine from cats with a history of urolithiasis than normal healthy cats, although the clinical significance of this observation has never been explored (Rhodes *et al.*, 1992; Rhodes *et al.*, 1993).

However, most human studies have failed to find differences in excretion rates of THP between healthy individuals and those that form calcium oxalate stones (Bichler *et al.*, 1976; Samuell, 1979), and some claim that stone-formers have lower values (Wikstrom and Wieslander, 1981). Even where an increased excretion rate was observed calcium oxalate precipitation was not promoted (Resnick *et al.*, 1982). Thus, the role of promoters and, more specifically that of THM in the formation of stones, is at best controversial. In 1993, Robertson concluded that the role of promoters in urolith formation is far from clear and their presence in urinary stones may even be adventitious.

Heterogeneous crystal nucleation may also be promoted by the presence of more than one crystal type within the urine; for example, calcium oxalate crystallisation may be promoted by the presence of calcium phosphate or uric acid crystals. These crystals may act as a trigger for heterogeneous crystallisation leading to the formation of a urolith of mixed composition (Ashby *et al.*, 1999; Hojaard *et al.*, 1999). This process is known as epitaxy (Ryall, 1993).

1.6.3.2 The 'Inhibitor' theory

An alternative theory of stone-formation suggests that there may be an absence or deficiency of certain protective substances in the urine of stone-formers, which are present in higher concentrations in the urine of normal individuals (Robertson, 1993). A large number of urine constituents claimed to have inhibitory properties against calcium oxalate, and to some extent calcium phosphate, have been isolated and partly characterised in human urine during the passed twenty years, although little work has concentrated on any of the other urolith types. These can be divided into two groups; the large macromolecular polyanions and micromolecular polyanions.

1.6.3.2.1 Low molecular weight polyanions

Although a number of low molecular weight polyanions have been reported to act as crystallisation inhibitors, citrate, magnesium and pyrophosphate are currently of greatest interest to researchers within the field of urolithiasis in humans (Table 1.1). Magnesium and citrate occur in human urine in millimolar concentrations, while pyrophosphate occurs in micromolar amounts (Robertson, 1993). Citrate and magnesium are known to be moderate inhibitors of both calcium phosphate and calcium oxalate crystallisation. They act partly through complexation with calcium ions (in the case of citrate) and oxalate and phosphate ions (in the case of

magnesium), and partly by adsorbing to the surface of crystals, where they behave like a 'crystal poison'. Pyrophosphate occurs in much smaller amounts, so can only function as a 'crystal poison' (Robertson, 1993). Their potential roles in preventing the recurrence of calcium oxalate urolithiasis are discussed below.

Table 1.1 Low molecular weight polyanion inhibitors of calcium oxalate formation (adapted from Robertson, 1993)

Inhibitor	**Strength and mechanism of inhibition**			
	Homogeneous nucleation	**Heterogeneous nucleation**	**Crystal growth**	**Crystal aggregation**
Magnesium	Moderate (complexation)	Moderate (complexation)	Moderate (complexation & adsorption)	No effect
Citrate	Moderate (complexation)	Moderate (complexation)	Moderate (complexation & adsorption)	Weak (charge repulsion)
Pyrophosphate	No effect	Strong (adsorption)	Strong (adsorption)	Strong (charge repulsion)

Citrate

Decreased urinary citrate has been reported as a major risk factor in the pathogenesis of calcium oxalate uroliths, with hypocitraturia being present in between 30 and 50% of human calcium oxalate stone-formers (Smith *et al.*, 1979; Nicar *et al.*, 1983; Cupisti *et al.*, 1992). Dietary potassium citrate has been used for the past decade in humans to help prevent the recurrence of calcium oxalate uroliths. A number of authors have shown that the concentrations of citrate in the urine can be manipulated through oral dosing with potassium citrate, and in some instances a relative reduction in the risk of urolith recurrence was observed, although the majority

of subjects studied were hypocitraturic (Sakhaee *et al.*, 1983; Pak *et al.*, 1985; Pak, 1987; Berg *et al.*, 1992; Caudarella *et al.*, 1996; Lindberg *et al.*, 1996). The beneficial effects are thought to be caused primarily by the alkalinising properties of dietary potassium citrate, although they may be partially attributable to concurrent advice for the patient to increase fluid intake (Robertson, 1993).

Dietary potassium citrate has also been advocated in the prevention of recurrence of calcium oxalate uroliths in dogs, although hypocitraturia has not been identified as a risk factor in dogs with calcium oxalate urolithiasis (Osborne *et al.*, 1986b; Lulich *et al.*, 1992a; Lulich and Osborne, 1995; Osborne *et al.*, 1995b). Potassium citrate has been permitted as a food preservative for all species including the dog, since 1991 and has been authorised for use as a urinary pH modifying substance in pet food since 1994 within Europe (Petfood Manufacturers Association, 1995). Within the United States, potassium citrate is listed as an acceptable mineral supplement in the American Association of Feed Control Officials Handbook, 1998, although no similar directives exist. Inclusion amounts of 0.2 to 0.5% in canned food and 1 to 2% in dry food have been recommended for modifying urine pH (Petfood Manufacturers Association, 1995). This amount of potassium citrate corresponds to approximately 150 mg/kg/d and, in dogs, has been reported to cause an increase in urine pH when fed to normal healthy dogs. However, no consistent increase in urinary citrate excretion was found, and no attempts were made to assess the risk of calcium oxalate formation during supplementation (Osborne *et al.*, 1986b; Lulich and Osborne, 1995; Osborne and Fletcher, 1995). It is not yet clear why there appears to be a difference between humans and dogs in their response to potassium citrate supplementation.

No research has been conducted examining the effect of potassium citrate on the risk of calcium oxalate formation in cats, although some authors have recommended its use based upon extrapolation of data from human and limited dog studies (Allen and Kruger, 2000; Osborne *et al.*, 2000c).

Magnesium

Urinary magnesium prevents calcium oxalate nucleation by complexing oxalate in the urine, to form soluble magnesium oxalate, thus reducing the concentration of supersaturation with respect to calcium oxalate, and the tendency towards nucleation (Meyer and Smith, 1975; Li *et al.*, 1985; Kohri *et al.*, 1988). Hypomagnesuria or an increased urinary calcium/magnesium ratio has been found in a number of human subjects with calcium oxalate urolithiasis, supporting the hypothesis that magnesium may play a role in preventing this disease (Smith *et al.*, 1979; Johansson *et al.*, 1980). In addition, dietary magnesium supplementation has been shown to increase urinary magnesium and to reduce the risk of recurrence when given to human stone-formers (Johansson *et al.*, 1980; Hallson *et al.*, 1982; Khan *et al.*, 1993).

Hypomagnesuria has not been identified as a risk factor for calcium oxalate formation in dogs or cats. Consequently, urinary magnesium and the potential benefits of dietary magnesium with respect to calcium oxalate formation have not been studied in these species. In addition, magnesium is a component of MAP uroliths and increased dietary magnesium is a risk factor for struvite formation (See Section 1.7.1.5). Supplementary dietary magnesium, therefore, may not be an option for use in the prevention of calcium oxalate stone-formation in cats and dogs.

Pyrophosphate

At concentrations found in normal human urine, pyrophosphate is a weak inhibitor of calcium oxalate crystal growth and agglomeration (Robertson, 1993). However, this low molecular weight polyanion does act as a potent 'crystal poison' at higher concentrations (Robertson and Scurr, 1986). Reduced urinary excretion of pyrophosphate has been observed in a few human calcium oxalate stone-formers (Russell *et al.*, 1964; Singh *et al.*, 1985), although most researchers believe that this is not a common contributing factor to the disease (Fleisch, 1978; Schwille *et al.*, 1988).

Urinary pyrophosphate can be increased through dietary manipulation. However, pyrophosphate given orally to human subjects appeared to be hydrolysed by acid in the stomach and did not appear in the urine (Fleisch *et al.*, 1964). In contrast, administration of inorganic orthophosphate in a neutral or mildly alkaline form has been associated with increased urinary pyrophosphate in humans as a result of competition between orthophosphate and pyrophosphate for reabsorption sites in the renal tubule. This leads to decreased reabsorption and renal leak of pyrophosphate (Fleisch *et al.*, 1964). In other studies, a reduction in calcium oxalate supersaturation was observed, and the risk of urolith formation was reduced during dietary orthophosphate supplementation (Burdette *et al.*, 1976; Pak *et al.*, 1978b). However, most orthophosphate preparations used in humans resulted in increased urinary phosphate and an alkaline urine pH, leading to an increased risk of struvite or calcium phosphate crystallisation (Pak *et al.*, 1978a). No studies have been reported that either recommend or quantify the effects of increasing urinary pyrophosphate on the risk of calcium oxalate prevention in dogs or cats.

1.6.3.2.2 Macromolecular polyanions

Recent research within the human field has concentrated on the role of macromolecules in urolith formation. It has been argued that the organic matrix may act as a heterogeneous nucleator promoting, rather than inhibiting crystallisation, or may sequester calcium and oxalate ions creating a high concentration of calcium oxalate (Section 1.6.3.1 The 'Promoter' theory). However, recent studies, conducted using the organic matrix of calcium oxalate crystals freshly precipitated from urine, have ensured that no macromolecules arising from cellular injury are incorporated into the crystals. This method allows the study of macromolecules occurring naturally in urine (Ryall, 1997). Many of these macromolecules have been shown *in vitro* to have inhibitory properties (Table 1.3). These include glycosaminoglycans (GAGs) (Hesse *et al.*, 1991; Ryall *et al.*, 1991), RNA-like molecules (Schrier *et al.*, 1979), non-polymerised THM (Grover *et al.*, 1990; Hess, 1994), nephrocalcin (Nakagawa *et al.*, 1981; Asplin *et al.*, 1991; Coe *et al.*, 1991), osteopontin (also known as uropontin) (Hoyer, 1994), prothrombin fragment F1 (originally called crystal matrix protein) (Ryall *et al.*, 1995), bikunin (Atmani *et al.*, 1993), fibronectin (Tsujihata *et al.*, 2000), and calprotectin (Umekawa and Kurita, 1994).

Limited research has been conducted examining the macromolecules present in the urine produced by cats and dogs, and their potential role in urolith formation. Nephrocalcin has been found in urine produced by a number of vertebrate species including the chicken, dog, mouse, pig, cow, rabbit, sheep and human (Nakagawa *et al.*, 1991). In humans, nephrocalcin has been shown to have several molecular abnormalities in calcium oxalate stone-formers, when compared to normal healthy subjects (Nakagawa *et al.*, 1985). This macromolecule has been shown to

demonstrate the same biochemical differences between healthy dogs and those with calcium oxalate uroliths, as those found in humans (Lulich *et al.*, 1999b). However, it remains unclear as to whether any sort of dietary intervention could influence the concentrations of these macromolecules in urine. Thus, within the context of this thesis, macromolecular inhibitors will not be investigated further.

Table 1.2 Macromolecular inhibitors of calcium oxalate formation

(adapted from Robertson, 1993*; Worcester, 1996**, Dussol and Berland, 1998***)

Inhibitor	**Strength and mechanism of inhibition**			
	Homogeneous nucleation	**Heterogeneous nucleation**	**Crystal growth**	**Crystal aggregation**
Non – polymerised THM*	No effect	Promotes nucleation (?)	Weak (complexation & adsorption)	Moderate (charge repulsion)
Nephrocalcin*	-	-	Moderate	Moderate-strong (charge repulsion)
GAGs*	No effect	Moderate (complexation & adsorption)	Weak to moderate (complexation & adsorption)	Very strong (charge repulsion)
RNA*	-	-	Strong (adsorption)	Strong (charge repulsion)
Prothrombin F1**			Moderate	Moderate
Calprotectin***			Moderate	
Bikunin***			Strong	Strong
Osteopontin (Uropontin)**	Moderate	Moderate	Moderate	Moderate

1.6.4 The concept of urinary supersaturation

Regardless of how urolith formation occurs, all researchers within the field of urolithiasis agree that urine must be supersaturated to some degree with at least one stone-forming salt for urolith formation to occur (Robertson *et al.*, 1968; Robertson and Nordin, 1976). When a sparingly soluble salt is allowed to dissolve in urine, dissolution will occur until the rate of dissolution comes into equilibrium with rate of return of the dissolved material to the solid (Robertson, 1993). The concentration of the dissolved substance at equilibrium is referred to as the solubility of the salt under the conditions described (Robertson, 1993). It is usual to express this solubility as the product of the constituent ion concentrations rather than the absolute solubility of the material in mass per unit volume (Robertson, 1993). The product of the constituent concentrations, each raised to the appropriate power corresponding to the number of ions in the single 'molecule' of the salt, is known as the solubility product (K_{sp}) (Robertson, 1993). This parameter is influenced by a range of factors including temperature, urine pH ionic strength, and ion complexation. However, correction for the last two parameters allows the thermodynamic K_{sp} to be determined for each mineral type (Werness *et al.*, 1985). This is a true constant under a wide range of conditions.

In normal, healthy humans urine tends to be undersaturated with respect to cystine and MAP. However, calcium oxalate has a low solubility and consequently even normal urine is nearly always supersaturated with respect to this salt (Robertson, 1993). It has been recognised for some time that oversaturated urine is not synonymous with crystal formation (Robertson and Nordin, 1976). Urine is more complex than water, containing ions and proteins that interact and complex with

minerals potentially involved in urolith formation, thus retaining them in solution above the solubility product, yet below the formation product (K_F) (Balaji and Menon, 1997). Such a solution is termed metastable, implying varying degrees of instability with respect to the potential crystallisation. If some nucleating material were added to such a solution heterogeneous nucleation followed by crystal growth may take place at a degree of supersaturation below the formation product (Robertson and Nordin, 1976). This concept is explained further in section 1.6.3.1.

As the stone-forming ion concentrations in the urine are increased further, a threshold is reached, above which the urine can no longer maintain these minerals in solution (Balaji and Menon, 1997). The upper limit of metastable supersaturation can be defined empirically and is termed the K_F of the salt. The K_F is not a thermodynamic constant, but covers a narrow band of high supersaturation concentrations where precipitation may take place over a relatively short time period (Robertson, 1993). Above the K_F band urine is oversaturated and unstable, allowing spontaneous homogeneous crystal precipitation, aggregation and growth.

1.6.4.1 Zones of urinary supersaturation

From the above chemical considerations it is evident that urinary supersaturation, falls within three definable zones of relative supersaturation, as shown in Figure 1.6 (Robertson and Nordin, 1976).

1.6.4.1.1 Zone of undersaturation

Urine below the solubility product for a given salt is termed undersaturated. Any crystals added to urine in this state will dissolve. If urine is maintained within this zone uroliths cannot form.

1.6.4.1.2. Zone of metastable supersaturation

Urine lying between the solubility product and the formation product can be considered metastable. As the degree of metastable saturation approaches the formation product the length of time before crystallisation commences is reduced. The time between urine formation and crystallisation may also be shortened by the presence of nucleating material such as cell debris or crystals of other mineral types; for example, calcium oxalate crystallisation may occur at a lower degree of supersaturation if other crystals such as brushite, apatite or urate are also present in the urine sample (Robertson and Nordin, 1976). This process is termed heterogeneous nucleation (Robertson, 1993). Any pre-existing crystals of a given salt are likely to grow in urine metastably supersaturated with that salt. The urine of normal healthy human subjects often falls within the zone of metastable saturation (Fleisch, 1978). Thus, crystalluria is a common finding in healthy subjects, although most crystals tend to be passed harmlessly in urine (Worcester, 1996).

1.6.4.1.3 Zone of oversaturation

Urine that reaches or exceeds the formation product falls within the zone of oversaturation. This is a highly unstable environment in which spontaneous homogeneous crystal formation, aggregation and growth occurs (Robertson and Nordin, 1976). Within this zone uroliths would be expected to form.

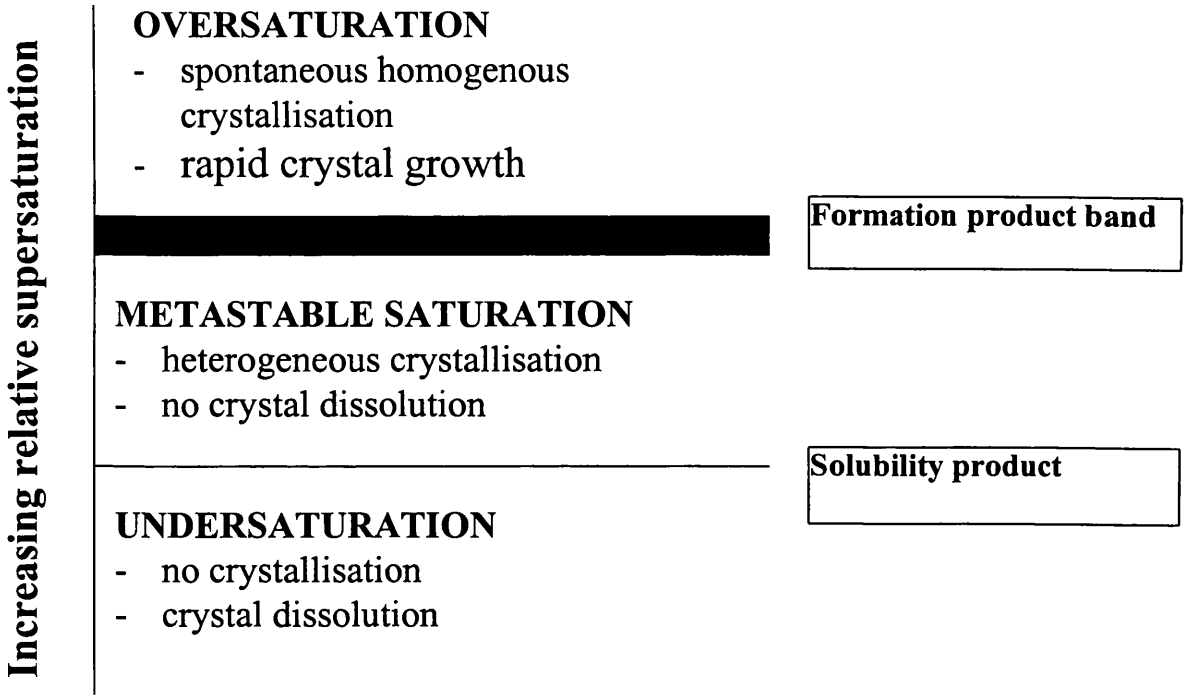


Figure 1.6 Zones of urinary relative supersaturation and the crystallisation processes that occur in each zone

(adapted from Robertson, 1976, 1993; Osborne 2000b, Bartges, 1999, Markwell *et al*, 2000)

1.6.5 Methods for measuring urinary relative supersaturation

The ability to predict the crystallisation potential of urine is a useful tool for clinicians and researchers who wish to develop or monitor therapeutic interventions for patients with urolithiasis, or to reduce the risk of urolith formation within susceptible groups and individuals. The risk of a given urolith component crystallising, and the rate and extent to which crystallisation will proceed, are largely dependent on the degree of supersaturation of that salt or acid in the urine concerned.

Within the human field, two methods of assessing the supersaturation of urine have been developed namely the relative supersaturation ratio (RSS) and the activity product ratio (APR), although over the years a number of variations upon these two methodologies have been published. Both of these methods have focused primarily on predicting the crystallisation potential of urine with respect to calcium oxalate as this is the most prevalent urolith type found in humans.

1.6.5.1 The relative supersaturation ratio (RSS)

The relative supersaturation ratio (RSS) is the algorithm used by most workers in the field of human urolithiasis when they wish to measure the supersaturation of urine. The original method for calculating RSS (SUPERSAT) was devised by Robertson (Robertson, 1969), and later developed by Werness *et al* in their widely used EQUIL 2 program (Werness *et al.*, 1985). These procedures require the measurement of urine pH and concentrations of calcium, magnesium, sodium, potassium, ammonium, phosphate, oxalate, citrate, sulphate, uric acid and chloride. The concentrations of the numerous soluble ion complexes formed between these various ions are calculated by means of an iterative computer program and finally the activity products of the various stone-forming salts, including calcium oxalate and MAP, are calculated. The ratios of these activity products to the thermodynamic solubility products of their respective salts yield the relative supersaturation (RSS) values of the various salts and acids on a scale where an RSS value of 1 defines a urine at the solubility product of the salt concerned and is therefore just saturated with respect to that salt. RSS values <1 define urine that is undersaturated and RSS values >1 urine that is supersaturated with respect to the salt concerned.

Since calcium oxalate is the most common stone type found in humans, both SUPERSAT and EQUIL computer programs have been used extensively to predict the crystallisation potential of calcium oxalate in human urine, with a high degree of accuracy (Pak *et al.*, 1977a). However, little research has been conducted to examine the accuracy of MAP RSS data generated by these programs. A recent study at The WALTHAM™ Centre for Pet Nutrition (in collaboration with Dr. Robertson, UCL, London) demonstrated that both SUPERSAT and EQUIL generated reasonably accurate calcium oxalate RSS results in urine of cats and dogs. In contrast, SUPERSAT was the only program which measured MAP RSS with a high degree of accuracy. EQUIL consistently overestimated MAP RSS and should not be used to predict the crystallisation potential of urine with respect to this mineral type (Robertson *et al.*, in press).

The main limitation of assessing urinary supersaturation by the above methodology is the assumption that there are no major urinary complexors of ions involved in urolith formation present that are not being measured, that may reduce the free ion activities of these constituents (Pak *et al.*, 1977a). In the case of human urine this assumption holds true, at least for calcium oxalate and MAP, and recent work from The WALTHAM™ Centre for Pet Nutrition also shows this to be the case in cat and dog urine (Robertson *et al.*, in press). In addition, this method involves a considerable amount of analytical work to measure the wide range of substance in each individual urine sample (Robertson and Markwell, 1999).

1.6.5.2 Activity product ratio (APR)

The activity product ratio (APR) is based upon the analysis of the total urinary concentrations of calcium, oxalate and phosphate and the measurement of urine pH.

APR is calculated by comparing the products of the concentrations of these ions (after taking into account the effect of pH on the ionisation of phosphate) prior to, and following a 48-hour incubation with seed crystals of calcium oxalate, struvite or brushite.

The benefit of using APR over RSS is the reduction in the number of analytes required to assess the supersaturation. However, the main limitation of this technique is the assumption that a steady state with respect to the solid phase will be reached by the end of the 48-hour incubation period (Pak *et al.*, 1977a). Research has shown that it may take urine longer than 48 hours to reach equilibrium, particularly when coming from oversaturation, because of the presence of inhibitors which slow down the approach to equilibrium (Pak *et al.*, 1977a; Robertson and Markwell, 1999). Recent research at The WALTHAM™ Centre for Pet Nutrition has shown this to be particularly relevant in the dog where urine was still approaching equilibrium after nine days of incubation (Robertson *et al.*, in press).

This variation in the time taken to reach equilibrium limits the usefulness of APR as a measure of supersaturation, and therefore RSS is the method currently used by the majority of workers within the human field to measure urinary saturation. Thus assessments of urinary supersaturation reported in this thesis will be made using the RSS methodology, together with the SUPERSAT computer program.

1.6.6 Composite theories of urolith formation

While it is accepted that there are factors in the urine that are able to modify the rate of growth and / or aggregation of crystals under the right urinary conditions, it is still not clear whether any one unique factor can distinguish individuals which form uroliths from those that do not (Batinic *et al.*, 2000). It is more likely that urine

contains both inhibitors and promoters, and the balance between positive forces (supersaturation and promoters) and negative forces (inhibitors) will determine whether an individual forms uroliths (Robertson, 1993).

Several authors have attempted to combine the various thermodynamic and kinetic factors in human urine that are thought to be involved in urolith formation into an index, which discriminates between stone-formers and normal subjects based on the urine biochemistry. Over the years a number of indices have been constructed including the 'saturation-inhibitor index' (Robertson *et al.*, 1976; Robertson, 1977), the 'crystal growth factor' (Smith *et al.*, 1980), the 'formation product ratio-activity product ratio discriminant (Pak and Galosy, 1980), 'urinary stone promoters/inhibitors ratio' (Batinic *et al.*, 2000) and several quotients which combine various urinary constituents in a number of arbituary ways (Tiselius, 1985; Tiselius, 1987; Tiselius, 1997).

One particularly interesting method is the risk assessment model constructed by Robertson *et al*, 1978, 1981 (Robertson *et al.*, 1978; Robertson *et al.*, 1981a). This method involves the use of overlapping frequency distributions of urinary factors, which have been found to be significantly different between human stone-formers and normal subjects. It ignores the excretions that are not different between the two groups. Initially the model included six factors: urinary volume, pH, and the excretions of calcium, oxalate, uric acid, and Alcian blue-precipitable polyanions (a measure comprising most of the macromolecular anions thought to influence calcium oxalate crystal growth and agglomeration at this time). The overlapping frequency distributions of the six factors were used to generate a set of risk curves, which define the contribution of each factor to the overall risk of forming uroliths. The risk curves

operate over the entire normal and abnormal range of each particular factor in urine. Using Bayes's theorem (a theorem of probability) (Lee, 1997), the contribution of each risk factor can be combined to give an overall measure of the relative risk of calcium urolith formation in an individual. Later on the model was adapted to incorporate some of the less important risk factors including citrate, pyrophosphate and magnesium (Robertson, 1993).

From all available evidence it is clear that, in most cases, urolith formation is a multifactorial disease that cannot usually be explained in terms of one single abnormality. A combination of several risk factors may be responsible for an increase in the supersaturation of the urine, and for subsequent crystal growth and aggregation. In addition, there may be other predisposing factors, which increase the risk of urolith formation within an individual. Such factors influencing urinary supersaturation with MAP and calcium oxalate in cats and dogs will now be discussed.

1.7 Factors influencing the urinary supersaturation in cats and dogs

1.7.1 Magnesium ammonium phosphate (MAP)

MAP (also known as struvite) was the most common urolith found in cats and dogs during the 1970-80s, and consequently, there is a substantial amount of literature documenting risk factors and prevention strategies for this mineral type in cats and dogs. As a result of the improved understanding of factors involved in MAP formation the incidence of this stone type has declined in recent years.

1.7.1.1 Age, breed, sex and sexual status

Epidemiological data available in the literature, mainly generated from two Stone Analysis Centres within the USA, indicate that MAP occurs across all age groups in cats and dogs, and is the most common urolith in dogs less than 1 year of age (Table 1.3) (Ling *et al.*, 1998c; Osborne *et al.*, 1999a). This mineral type also appears to occur more commonly in females. Cats do not appear to have any breed predisposition to MAP urolith formation, while a number of dog breeds appear to present more commonly with MAP (Table 1.3). It has been hypothesised that susceptible breeds may inherit an abnormality in host defences that increase susceptibility (Osborne *et al.*, 1999a).

1.7.1.2 Urine pH

Urine pH is *the* most important factor controlling MAP supersaturation in dogs and cats. The production of alkaline urine (as a result of diet or other factors such as urinary tract infection, see section 1.7.1.3) increases the risk of MAP crystallisation and subsequent urolith formation. The urine pH determines the proportion of urinary phosphate present as the trivalent anion, the form of phosphate necessary for MAP formation. Increasing the urine pH increases logarithmically the amount of the trivalent ion that is present, as the divalent ion is deprotonated (Boistelle *et al.*, 1983). The relatively greater importance of changing urine pH compared with other factors such as urinary magnesium can be demonstrated theoretically (Marshall and Robertson, 1976; Buffington, 1988), and has been shown experimentally (Buffington, 1988; Buffington *et al.*, 1992). Reduction of urine pH through dietary manipulation is thought to be the most reliable means of both preventing and managing MAP urolithiasis (Markwell and Buffington, 1994).

Table 1.3 Age, breed and sex as risk factors for MAP urolith formation in cats and dogs.

Factor	Cat*	Dog**
Age	May be detected at any age Most commonly 1-10 years	Mean age 6.0 years Age range 1 month –19 years
Breed	No breed predisposition	Miniature schnauzer Shih Tzu Bichon Frise Miniature Poodle Lhasa Apso
Sex	More common in females	More common in females

(adapted from Osborne *et al*, 1995a*; and Osborne *et al*, 1999b**)

1.7.1.3 The role of urinary tract infections (UTIs)

One important difference between cats and dogs is that most MAP uroliths that form in cats do so in the absence of a bacterial UTI. This is in contrast with the situation in dogs (and humans), where it is estimated that more than 85% occur as a consequence of UTI with urease-producing bacteria such as *Staphylococcus intermedius* or *Proteus* spp. (Goulden, 1968; Clark, 1974; Klausner *et al.*, 1980; Markwell *et al.*, 2000). Hydrolysis of urea by the enzyme urease results in the formation of ammonia and carbon dioxide. The ammonia reacts with water, the result of which is an increased ammonium concentration and alkalinisation of the urine. As discussed in Section 1.7.1.2, an alkaline urine pH will increase the MAP crystallisation potential. Thus, appropriate treatment of any underlying UTI is essential in the management of MAP formation in the majority of dogs. The low incidence of infection-induced uroliths in cats probably reflects the high resistance of this species to ascending UTIs (Lees *et al.*, 1979).

1.7.1.4 Increased urine volume

Increasing water intake is the oldest existing treatment for kidney stone prevention in humans, among whom the majority of stones are composed primarily of calcium salts (Borghi *et al.*, 1999). In theory, this preventative measure should reduce urinary supersaturation by decreasing the concentrations of magnesium, ammonium and phosphate that contribute to MAP formation. In addition, increasing urine volume may influence crystal transit time through the urinary tract, thus reducing the potential for crystal growth and aggregation (Markwell *et al.*, 2000). Humans can be encouraged to drink more water through verbal communication and reasoning. However, this method is not an option for cats and dogs. Thus, any increase in water consumption has to be implemented through dietary measures, and voluntary intake.

One of the most effective ways to increase water intake in cats is through feeding canned foods. Many studies conducted in cats show that cats fed dry foods (which typically contain <10% moisture) take in less total moisture than cats fed wet foods (typically containing >70% moisture). In a study conducted by Burger *et al*, 1980, the total water intake for cats fed dry food was only 46% of that when cats received canned food. Similar studies conducted in Labrador retrievers (LR) suggested that total moisture intake did not differ even when foods with markedly different moisture contents were fed (Burger *et al.*, 1980). However, this study was conducted in a breed of dog that does not commonly present with urolithiasis. No studies appear to have been conducted comparing the effect of dietary moisture on urinary supersaturation in cats or dogs. Additionally, because the appropriate treatment of UTIs is the most important factor in controlling MAP formation in the

majority of dogs, possible additional benefits of increasing urine volume are not easy to quantify.

1.7.1.5 Dietary magnesium and phosphorus

For MAP urolith formation, alteration of urinary crystalloid concentrations is relatively less important than controlling urinary pH. Nevertheless, studies have shown that increasing dietary minerals can bring about formation of crystalline material and/or obstruction of the urinary tract in cats (Rich *et al.*, 1974; Lewis *et al.*, 1978). However, careful interpretation of results is required since some of the magnesium supplements used in experimentally induced MAP urolithiasis created alkaline urine, which may have had a greater influence over the MAP formation than the dietary magnesium.

Phosphorus restriction is of theoretical value in cats as a reduction of urinary phosphate reduces the MAP activity product (Buffington *et al.*, 1992). Because of the role infection plays on MAP formation in dogs, the role of dietary minerals is not clearly defined (Osborne *et al.*, 1999b). Some authors believe that sterile MAP uroliths do form in dogs, in which instance dietary minerals may become more important (Weaver and Pillinger, 1975). However, these observations are not supported by any experimental or clinical studies in dogs (Osborne *et al.*, 1999b).

1.7.2 Calcium oxalate

In contrast to the prevalence of MAP uroliths, the incidence of calcium oxalate uroliths removed and analysed from cats and dogs appears to have increased over time. At one Urolith Analysis Centre within the USA, calcium oxalate urolithiasis in dogs increased from 5% of uroliths analysed in 1981 to 35% in 1997 (Lulich *et al.*,

1999b). Likewise, uroliths removed from cats and analysed at the same centre show a similar increase from 3% in 1981 to over 50% of total uroliths analysed in 1997 (Osborne *et al.*, 1999a).

1.7.2.1 Age, breed, sex and sexual status

Unlike MAP, calcium oxalate uroliths appear to occur more commonly in older male cats and dogs. There also appear to be breeds of cat and dog that more commonly present with this type of urolith (Table 1.4).

Table 1.4 Age, breed and sex as risk factors for calcium oxalate urolith formation in cats and dogs.

Factor	Cat*	Dog**
Age	Mean age 7 years Age range <1 to >20years	Mean age 8.5 years Age range <1 to >15 years
Breed	Burmese Himalayan Persian	Miniature schnauzer Lhasa apso Yorkshire terrier Bichon frise Shih tzu Miniature poodle
Sex	More common in males	More common in males

(adapted from Osborne *et al*, 1995a*, and Osborne *et al*, 1999b**)

1.7.2.2 Increased urine volume

As discussed in section 1.7.1.4, increasing urine volume may effectively reduce urinary supersaturation. There is a great deal of evidence to show that low urine volume is a risk factor for calcium oxalate formation, and a simple increase in water intake has been shown to significantly reduce recurrence rates in human calcium oxalate stone-formers (Borghi *et al.*, 1993; Borghi *et al.*, 1999). Robertson

(1993) identified low urine volume as the most important risk factor for calcium oxalate formation in humans.

As previously mentioned, increased water intake can only be achieved though dietary means in cats and dogs. One method may be to feed a diet containing a high moisture content, although the benefits are yet to be quantified in terms of a reduction in urinary supersaturation. A second option for increasing water intake in dogs and cats is to increase dietary sodium. The link between dietary sodium and water intake has been well documented in cats and dogs as well as many other mammalian species (Cizek, 1959; Hamar *et al.*, 1976). It is known that sodium chloride stimulates thirst in cats and dogs, as well as humans, although the mechanisms by which this occurs are complex. However, excess dietary sodium has also been shown to cause an increase in urinary calcium in both dogs and humans leading to recommendations that sodium should be restricted in individuals predisposed towards calcium oxalate formation (Kok *et al.*, 1990a; Lulich *et al.*, 1999b). Increased dietary sodium results in increased urinary sodium, which could encourage urolith formation by inhibiting the renal tubular reabsorption of calcium, thereby resulting in hypercalciuria (Sakhaee *et al.*, 1993).

This is a controversial issue as other studies have shown that supplemental dietary sodium had no effect on urinary calcium (Allen *et al.*, 1989; Stevenson *et al.*, 2000c), or oxalate (Lulich *et al.*, 1992b; Stevenson *et al.*, 2000c). However, no studies have been conducted examining the direct effect of supplementary dietary sodium on urinary calcium oxalate supersaturation in cats or dogs.

1.7.2.3 Calcium

1.7.2.3.1 Calcium metabolism

In humans, urinary calcium excretion largely depends upon dietary calcium intake (Menon *et al.*, 1998). On average, 25-30% of daily calcium intake is absorbed, although intestinal absorption changes inversely with dietary calcium intake (Wilkinson, 1976). Thus, percentage absorption of calcium is greater on low calcium diets than on high calcium diets (Menon *et al.*, 1998). Intestinal calcium absorption is mediated via cellular and pericellular pathways. At high luminal concentrations, absorption is primarily through diffusion along pericellular pathways, whereas at low concentrations, cellular pathways become more important (Menon *et al.*, 1998). Calcium is absorbed in the ionic state. Therefore substances that complex with calcium, such as phosphate, citrate, sulphate, oxalate and fatty acids decrease the amount of calcium available for absorption (Menon *et al.*, 1998). If dietary calcium is restricted without a concomitant decrease in dietary oxalate, passive intestinal absorption of oxalate in the colon followed by an increase in urinary oxalate may occur (Hodgkinson, 1978). Thus, in human calcium oxalate stone-formers severe calcium restriction is not recommended (Bataille *et al.*, 1983; Jaeger *et al.*, 1985). Calcium is absorbed throughout the entire length of the gastrointestinal tract, although it has been estimated that five times as much calcium is absorbed in the dog ileum when compared to the jejeunum, which in turn absorbs four times more calcium than the duodenum (Cramer, 1965).

The most important factor that mediates active or transcellular absorption of calcium is 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2$.vitamin D_3]. This compound increases calcium absorption by the brush border membranes of the intestinal mucosa.

It may alter membrane lipid concentration, allowing for rapid increase in membrane permeability to calcium. When the amount of dietary calcium is low, production and serum concentrations of 1,25-$(OH)_2$.vitamin D_3 are high. Hence calcium is absorbed through the duodenum, jejunum, ileum and colon (Menon *et al.*, 1998).

1.7.2.3.2 Urinary calcium excretion

Urinary calcium excretion is regulated by a combination of filtration, and partial reabsorption, where urinary calcium is the fraction of the filtered load not reabsorbed (Robertson and Nordin, 1976). When comparing the diet of human calcium oxalate stone-formers with that of normal healthy controls, studies have shown no difference in the amount of calcium ingested between the two groups, yet the urinary excretion of calcium was higher in the stone-forming group, indicating that calcium absorption was higher in this group (Fellstrom *et al.*, 1989).

Hypercalciuria is a common disorder encountered in around 60% of human subjects with urolithiasis (Pak *et al.*, 1974). It is important in the formation of calcium uroliths (oxalate and phosphate) since it will result in increased urinary supersaturation with respect to both calcium oxalate and calcium phosphate (Pak *et al.*, 1974; Menon *et al.*, 1998). Pak (1974) proposed that three types of hypercalciuria existed in humans, including absorptive hypercalciuria in which the primary abnormality is an increased intestinal absorption of calcium, renal hypercalciuria, characterised by a primary renal leak of calcium, and resorptive hypercalciuria, characterised by increased bone demineralisation (Pak *et al.*, 1974). Both absorptive and renal leak hypercalciuria are associated with normocalcaemia, whereas hypercalcaemia is normally present in association with resorptive hypercalciuria (Lulich *et al.*, 1991a; Menon *et al.*, 1998).

Increased urinary calcium is also known to be a factor in the formation of calcium oxalate uroliths in dogs. One study, examining the urine produced by miniature schnauzers with calcium oxalate uroliths, showed that urinary calcium was elevated compared to that of normal healthy beagles, while the affected dogs remained normocalcaemic (Lulich *et al.*, 1991a). The authors suggested that the findings were consistent with absorptive hypercalciuria, because urinary calcium excretion when food was withheld was significantly lower than when dogs consumed (Lulich *et al.*, 1991a). Although similar studies examining both urine and serum parameters have not been conducted in cats with calcium oxalate uroliths, it is estimated that around 35% of affected cats will have mild hypercalcaemia (Osborne *et al.*, 1996b). The significance of this observation, and the underlying mechanisms responsible are not yet known.

1.7.2.4 Oxalate

1.7.2.4.1 Oxalate metabolism

Oxalate is the divalent anion derived from the simple organic dicarboxylic acid present in many foods as oxalic acid. The main sources of dietary oxalate are plant products, principally cereals and leafy plants (Holmes and Kennedy, 2000). The exact physiological role of oxalate in plants is not known although it has been suggested that it is involved in seed germination, calcium storage and regulation, ion balance, detoxification, structural strength and insect repulsion (Horner and Wagner, 1995). Humans cannot metabolise oxalate directly, and thus renal excretion becomes the sole source of oxalate elimination (Asplin *et al.*, 2000). In normal human subjects, oxalate is poorly absorbed from the intestine. The entire intestinal tract is capable of absorbing oxalate, although the stomach and the distal bowel are thought to

be the primary sites (Menon *et al.*, 1998). Although most oxalate is absorbed by passive diffusion, facilitated diffusion has been demonstrated across brush border membrane vesicles of the ileum (Knickelbein *et al.*, 1968).

In humans, approximately half of ingested oxalate is destroyed by bacterial action (Menon and Mahle, 1983). Two bacteria *Oxalobacter formigenes* and *Pseudomonas oxalaticus*, contain enzymes that degrade oxalate within the physiological pH range (Allison *et al.*, 1986; Dawson *et al.*, 1988). The role of these bacteria in preventing oxalate absorption is unknown, although it has been suggested that a deficiency of *O. formigenes* may increase absorption of oxalate leading to mild hyperoxaluria (Allison *et al.*, 1986). Around 25% of oxalate is excreted unchanged in the faeces, and the remainder is excreted in the urine (Menon *et al.*, 1998). Although a range of herbivorous species, laboratory rodents and guinea pigs are known to be colonised with oxalate-degrading bacteria (James and Butcher, 1972; Allison and Cook, 1981; Argenzio *et al.*, 1988), it is not currently known whether normal healthy cats and dogs are so colonised.

A large amount of the oxalate found in human urine (up to 80%) is derived from endogenous production (Figure 1.7) (Menon *et al.*, 1998; Lulich *et al.*, 1999b). Oxalic acid is the metabolic end product of glyoxylate metabolism (Lulich *et al.*, 1999b). Approximately 40% of endogenous oxalate is derived from the metabolism of ascorbic acid and the remainder is derived from the conversion of glycine to glycollate. Vitamin B_6 deficiency also results in increased oxalic acid formation and hyperoxaluria (Lulich *et al.*, 1999b). No research has been conducted examining the relative contribution of endogenous and exogenous oxalate production on urinary oxalate content in cats and dogs.

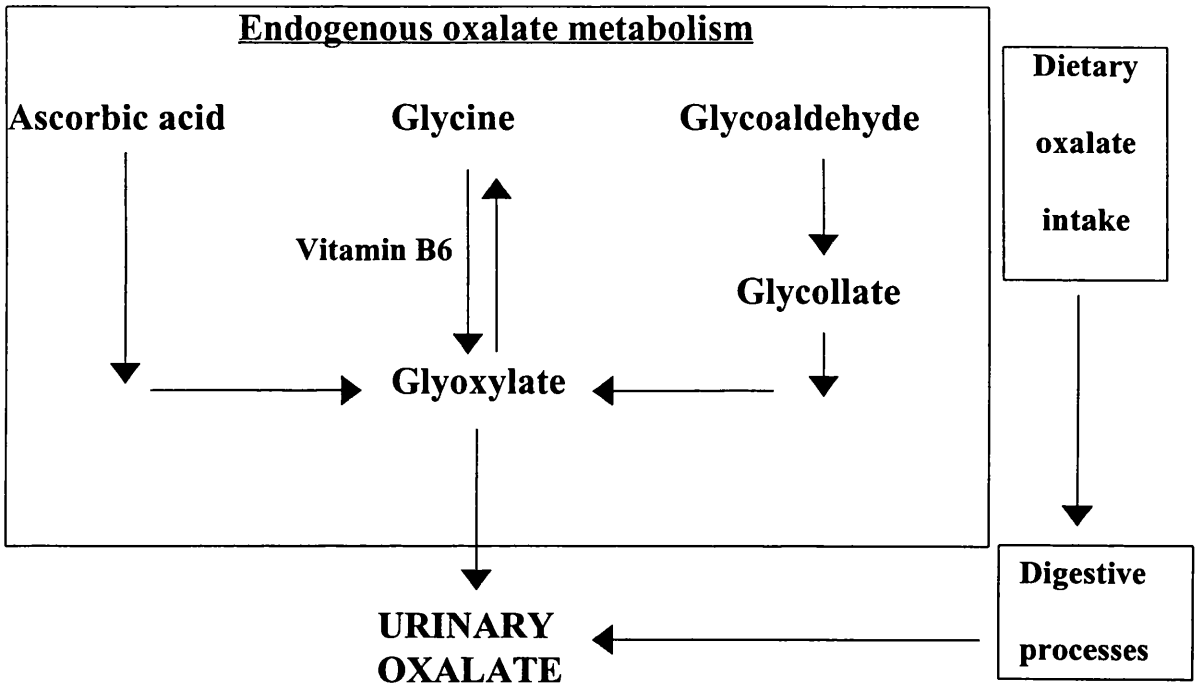


Figure 1.7 Pathways of oxalate biosynthesis

(adapted from Lulich *et al*, 1999)

1.7.2.4.2 Urinary oxalate excretion

The renal handling of oxalate is poorly understood, even in human subjects. However, it is known that in humans oxalate is freely filtered at the glomerulus and is secreted along the entire length of the proximal tubule (Williams and Wilson, 1990). In the human kidney, oxalate undergoes bidirectional transport in the renal tubules (Menon and Mahle, 1982).

In humans, hyperoxaluria can occur as a result of two types of errors of metabolism, inherited through an autosomal recessive pattern (Williams and Wilson, 1990). Both types are associated with increased oxalate production, and recurrent calcium oxalate urolithiasis, which often leads to early death through renal failure (Menon and Mahle, 1982). This inherited disorder has only been reported once within a closed cat colony, where the disease was described as a feline analogue of the

human disorder (McKerrell *et al.*, 1989). At present, there is no evidence to suggest that dogs inherit the same inborn error of metabolism.

Of more relevance to this thesis is the potential impact of mild hyperoxaluria on the urinary supersaturation of idiopathic human calcium oxalate stone-formers. A number of workers within the human field have proposed that mild hyperoxaluria is the second most important risk factor for calcium stone-formation in humans (after low urine volume) (Robertson *et al.*, 1978; Robertson and Peacock, 1980; Bataille *et al.*, 1983). In a study comparing the relative influence of mild hyperoxaluria and hypercalciuria on a number of parameters in stone-formation, mild hyperoxaluria had a far more marked effect on urinary supersaturation with respect to calcium oxalate, and on the maximum amount of crystalluria produced, than hypercalciuria (Robertson and Peacock, 1980). No such relationships were seen with hypercalciuria, except at very low urinary oxalate concentrations (Hallson, 1988). This is likely to be because oxalate is present in human urine at far lower concentrations than calcium.

In humans, urine calcium ions tend to outnumber oxalate ions by a factor of 10 or more (Brown and Purich, 1992). One study reported a calcium to oxalate molar ratio of 17.6 in human subjects (Markwell *et al.*, 2000). Thus, urinary oxalate is the limiting factor in the crystallisation process, and small increases in urinary oxalate will increase the urinary crystallisation potential. The same is probably not true for cats and dogs because their urinary molar calcium to oxalate ratio is closer to 1. Thus in these species changes in either urinary calcium or oxalate would be predicted to have a similar effect on the amount of precipitate that can potentially form (Markwell *et al.*, 2000). However, the relative importance of dietary calcium and dietary oxalate on urinary supersaturation has yet to be established in cats and dogs. In addition, one

study comparing the urine from healthy dogs with urine from dogs with calcium oxalate urolithiasis did not find hyperoxaluria a significant formation factor. In contrast, urine from the stone-forming dogs contained less oxalate than the clinically normal dogs (Lulich *et al.*, 1991a).

1.7.2.5 Other dietary factors

Other dietary factors that have been found to increase the risk of calcium oxalate formation in humans include increased intake of animal protein, reduced dietary fibre, decreased vitamin B_6 and increased vitamin C. Many of these findings have been extrapolated into dietary advice for cats and dogs with calcium oxalate urolithiasis, with little or no scientific basis.

1.7.2.5.1 Protein

In humans, a diet particularly high in animal protein, and therefore purine, results in passage of urine increased in uric acid and more acidic than normal (Coe *et al.*, 1976; Robertson *et al.*, 1979a). Although this can lead directly to formation of uric acid uroliths, uric acid crystals may also trigger heterogeneous crystallisation with calcium oxalate as previously discussed, leading to formation of mixed composition uroliths (Coe *et al.*, 1980). Another study examining the effect of protein on urine parameters in humans, found that increasing vegetable protein correlated with increased urinary oxalate, while increased animal protein resulted in increased urinary calcium and urate (Brockis *et al.*, 1982).

With reference to cats and dogs, no studies have been conducted comparing the effects of animal and vegetable protein on the risk of calcium oxalate formation in healthy or stone-forming animals. The effect of both source and amount of protein on uric acid and urate formation in dogs has been investigated (Bartges *et al.*, 1995a;

Bartges *et al.*, 1995b). In these studies a diet with restricted protein was found to reduce urinary uric acid and ammonia excretion, although the source of protein did not significantly affect urinary supersaturation with uric acid, sodium urate or ammonium urate. The cat is an obligate carnivore dependent on a supply of at least some animal-derived tissue in its diet (Burger, 1995a; Wills, 1996). The cat requires a higher amount of dietary protein than the dog, because its ability to regulate amino acid catabolism is limited (Burger, 1995a). Consequently, it is unable to adapt to diets very low in animal protein, and tends to find these diets unpalatable. Thus, vegetarian diets or those severely restricted in protein are not an option for cats, and consequently no research has been conducted to establish the effects of these diets on cat urine.

1.7.2.5.2 Fibre

Increased dietary fibre through supplementation with rice bran significantly reduced the calcium excretion of rats fed a high calcium diet, and reduced calcium excretion in the majority of human calcium oxalate stone-formers in one study (Ohkawa *et al.*, 1984). However, other studies have shown that a high fibre diet also results in a concomitant increase in urinary oxalate (Jahnen *et al.*, 1992), or phosphate (Ebisuno *et al.*, 1991). No research has been done to establish the effect of dietary fibre on the risk of calcium oxalate formation in cats and dogs.

1.7.2.5.3 Vitamin B_6

The active form of vitamin B_6 (pyridoxal-5-phosphate) is the coenzyme responsible for the conversion of glyoxylate to glycine, within the pathway of endogenous oxalate metabolism (Mitwalli, 1989). It has been shown experimentally that a deficiency in dietary vitamin B_6 causes increased endogenous production of

oxalate, in amounts large enough to cause marked renal damage in rats (Agnew, 1951; Hauschildt *et al.*, 1972), cats (Gershoff *et al.*, 1959), and humans (Faber *et al.*, 1963). However, the benefits of supplementary vitamin B_6 above minimum requirements are more controversial. Some studies dosing human subjects with supplementary vitamin B_6 found no effect (Revusova *et al.*, 1977; Tiselius and Almgard, 1977), while others found a significant reduction in urinary oxalate (Gibbs and Watts, 1970; Harrison *et al.*, 1981; Mitwalli, 1989). Supplementary vitamin B_6, above the minimum requirements for cats and dogs may have no further beneficial effects in reducing urinary oxalate excretion. Research conducted in normal healthy cats at The WALTHAM™ Centre for Pet Nutrition showed no change in calcium oxalate supersaturation, or urinary oxalate excretion, when the cats were supplemented with vitamin B_6 at ten times the amount required for adult maintenance (Wrigglesworth *et al.*, 1999).

1.7.2.5.4 Vitamin C

Oxalate is the endpoint of vitamin C metabolism (Hellman and Burns, 1958). Thus, increased amounts of dietary vitamin C may logically be expected to increase the excretion of urinary oxalate. Vitamin C is known to be a powerful antioxidant (Niki, 1990), and is commonly taken as a supplement within the human diet (Wilson *et al.*, 1973). Many studies have been conducted examining the potential effects of vitamin C on urinary oxalate excretion in human subjects. The results of these studies are inconsistent, with some showing elevated concentrations of urinary oxalate (Tiselius and Almgard, 1977; Hatch *et al.*, 1980; Hughes *et al.*, 1981), and others showing no effect (Wanzilack *et al.*, 1994; Gerster, 1997; Auer *et al.*, 1998). No consensus over this issue has yet been reached. In humans, vitamin C is classed as an

essential component of the diet. However, in cats and dogs vitamin C is synthesised within the body from glucose, and is thus, not classified as essential (Case *et al.*, 1995). No published research has established potential effects of supplementary vitamin C on the risk of calcium oxalate formation in cats and dogs.

1.8 Rationale and aims of the study

Urolithiasis is a significant disease within cats and dogs. In the majority of cases the condition is not life threatening and animals can continue to live for many years after the initial stone episode. Calcium oxalate stone-formation, in particular, appears to be growing in prevalence, yet many of the risk factors involved in the formation of this stone type, or measures that may be implemented to prevent it, are poorly understood. The majority of recommendations available in the current literature around the prevention of calcium oxalate formation have largely been extrapolated from human studies, some of which may not be appropriate for use within these animal groups.

Thus, this thesis aims to:

1. confirm the main types of urolith that form in dogs and cats (through quantitative urolith analysis), and correlate urolith type with epidemiological data including age, sex, sexual status and breed distribution.
2. increase the understanding of some of the processes involved in calcium oxalate stone-formation in dogs through:

   - identifying differences between dogs with calcium oxalate uroliths and matched healthy control dogs.

   - establishing the effect of potassium citrate therapy on urine parameters of dogs.

   - establishing the relative impact of dietary calcium and dietary oxalate on urinary parameters of the healthy dog.

   - identifying risk factors for urolith formation and establishing whether they can be manipulated through diet.

CHAPTER 2. MATERIALS AND METHODS

A number of methodologies were used in all studies reported in this thesis, and these are described below:

2.1 Housing conditions

Housing conditions and procedures were within the requirements of the Animals (Scientific Procedures) Act, 1986, and conformed to the guidelines of the WALTHAM™ Ethical Review Comittee at all times.

2.1.1 Cat housing specifications

Cats were housed individually in environmentally controlled two-roomed lodges with a total floor area of 2.6m^2, consisting of an inner room entered from a central court and an outer conservatory (Loveridge, 1994; Stevenson *et al.*, 1996). The floor covering in both rooms was vinyl, extending 40cm up the walls, which were glazed above the vinyl to the ceiling for 2m. There was a separate air supply and extract for each lodge, with warm air supplied to the inner room, and extracted from the conservatory. This allowed temperature to be controlled in the inner room, while the outer conservatory was affected by fluctuations in outside temperatures (Loveridge, 1994; Stevenson *et al.*, 1996).

2.1.2 Dog housing specifications

Dogs were individually housed in an environmentally controlled two-roomed pen consisting of an inner room (3.75m^2) entered from a central corridor and an outer room (2m^2) (Stevenson *et al.*, 1998b). The floor covering in the inner room was heat-sealed vinyl extending 40cm up the walls; the outer room consisted of a fibreglass

tray. A section of the inner pen had a warm sleeping area. There was a separate air supply and extract for each pen, with 12 air changes per hour. Warm air was supplied to the inner room and extracted from the outer conservatory. The temperature of the inner room was maintained at 22±2°C (Stevenson *et al.*, 1998b).

2.2 Urinalysis

Cats were trained to urinate into angled litter trays situated in one corner of the inner room. Urine samples were obtained from dogs by training them to urinate in the fibreglass tray in the outer pen area.

2.2.1 Urine pH

Urine from both cats and dogs drained rapidly into a glass U-tube housing a combined pH and temperature electrode (Figure 2.1). The pH probe was attached to a pH meter, which was checked daily by a three-point calibration to pH buffers 4, 7 and 9. The computer software (Signal Centre, Computer Park Software Ltd, Broughton Grange, Headlands, Kettering, Northants) requested urine pH and temperature data from each pH meter every 30 seconds. At the end of each day the data were analysed by the software, which recognised urinations by a temperature increase in the U-tube of at least 2°C. In this way pH values were measured within 30 seconds of voiding (Stevenson *et al.*, 1996; Stevenson *et al.*, 1998b). Data collected in this way can be analysed to provide a mean daily urine pH for each animal, and can also be collated to show diurnal profiles.

2.2.2 Urine volume and specific gravity

After urine pH was measured, as described above, all urine was collected in a glass bottle. Once daily at 9am, the 24-hour urine volume and specific gravity of each 24-hour urine sample were measured.

2.2.3 Urinary relative supersaturation (RSS)

2.2.2.1 Sample collection

During each feeding study the above collection system was modified for a 48-hour period to allow urine to be frozen upon voiding. The U-tube and electrode were replaced by a Dewar flask containing a glass bottle surrounded by dry ice (Figure 2.2) (Stevenson *et al.*, 1996; Stevenson *et al.*, 1998b). Samples collected in this way were defrosted in a fridge and homogenised, after which urine volume and pH were measured. The samples were then acidified to pH2 with a 37% solution of hydrochloric acid (BDH Laboratory supplies, Poole, UK), and refrozen at –20°C whilst awaiting analysis. Although urinary acidification may affect precipitation of some urinary constituents, including citrate, pyrophosphate and uric acid, previous work at the WALTHAM™ Centre for Pet Nutrition has shown that, at the concentrations found in dog and cat urine, these effects are not significant (Reed, 2001). The above frozen urine collection system can also be used for storage and preservation of freely voided urine samples freshly collected from dogs within a home environment.

2.2.2.2 Sample analysis

Urine samples were subsequently defrosted at 4°C overnight and sonicated (Ultrawave Ltd, Cardiff) at room temperature for 5 minutes at 50 hertz. Samples were then filtered using a 10ml syringe fitted with a 0.2μm filter to remove animal hairs from the samples. Thereafter samples were diluted with deionised water tenfold for the determination of potassium, magnesium, calcium, sodium, ammonium, chloride, sulphate and phosphate and urate. The cations were analysed using Dionex DX120 ion-exchange high performance liquid chromatograph (Dionex UK Ltd, Camberley, Surrey, UK (Markwell *et al.*, 1999). This was a single eluent system using 1.6ml/l methanesulphonic acid at a flow rate of 1ml/min. Diluted samples (25 μl) were injected automatically using a AS3500 autosampler on a Dionex CS12A column (4mm bore, 10cm length) fitted with a CG12 A guard column (4mm bore, 25cm length) using a Dionex chemical suppressor CSRS-11 to reduce background conductivity detection chromatograph (Dionex UK Ltd, Camberley, Surrey, UK). (Rabin *et al.*, 1993) The maximum run time for these samples was ten minutes.

Urine samples were analysed for anions using a Dionex ion-exchange Chromatograph series 4500I chromatograph (Dionex UK Ltd, Camberley, Surrey, UK) operated on a gradient system. Diluted samples (25 μl) were injected automatically using an AS3500 autosampler on a Dionex AS11 column (4mm bore, 10cm length) fitted with an AG11 guard column (4mm bore, 25cm length) using a Dionex chemical suppressor ASRS to reduce background (Markwell *et al.*, 1999). Components were eluted using a gradient of 1 ml/min of 10 mM to 80 mM sodium hydroxide and identified using conductivity detection. The gradient was run over ten minutes for the elution of chloride, sulphate and phosphate and over 22 minutes for

the elution of oxalate, citrate and pyrophosphate. On both of the described systems helium was used for degassing the eluents and pressurising the reservoirs at an operating pressure not exceeding 0.56 kg / cm^2. Creatinine was also measured by high performance liquid chromatography using a variable wavelength detector at 235nm (UV). The eluent used was di-hydrogen phosphate buffer on a C18, 50DS reverse phase analytical column.

In order to ensure accuracy and reproducibility of results a number of quality control measures were routinely used. Chromatographs were examined and peak area was compared with the calibrated linearity range and checked for reproducibility, response and resolution. The results of analysis of quality control standard samples were assessed, including a standard cat urine sample of known composition, audit samples re-analysed from a previous batch, and a commercially available human urine standard (Sigma-Aldrich Chemical Company Ltd, Poole, Dorset, UK). The data from the cat urine standard was compared against historical data. The analytical procedures were concluded to be in control and stable when 66% of analyses were within one standard deviation of the historical mean, 95% within two standard deviations and 99.9% within three standard deviations of the historical mean. Audit samples were compared statistically with the previous analysis of the same sample. The human urine standard was compared with stated mean and tolerence values for each component as supplied by Sigma-Aldrich. If one or more of the above quality control measures failed samples were re-submitted for analysis.

The concentrations of analytes determined by these procedures were entered into a microcomputer based program, SUPERSAT, obtained from Dr. WG Robertson, at the Centre for the Prevention of Urinary Stones, Institute of Urology and

Nephrology, University College London. This program calculates urinary relative supersaturations (activity product / solubility product) for struvite, calcium oxalate and brushite (calcium hydrogen phosphate dihydrate) (Robertson *et al.*, in press; Markwell *et al.*, 1999).

2.3 Dietary nutrient analysis

All diet nutrient analyses were conducted by the Central Nutritional Laboratory, Pedigree Petfoods, Melton Mowbray. All dietary nutrients, with the exception of protein and oxalate were measured using modified standard methods (Her Majesty's Stationery Office, 1991). Protein was analysed by a modified method based on the Dumas principle, using a nitrogen analyser (LECO, Cheshire) (Sweeny, 1989). Oxalate was analysed using high performance liquid chromatography (Spectro-physics Ltd., Hemel Hempsted) with conductivity detection against an oxalic acid calibration standard (Sigma-Aldrich Chemical Company Ltd, Poole, Dorset, UK).

2.4 Data analysis

Unless otherwise stated data were expressed as means±standard deviations. Specific statistical methods are described in the Materials and Methods section in each chapter. For all methods the level of significance was taken as $P<0.05$. Significant differences between tabulated data were indicated (at $P<0.05$) by using different superscript letters.

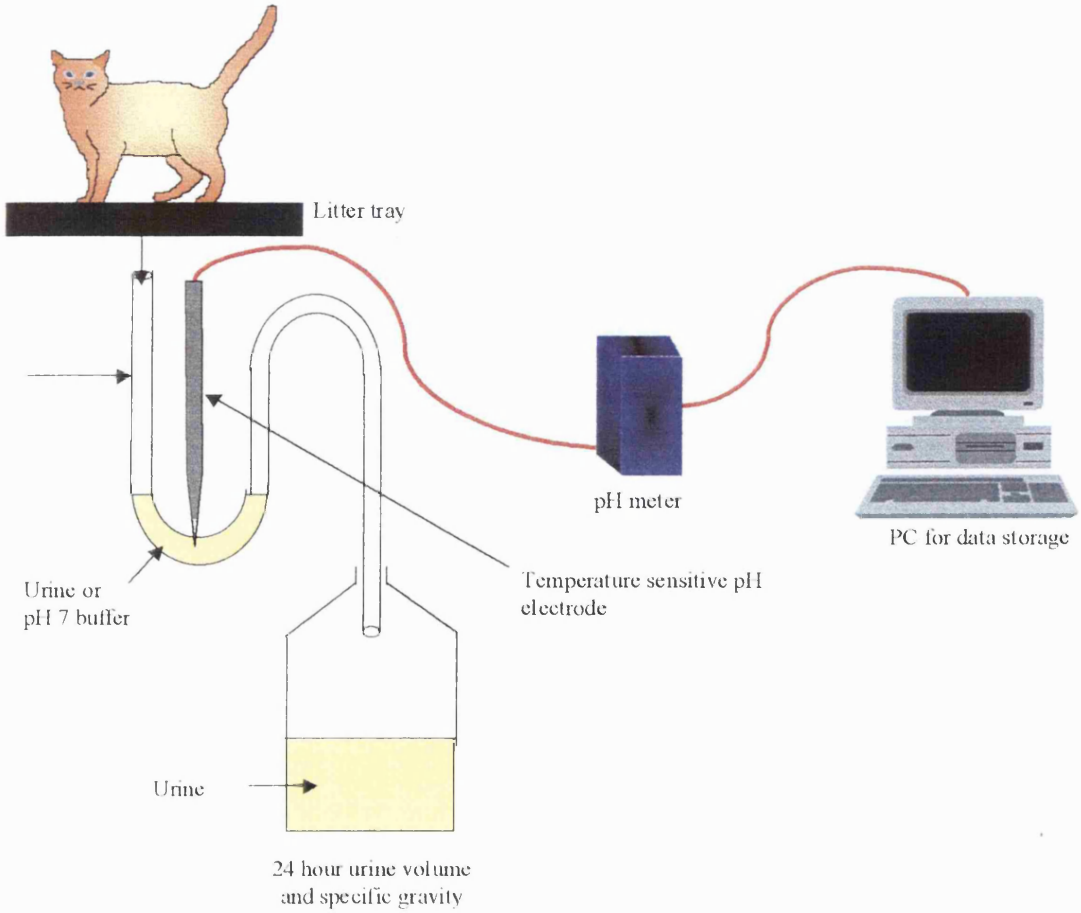


Figure 2.1 The automated urine pH monitoring system

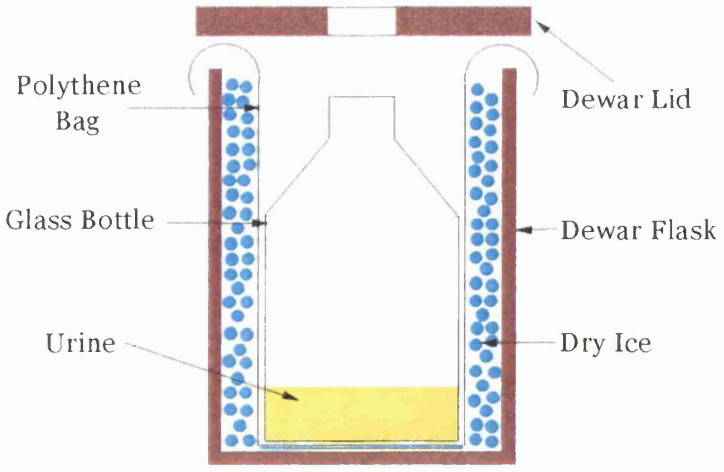


Figure 2.2 The frozen urine collection system for RSS assessment

CHAPTER 3. QUANTITATIVE ANALYSIS OF UROLITHS SURGICALLY REMOVED FROM CATS AND DOGS

"The study of the causes of things must be precede by the study of things caused"

Hughlings Jackson

3.1 Introduction

Urolithiasis continues to be an important clinical problem in dogs and cats. This condition may affect 1.5 to 3% of all dogs admitted for medical care in the USA (Osborne *et al.*, 1995b). Similar figures have also been quoted for Germany (0.5-1.0%) (Hesse, 1990), although these figures are proportional morbidity ratios (i.e. proportion of a hospital population) rather than a percentage of the population as a whole. In addition, the incidence of lower urinary tract diseases in cats within Europe and North America was reported in the 1970s and 1980s to be approximately 0.5-1% of the population (Walker *et al.*, 1977; Lawler *et al.*, 1985). More recent data are not available, although a proportional morbidity rate of approximately 7% of feline cases was reported from a survey of North American veterinary schools between 1980 and 1993 (Lulich and Osborne, 1996). In a group of cats evaluated between 1993 and 1995, causes of signs were reported for 109 non-obstructed cats. In this study it was found that urolithiasis occurred in around 15% of cats (Buffington *et al.*, 1997).

Effective management of urolithiasis depends upon identification and manipulation of factors contributing toward the initial stone-formation. This process, in turn relies upon accurate identification of the type of urolith formed. Over the

years many canine and feline urolith analysis series have been published (Walker *et al.*, 1977; Hesse and Sanders, 1985; Ling and Ruby, 1986; Osborne *et al.*, 1989b; Escolar, 1990; Hesse, 1990; Ling *et al.*, 1990; Wallerstrom and Wagberg, 1992; Osborne *et al.*, 1995b; White, 1996; Hesse *et al.*, 1997; Ling *et al.*, 1998c; Osborne *et al.*, 1999a; Hesse *et al.*, 2000; Osborne *et al.*, 2000a; Osborne *et al.*, 2000b; Osborne *et al.*, 2000c). However, many of the early reports include analysis by qualitative methods, which involve placing crushed urolith in contact with various chemicals supplied as a kit, and examining the colour change to indicate the mineral types present (Ruby and Ling, 1986).

Qualitative methods are reported to be far less accurate than quantitative techniques (Bovee and McGuire, 1984; Osborne *et al.*, 1986a). One study comparing a qualitative chemical procedure with a quantitative crystallographic method found that the qualitative method failed to detect 62% of the calcium-containing uroliths, and gave false-positive results for uric acid in 55% of cystine uroliths (Bovee and McGuire, 1984). In several recent reports, uroliths were analysed quantitatively using either optical crystallography or infrared spectroscopy. Although the majority of studies have been conducted in North America (Ling and Ruby, 1986; Ling *et al.*, 1990; Ling *et al.*, 1998c; Osborne *et al.*, 1999a), similar studies have also been reported in Germany (Hesse and Sanders, 1985; Hesse, 1990; Hesse *et al.*, 1997; Hesse *et al.*, 2000), and Canada (Houston *et al.*, 2000). Different laboratory techniques have been used between studies and thus, no attempts have been made to compare urolith analysis profiles between countries. Thus, the purpose of this study was to compile, analyse and compare data on uroliths analysed from dogs and cats in

four different countries; United Kingdom, Italy, Netherlands and Hong Kong, using an accurate quantitative urolith analysis technique.

3.2 Materials and Methods

3.2.1 Urolith analysis

A total of 1101 uroliths, surgically removed from dogs (n=912) and cats (n=189) between the years of 1998 and 2000, were analysed quantitatively by infrared spectroscopy and photo-acoustic detection (Manning and Blaney, 1986; Gould *et al.*, 1995) at UCL Hospitals, Department of Chemical Pathology, London. This approach has principal spectral analysis programmes for reliable measurement of common components in both single and multi-component uroliths containing one or more of the following - calcium oxalate, calcium phosphate (CAP), magnesium ammonium phosphate (struvite) [MAP], uric acid and cystine. Interbatch coefficients of variation for these common components are consistently less than 10% when analysed in mixed uroliths. Identification of rarer components (such as xanthine, dihydroxyadenine, ammonium urate, calcium carbonate, silica and drug metabolites) are also easily attainable with this analytical approach. This technique was chosen because it offered a number of advantages over other currently available methods such as crystallography and x-ray diffraction. Infra-red spectroscopy is an accurate non-destructive technique that allows quick and repeatable analysis of very small samples (down to 1mg in weight) with minimal sample preparation (Otnes, 1983; Gould *et al.*, 1995). It is also relatively easy to learn and can reliably be reproduced by different workers with relatively little experience (Otnes, 1983).

3.2.2 Patient information

Uroliths were submitted for analysis from four countries; Hong Kong, Italy, the Netherlands and Great Britain. Information regarding the cat or dog was collected at the time of urolith removal and submitted with the urolith, including age, breed and sex of the animal.

3.2.3 Data analysis

3.2.3.1 Mineral type

Patient information was used to determine any trends towards particular urolith mineral types for each age group, breed and sex. Urolith analysis profiles were compared between countries. The prevalence of each mineral type was compared by converting the total number of stones of each mineral type into a percentage of the total number of stones (overall), and for each country individually. A comparison of the percentage occurrence of each stone type was made between countries using a multiple comparisons test for proportions (Zar, 1999). The numbers of each mineral type were then subdivided by sex and by age.

3.2.3.2 Age profiles

The median and mean age (±standard deviation) of stone-formation were calculated for each stone type overall and by individual country. Since age was found to have a normal distribution, comparisons of the age of stone removal were made by stone type within each country and the age of stone removal for each mineral type was compared between countries using analysis of variance.

3.2.3.3 Gender distribution

The percentage of male and female animals forming uroliths of each mineral type were calculated.

3.2.3.4 Breed distributions

The percentage of each breed represented within each mineral type was calculated, both overall and within each country. Data were obtained from the British Kennel Club (1-5 Clarges Street, London, W1J 8AB), and the Governing Council of the Cat Fancy (4-6 Penel Orlieu, Bridgewater, TA6 3PG), from which the relative popularity of each dog and cat breed within Great Britain was established, as a percentage (Appendices 3.2 and 3.4). Using only data from pedigree cat and dog breeds, a measure of the relative risk of forming a particular urolith type (RF) was calculated by dividing the percentage of each stone type formed by a particular breed by the percentage of that breed within the pedigree population in Great Britain. A figure greater than one indicated that the breed was over-represented compared to the percentage of this breed in the dog population, while a figure of less than one indicated that the breed was under-represented compared to the percentage within the dog population of Great Britain.

For all statistical methods $P<0.05$ was considered significant.

3.3 Results

3.3.1 Canine urolith analysis across all countries

A total of 912 uroliths were analysed from dogs between 1998 and 2000. These made up 83% of the total number of uroliths analysed from cats and dogs taken together.

3.3.1.1 Mineral composition

Of the canine uroliths analysed 47% were found to be MAP, 32% calcium oxalate, 8% ammonium urate/uric acid, 1% CAP, 3% cystine and 9% "mixed" (<70% of any one mineral type), (Table 3.1, Figure 3.1).

3.3.1.2 Sex distribution

MAP was more common in female dogs making up 74% of the MAP uroliths analysed (Table 3.1, Figure 3.2). In contrast, all the other mineral types occurred more commonly in males.

3.3.1.3 Age distribution

MAP occurred across all age groups with a minimum age of 0.2 years and a maximum of 19 years (Table 3.1), although it was more common between the age of 4 and 8. The mean age (±SD) of struvite formation was 6.3±3.2 years, with a median of 6 years. Calcium oxalate uroliths were not found in dogs of less than 1 year of age with a minimum of 1.5 years and a maximum of 15 years. This mineral type appeared to increase in prevalence with age up to around 10 years, after which time the occurrence declined with age (Table 3.1, Figure 3.4). The mean age (±SD) of calcium oxalate formation in dogs was 8.5±2.9 years, with a median of 8 years.

All other mineral types were more common during middle age. The mean age of ammonium urate / uric acid formation was 4.8±2.6 years (median 4 years, minimum 1 year, maximum 12 years), that of calcium phosphate was 7.8±2.76 years (median 8 years, minimum 3 years, maximum 12 years), and the mean age for cystine formation was 5.8±2.9 years (median 6 years, minimum 0.1 years, maximum 11 years). Mixed uroliths occurred across all age groups with a mean of 7.3±2.9 years, median of 7 years, minimum of 0.8 years and maximum of 13 years. When comparing the age profiles of all stone types, ammonium urate/uric acid and cystine were removed from dogs at a significantly younger age than either calcium-containing stones (both phosphate and oxalate), or mixed stones ($P<0.0001$). MAP uroliths were also removed at a significantly lower age than calcium oxalate stones ($P<0.0001$), (Table 3.3).

3.3.1.4 Breed distribution

Sixty-one different breeds presented with MAP urolithiasis; 7% of the total number of MAP uroliths were removed from both Yorkshire terriers and Miniature schnauzers, 4% from Pekingese, 3% Labrador retriever, 3% Cavalier King Charles spaniel, 3% Cocker spaniel, 3% Maltese terrier, 3% Pomeranian and 13% from cross breeds (Figure 3.6).

Fewer breeds presented with calcium oxalate urolithiasis (n=41) including the Yorkshire terrier (23% of the total number of calcium oxalate stones analysed), Shih tzu (14%), Doberman (5%), Miniature schnauzer (4%), Pomeranian (4%), West Highland white terrier (4%) and Jack Russell terrier (3%), (Figure 3.7). A further 12% were removed from cross breed dogs. A total of 89% of calcium oxalate uroliths

removed from pure breed dogs were from breeds classed as toy or terrier by the British Kennel Club.

Ammonium urate/uric acid stones were predominantly found in Dalmatian dogs (44%), although the Shih tzu (13%), Miniature schnauzer (6%), Pug (6%), and Cocker spaniel (6%) also presented with this urolith type (Figure 3.8). Calcium phosphate uroliths were found in 11 breeds, although 23% of this stone type were removed from the Shih tzu. Cystine stones were found in 14 breeds but most commonly in the Bulldog (13%), Staffordshire bull terrier (10%), Jack Russell terrier (10%). Mastiff (7%) and Labrador retriever (7%), (Figure 3.9).

3.3.2 Feline urolith analysis across all countries

A total of 189 uroliths were analysed from cats between 1998 and 2000. These made up 17% of the total number of uroliths analysed from both cats and dogs.

3.3.2.1 Mineral composition

Of the feline uroliths analysed 56% were found to consist of MAP, 33% calcium oxalate, 4% ammonium urate/uric acid, 4% calcium phosphate and 3% "mixed" (<70% of any one mineral type), (Table 3.2, Figure 3.1). No cystine uroliths were analysed from cats.

3.3.2.2 Sex distribution

MAP, calcium oxalate and ammonium urate/uric acid were equally common in males and females (Table 3.2, Figure 3.3). Although only making up 7% of the total number of uroliths analysed calcium phosphate and "mixed" stones were more common in male cats.

3.3.2.3 Age distribution

MAP and calcium oxalate uroliths occurred across all age groups (Table 3.2), although both were more common between the ages of 3 and 9 (Figure 3.5). The mean age (±SD) of MAP formation was 6.8±3.7 years, with a median of 7 years, a minimum of 1 year and maximum of 18 years. Mean age (±SD) of calcium oxalate formation was 6.8±3.5 years, with a median of 6 years, a minimum of 1 year and a maximum of 19 years. All other mineral types occurred most commonly during middle age. The mean age of ammonium urate / uric acid formation was 4.4±2.0 years; for calcium phosphate it was 7.1±3.6 years, and the mean age for "mixed" stone-formation was 6.0±2.2 years. Mineral type was not significantly affected by age in cats (P=0.52), (Table 3.4).

3.3.2.4 Breed distribution

The domestic shorthaired cat (DSH) was the predominant breed presenting with all mineral types (Figures 3.10, 3.11). However, the Persian contributed 14% of struvite uroliths (Figure 3.10) and 27% of calcium oxalate uroliths (Figure 3.11). In addition, 3% of calcium oxalate uroliths were removed from Burmese cats (Figure 3.11). The numbers of other urolith types were too small to draw any conclusions regarding possible breed predisposition.

3.3.3 Comparison of uroliths removed from cats and dogs in Hong Kong, Italy, Netherlands and Great Britain

3.3.3.1 Mineral type

A comparison of the urolith types removed from dogs in Hong Kong, Italy, Netherlands and Great Britain (Figure 3.12) showed that significantly fewer MAP uroliths were removed from dogs in Italy, while a significantly larger percentage of calcium oxalate uroliths were found compared with that in the other three countries. Italy was the only country in which the calcium oxalate uroliths outnumbered the MAP uroliths. Other significant differences between the countries included a lower percentage of ammonium urate/uric acid uroliths in the Netherlands compared to Italy, a lower percentage of calcium phosphate uroliths in Hong Kong and the Netherlands compared to Italy, and a larger percentage of cystine stones in Italy and Great Britain compared to Hong Kong.

Examination of the uroliths removed from cats (Figure 3.13) showed that in the Netherlands there was a significantly lower percentage of MAP stones and a significantly larger percentage of calcium oxalate stones than in the other three countries. All other mineral types did not occur in large enough numbers for valid statistical analysis.

3.3.3.2 Sex distributions

The sex distributions observed in the overall data (sections 3.3.1.2 and 3.3.2.2) remained consistent across the four countries.

3.3.3.3 Age distributions

A comparison of the age of urolith removal for each mineral type, across the four countries revealed some significant differences in dogs (Table 3.3). Both MAP ($P<0.0001$) and calcium oxalate ($P<0.0001$) were removed from dogs at a significantly younger age in Hong Kong than in the other three countries. In addition, MAP occurred at an earlier age in the Netherlands than in Great Britain. No significant differences in age of stone removal were observed for cats (Table 3.4).

3.3.3.4 Breed distributions

3.3.3.4.1 Dogs

When examining differences in breed distributions between countries a number of differences were observed. In Hong Kong, the most common dog to form either MAP (42% of struvite stones analysed), or calcium oxalate (32%) or ammonium urate (44%) was the Shih tzu. Unlike the situation in the other three countries, no ammonium urate/uric acid stones were removed from Dalmatians in Hong Kong, and cross-breeds only made up 3% and 6% of the MAP and calcium oxalate stones respectively. Of all four countries studied, Hong Kong had the lowest percentage of cross-breeds (28% of dog population) (Appendix 3.1). In Italy, the Shih tzu commonly formed MAP (17%) and calcium oxalate uroliths (12%), with the Yorkshire terrier also well represented (10% MAP; 28% calcium oxalate). Italy also had a high percentage of cross-breeds with stones, 30% of MAP and 26% of calcium oxalate uroliths coming from this group. However, cross-breed dogs make up around 80% of the dog population in that country. In addition, 83% of the ammonium urate/uric acid stones were removed from Dalmatians. In the Netherlands, 13% of MAP stones came from Maltese terriers, while 29% of calcium oxalate stones were

removed from Yorkshire terriers. The relative popularity of breeds in each of the above countries could not be established and therefore a measure of the relative risk of certain breeds forming uroliths of a specific mineral type could not be calculated.

In Great Britain, the most common pedigree breed is the Labrador retriever. Thus, although this breed was the most common dog to form MAP uroliths in this country it was under-represented when compared with the breed popularity, since the relative frequency (RF) was less than 1 (Table 3.5). The German shepherd (RF=0.7) and Golden retriever (RF=0.8) are also popular breeds in Great Britain and were also under-represented in the MAP forming group. The only breed whose MAP RF was equivalent to the popularity of the breed was the Cocker spaniel. A number of breeds were over-represented compared with the popularity of the breed, including the Border collie (RF=3), Scottish terrier (RF=8), Lhasa apso (RF=4) and Miniature schnauzer (RF=4), although in no breed was the RF above 10. A number of the top 20 breeds in Great Britain did not present with MAP in this data series including the West Highland white terrier, English springer spaniel, Staffordshire bull terrier and Boxer.

The top three most popular dogs in Great Britain (Appendix 3.2) were not represented in the calcium oxalate breed distribution. All breeds found to form calcium oxalate produced an RF of one or greater, with the Fox terrier producing the highest calcium oxalate RF at 25 (Table 3.6). Other breeds producing a high RF were Yorkshire terrier (RF=9), Lhasa apso (RF=5), Miniature schnauzer (RF=5) and Doberman (RF=5). A total of 89% of calcium oxalate uroliths removed from pedigree dogs came from small breeds, the Doberman being the only large breed that contributed 2% or more of calcium oxalate uroliths within Great Britain. The Jack

Russell terrier presented with both MAP and calcium oxalate uroliths, although an RF could not be calculated for this breed as it is not listed by the British Kennel Club.

Ammonium urate/uric acid (Table 3.7) and cystine uroliths (Table 3.8) were all removed from breeds with a high RF. The Dalmatian produced an RF of 62 for ammonium urate/uric acid, while the Bull terrier (RF=19), Bulldog (RF=24) and Mastiff (RF=95) were all over-represented in the cystine group.

3.3.3.4.2 Cats

Cat breed distributions were consistent across the four countries with Persians being the most common pure breed represented for both MAP and calcium oxalate uroliths. However, in all countries, the majority of stones, regardless of mineral type, were removed from DSH cats.

When examining the RF for pedigree cats in Great Britain, compared to the popularity of the breed the Persian cat was over-represented (Appendix 3.4), with an RF of 2.3 for MAP and 3.6 for calcium oxalate (Table 3.9).

Table 3.1 Quantitative analysis of uroliths surgically removed from dogs between 1998 and 2000 (n=912)

Mineral type	Total analysed [% (no.)]	Percentage		Percentage in age range (years)			
		male	female	≤1.0	1.1 – 5.0	5.1 – 10.0	10.1+
MAP	47 (427)	26	74	3	43	43	11
Calcium oxalate	32 (292)	86	14	0	16	59	25
Ammonium urate / uric acid	8 (68)	93	7	7	60	30	3
Calcium phosphate	1 (13)	92	8	0	33	50	17
Cystine	3 (30)	100	0	7	38	52	3
Mixed*	9 (82)	62	38	1	28	58	13

* Mixed uroliths contain less than 70% of any one mineral type

Table 3.2 Quantitative analysis of uroliths surgically removed from cats between 1998 and 2000 (n=189)

Mineral type	Total analysed [% (no.)]	Percentage		Percentage in age range (years)			
		male	female	≤1.0	1.1 – 5.0	5.1 – 10.0	10.1+
MAP	56 (105)	56	44	2	39	42	17
Calcium oxalate	33 (63)	52	48	2	38	43	17
Ammonium urate / uric acid	4 (8)	50	50	0	86	14	0
Calcium phosphate	4 (8)	75	25	0	38	50	12
Cystine	0 (0)						
Mixed*	3 (5)	100	0	0	25	75	0

* Mixed uroliths contain less than 70% of any one mineral type

Table 3.3 The mean age (±SD) of urolith removal for each mineral type found in dogs from Hong Kong, Italy, Netherlands, Great Britain and overall

Mineral type	Country				
	Hong Kong	Italy	Netherlands	Great Britain	Overall
MAP	5.0±2.4	6.8±3.1	6.2±3.2	6.9±3.6	6.3±3.2
Calcium oxalate	6.8±2.9	8.9±2.6	8.8±3.1	9.4±2.8	8.5±2.9
Ammonium urate / uric acid	5.2±2.7	4.1±1.7	5.5±4.9	4.8±2.9	4.7±2.6
Calcium phosphate		5.5±4.9		8.2±2.5	7.8±2.7
Cystine	5.0±1.4	6.8±1.8	1.8±2.4	5.9±3.1	5.8±2.9
Mixed	6.7±2.8	9.0±2.5	8.0±2.6	6.9±3.1	7.3±2.9

Table 3.4 The mean age (±SD) of urolith removal for each mineral type found in cats from Hong Kong, Italy, Netherlands, Great Britain and overall

Mineral type	Country				
	Hong Kong	Italy	Netherlands	Great Britain	Overall
MAP	5.7±1.9	6.2±3.8	7.0±3.9	7.4±3.7	6.8±3.7
Calcium oxalate	3.7±0.8	8.5±4.0	7.4±3.9	6.4±3.2	6.8±3.5
Ammonium urate / uric acid		4.0±1.4	5.5±3.5		4.4±2.0
Calcium phosphate		7.3±3.1		6.9±4.9	7.1±3.6
Mixed				5.3±2.1	6.0±2.2

Table 3.5 The relative frequency of forming MAP uroliths compared with the popularity of the breed in Great Britain

Breed	Percentage of dog population	Percentage of MAP uroliths from this group	Relative frequency
Labrador retriever	14	10	0.7
Yorkshire terrier	3	7	2.3
Shih tzu	2	6	3
German shepherd	7	5	0.7
Border collie	1	5	5
Cocker spaniel	5	5	1
Lhasa apso	1	4	4
Miniature schnauzer	1	4	4
Scottish terrier	0.5	4	8
Golden retriever	5	4	0.8
Bichon frise	1	4	4
Cavalier King Charles spaniel	5	8	1.6

Table 3.6 The relative frequency of forming calcium oxalate uroliths compared with the popularity of the breed in Great Britain

Breed	Percentage of dog population	Percentage of calcium oxalate uroliths from this group	Relative frequency
Yorkshire terrier	3	28	9
West Highland white terrier	5	11	2
Shih tzu	2	6	3
Lhasa apso	1	5	5
Cavalier King Charles spaniel	5	5	1
Doberman	1	5	5
Miniature schnauzer	1	5	5
Fox terrier	0.2	4	25
Miniature dachshund	1	4	4
Bichon frise	1	4	4
Cairn terrier	0.75	2	3
Miniature poodle	0.4	2	5

Table 3.7 The relative frequency of forming ammonium urate/uric acid uroliths compared with the popularity of the breed in Great Britain

Breed	Percentage of dog population	Percentage of ammonium urate/uric acid uroliths from this group	Relative frequency
Dalmatian	1	62	62
Shih tzu	2	8	4

Table 3.8 The relative frequency of forming cystine uroliths compared with the popularity of the breed in Great Britain

Breed	Percentage of dog population	Percentage of cystine uroliths from this group	Relative frequency
Staffordshire bull terrier	5	19	4
Bull terrier	1	19	19
Bulldog	0.8	19	24
Mastiff	0.2	19	95

Table 3.9 The relative frequency of forming uroliths compared with the popularity of Persians in Great Britain

Stone type	Percentage of pedigree cat population	Percentage of uroliths from this group of pedigree cats	Relative frequency
MAP	19	45	2.3
Calcium oxalate	19	69	3.6

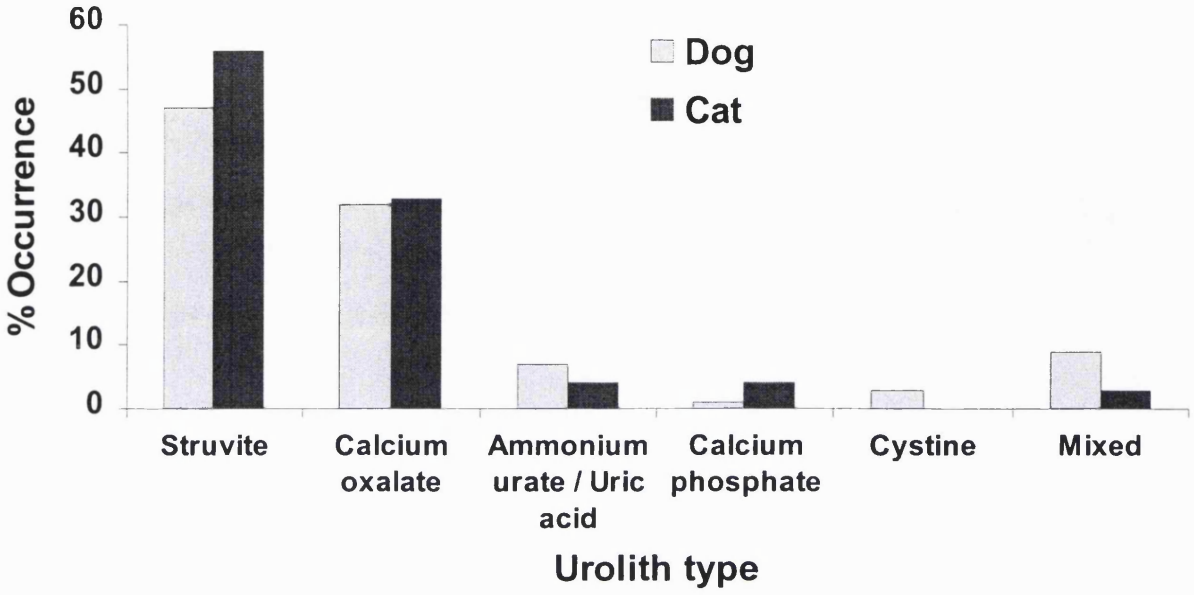


Figure 3.1 The relative proportions of each urolith mineral type found in cats and dogs across all countries

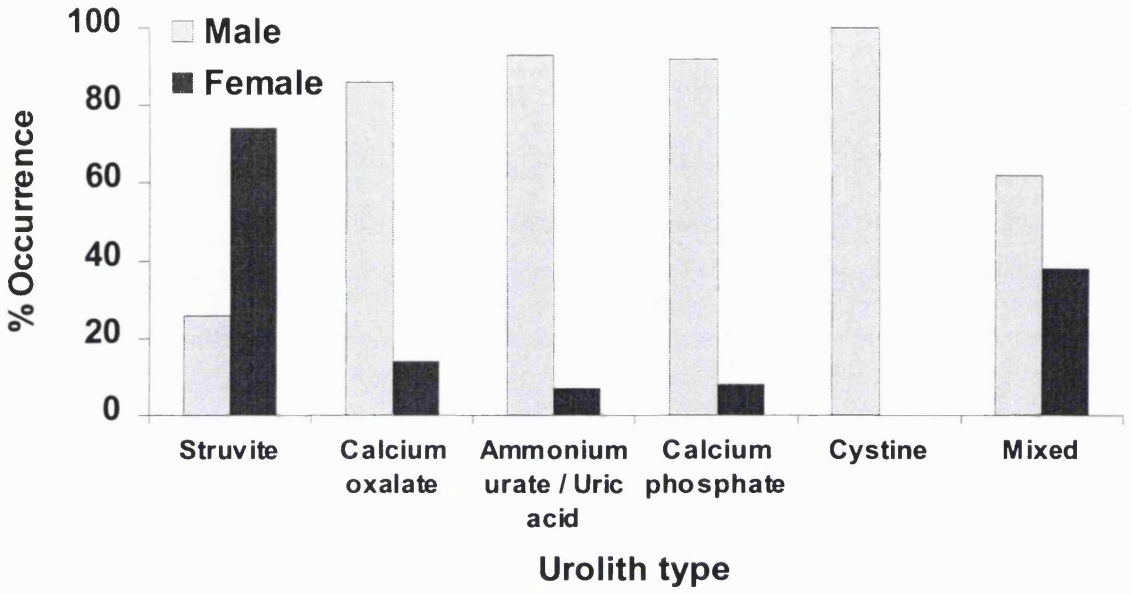


Figure 3.2 Sex distributions for urolith formation in dogs

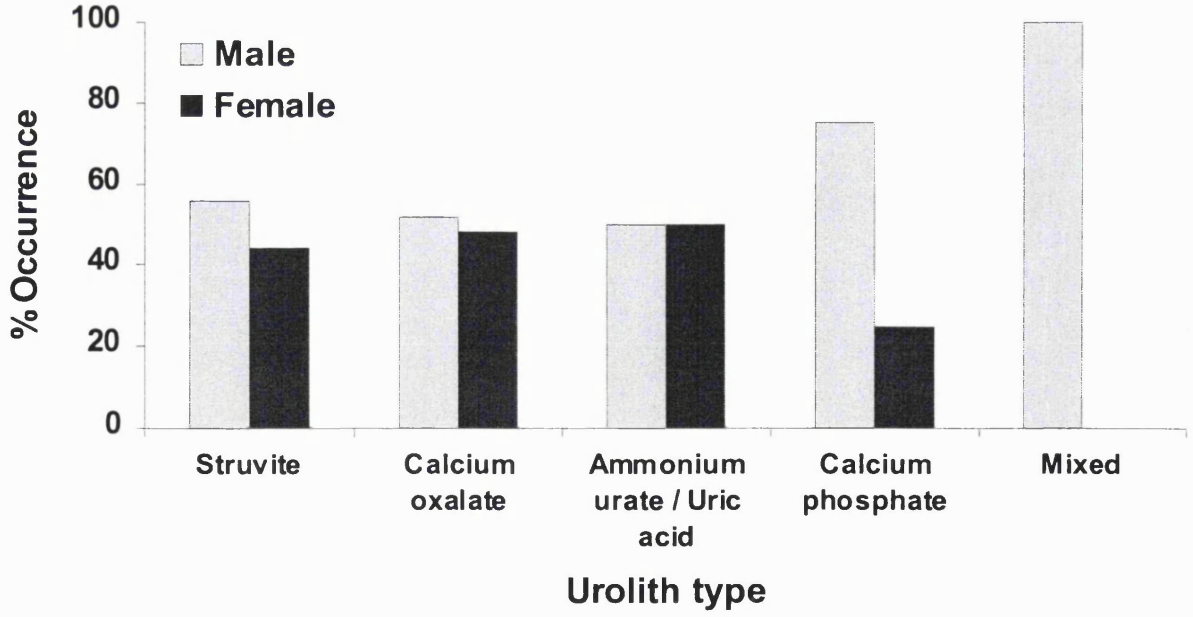


Figure 3.3 Sex distributions for urolith formation in cats

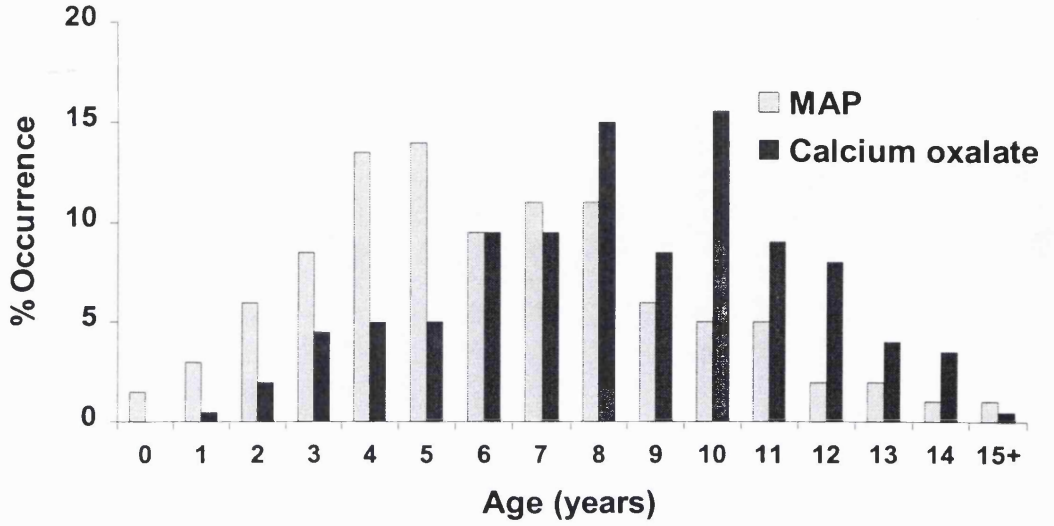


Figure 3.4 Magnesium ammonium phosphate (MAP) and calcium oxalate age profile in dogs

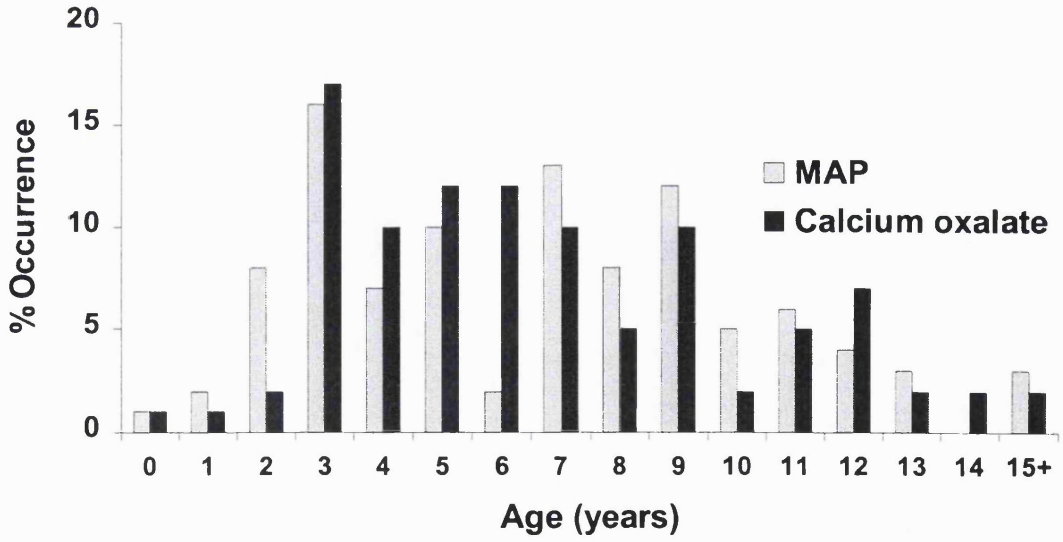


Figure 3.5 Magnesium ammonium phosphate (MAP) and calcium oxalate age profile in cats

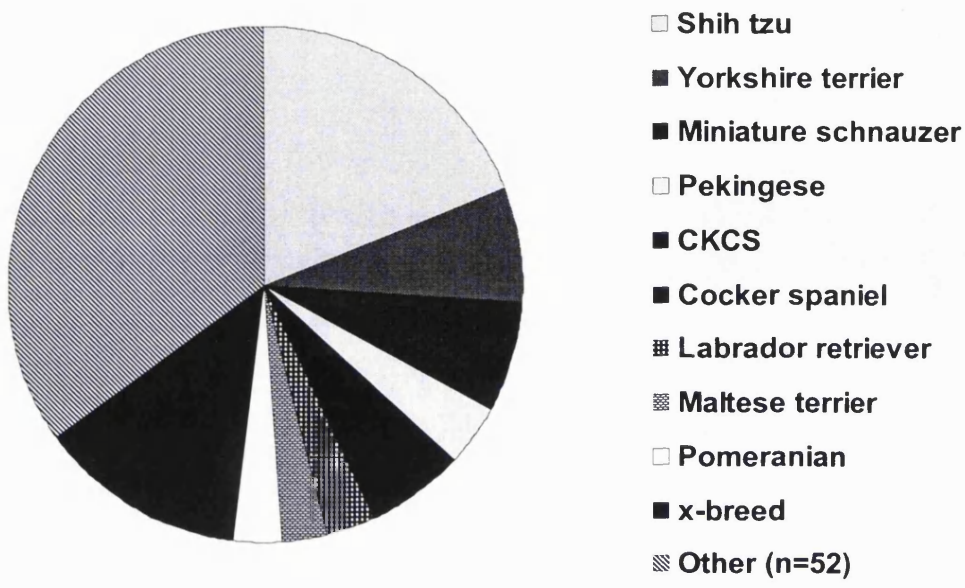


Figure 3.6 The breed distribution (%) of canine MAP urolithiasis across all countries (Hong Kong, Italy, Netherlands and Great Britain)

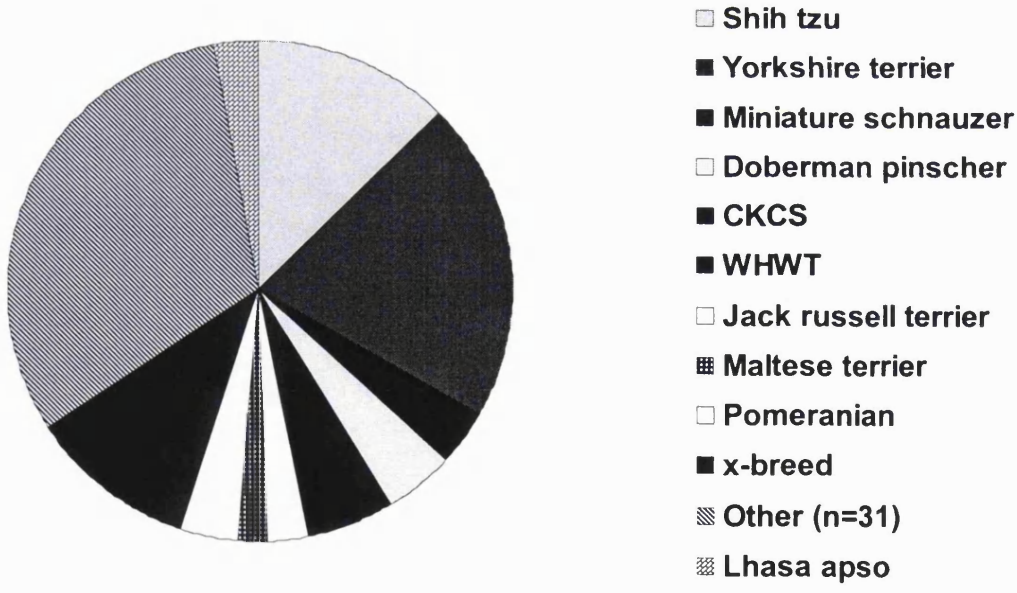


Figure 3.7 The breed distribution (%) of canine calcium oxalate urolithiasis across all countries (Hong Kong, Italy, Netherlands and Great Britain)

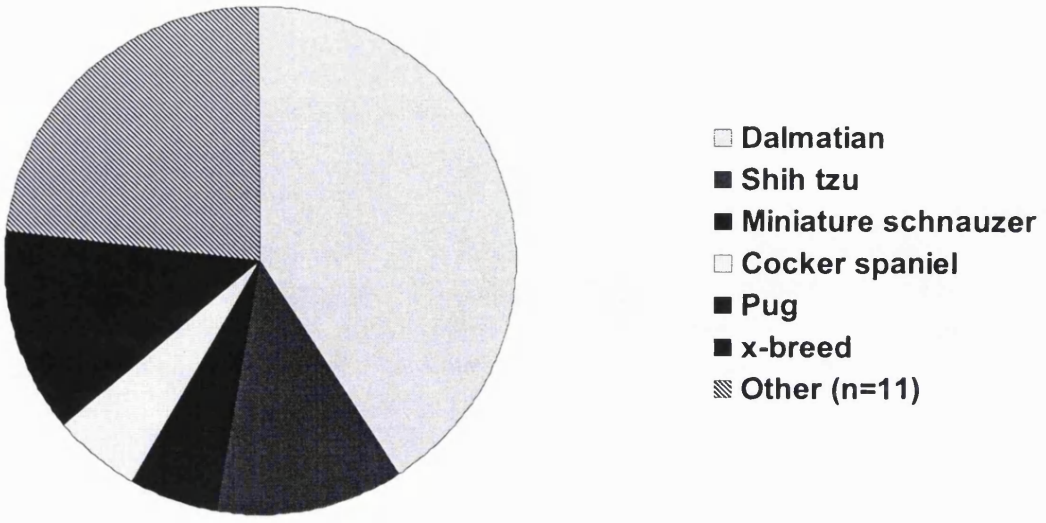


Figure 3.8 The breed distribution (%) of canine ammonium urate/uric acid urolithiasis across all countries (Hong Kong, Italy, Netherlands and Great Britain)

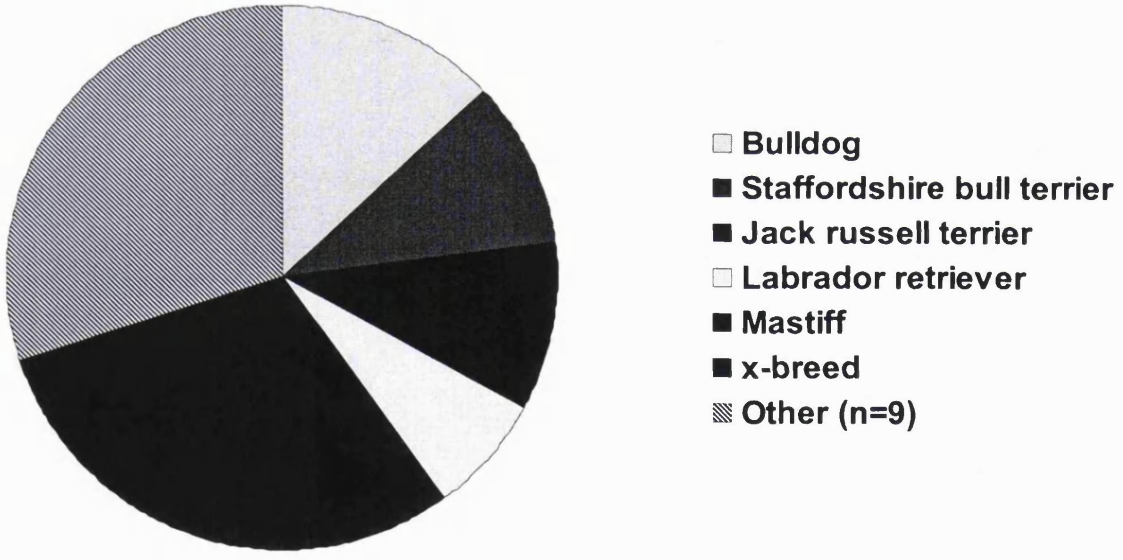


Figure 3.9 The breed distribution (%) of canine cystine urolithiasis across all countries (Hong Kong, Italy, Netherlands and Great Britain)

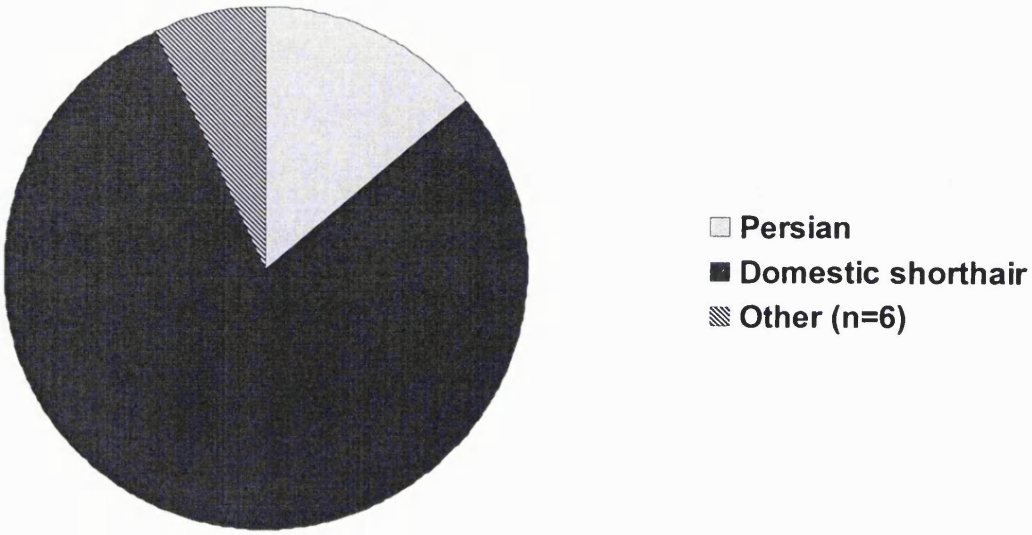


Figure 3.10 The breed distribution (%) of feline MAP urolithiasis across all countries (Hong Kong, Italy, Netherlands and Great Britain)

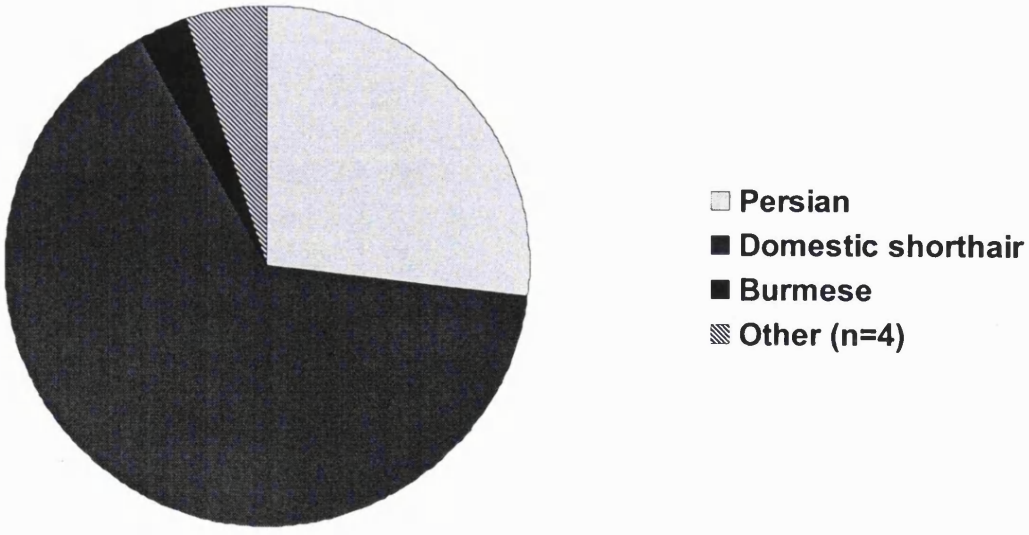


Figure 3.11 The breed distribution (%) of feline calcium oxalate urolithiasis across all countries (Hong Kong, Italy, Netherlands and Great Britain)

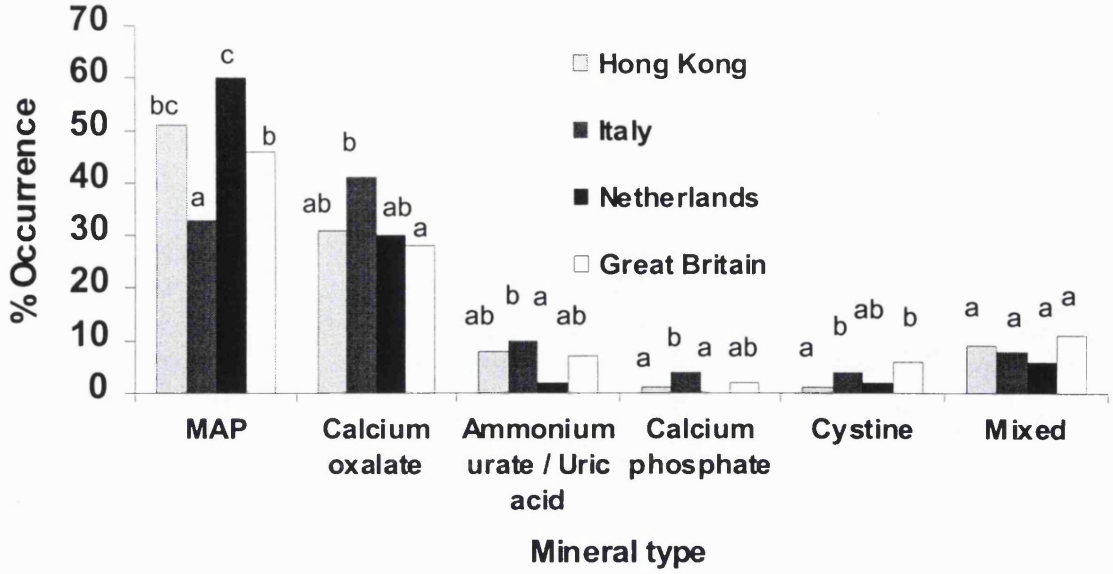


Figure 3.12 A comparison of the urolith mineral types removed from dogs in Hong Kong, Italy, Netherlands and Great Britain

Different superscript letters within a mineral type indicate a significant difference

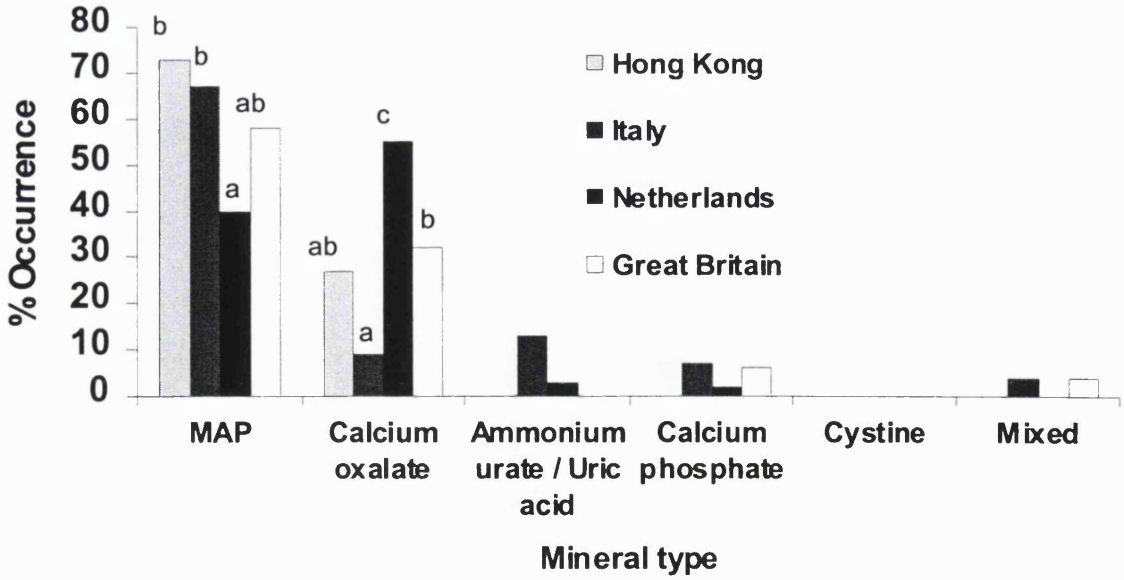


Figure 3.13 A comparison of the urolith mineral types removed from cats in Hong Kong, Italy, Netherlands and Great Britain

Different superscript letters within a mineral type indicate a significant difference

3.4 Discussion

The data presented in this study show that MAP was the most common mineral type found in uroliths removed from cats and dogs, when data from all countries was examined as one series. This is consistent with the findings of other canine studies in which MAP was also the most common mineral type found across a number of countries including North America (Ling and Ruby, 1986; Ling *et al.*, 1998c; Osborne *et al.*, 1999a; Houston *et al.*, 2000), Canada (Houston *et al.*, 2000), Germany (Hesse, 1990; Hesse *et al.*, 1997), Great Britain (White, 1996) and Sweden and Norway (Wallerstrom and Wagberg, 1992).

Published urolith analysis data from cats was less consistent between countries than dogs, with fewer countries represented. One urolith analysis centre in North America documented an increase in the prevalence of calcium oxalate uroliths in cats, associated with a decline in MAP uroliths, between 1981 and 1997, so that by 1997 calcium oxalate uroliths were more prevalent (55% of analysed uroliths) than MAP (36%) (Osborne *et al.*, 1997). Although a similar increase was also noted by Hesse *et al*, (2000) in Germany and surrounding countries, between 1984 and 1999, MAP uroliths still remained the predominant mineral type found in cats (61% of analysed uroliths between 1997-99), as also found in this study. Other minerals found in uroliths, including cystine, ammonium urate/uric acid and calcium phosphate occurred far less frequently than MAP and calcium oxalate. Percentages in this study were consistent with other reported urolith analysis data series with the exception of cystine uroliths in cats. This mineral type was not found in any cat samples examined in this study, while in one North American data series 22 cystine uroliths were analysed out of a total of 6335 feline uroliths (0.3%) (Osborne *et al.*, 1995b).

When the data series were split by country, the canine urolith analysis data showed that, in contrast to the other three countries, in Italy calcium oxalate uroliths were more prevalent than MAP. Italy also tended to have a higher percentage of cystine and ammonium urate/uric acid uroliths than the other countries. In contrast, the feline data series showed that calcium oxalate uroliths were more prevalent than MAP uroliths in the Netherlands, while MAP remained the most prevalent urolith type in the other three countries. Within the scope of this study it was not possible to explore fully the reasons behind the differences in prevalence of the various mineral types between countries.

As discussed in Chapter 1, section 1.5.3, cystine and ammonium urate/uric acid uroliths tend to form in dogs as a result of a genetically inherited error of metabolism. It is possible that differences observed in the occurrence of these stone types between countries may be due to the relative incidence of these metabolic errors in the dog populations within each country.

The observed differences in the proportions of calcium oxalate and MAP between countries may be linked to factors such as climate and lifestyle. Demographic risk factors such as climate, occupation, fluid intake, social class and affluence have been examined as contributing factors for stone-formation in humans, particularly with respect to calcium oxalate. The incidence of stone disease in humans tends to be higher in hot, dry climates compared to a more temperate one (Pierce and Bloom, 1945; Prince *et al.*, 1956; Bateson, 1973). This is partly due to the effect of ambient temperature on urine volume, but may also be due to the effect of increased sunlight on calcium excretion. The increased exposure to UV light may stimulate production of vitamin D in the skin, subsequently increasing absorption of calcium in

the intestine (Robertson, 1993). Calcium stone disease is also more prevalent within higher socio-economic groups than in less affluent ones (Robertson *et al.*, 1979b; Robertson *et al.*, 1981b; Asper, 1984), and among the more wealthy industrialised countries than poorer, developing countries (Robertson, 1993). In addition, a sedentary lifestyle has also been linked to increased stone prevalence (Robertson, 1993) as has working in a hot environment (Borghi *et al.*, 1993).

It is logical to postulate that climate and lifestyle may also affect urine composition and therefore the risk of urolith formation in cats and dogs. One study examining risk factors for feline lower urinary tract diseases in New Zealand identified a number of contributing demographic factors (Jones *et al.*, 1997). Reported cases of urolithiasis increased following periods of high rainfall, and the authors suggest that during wetter, cooler periods of the year, cats remained indoors for longer periods of time. Inactivity and sleeping inside were also contributing factors in cats both in the study by Jones *et al*, 1997 and in previously published findings (Willeberg, 1975; Willeberg, 1976; Willeberg and Priester, 1976; Walker *et al.*, 1977). Another factor that has been extensively discussed is the role of diet in urolith formation. In the study by Jones *et al* (1997), diets with a high proportion of dry food, and therefore a low moisture content, were identified as a risk factor for the development of lower urinary tract diseases. A similar study conducted in dogs in North America found that dogs which were overweight, those classed as pets rather than working dogs or those that lived in the city were at increased risk of calcium oxalate formation (Lekcharoensuk *et al.*, 2000b). It is thus possible that differences in the way owners feed and house cats and dogs between countries may influence both the number and type of urolith formed.

Other factors that may contribute to the formation of uroliths of a certain mineral type include the gender, age profile and breed distribution within each country. Although the gender distributions were not available within each of the countries examined, in dogs, MAP was found more commonly in females, while all other urolith types were more common in males across all countries. This is consistent with the findings of most other canine urolith analysis data series (Brodey, 1955; Hesse, 1990; Ling *et al.*, 1998c; Osborne *et al.*, 1999a; Houston *et al.*, 2000; Lekcharoensuk *et al.*, 2000b). As discussed in Chapter 1, section 1.7.1.3, MAP uroliths are generally linked to the presence of a urinary tract infection (UTI) with a urease-producing organism in dogs. UTIs are more frequently found in female dogs predominantly because of anatomical differences between the sexes; the female having a shorter urethra which may increase the chances of infections moving up the urethra to the bladder (Ling *et al.*, 1998b). A recent study examining the most common types of bacteria associated with UTIs in dogs found that urease-producing bacteria specifically, were more common in female dogs (Norris *et al.*, 2000). All other urolith types (calcium oxalate, ammonium urate/uric acid, cystine and calcium phosphate) generally occur in the absence of a UTI and are more commonly found in males than in females (Osborne *et al.*, 1999a). It is likely that anatomical differences also contribute to the increased prevalence of these uroliths in male dogs. The male, having a longer, narrower urethra, may be at increased risk of crystal retention and subsequent urolith formation in the bladder.

Additional research in other species specifically with respect to calcium oxalate suggests several other possible reasons why this mineral type may be more common in males. In rats, testosterone enhances and oestrogen suppresses enzymes

involved in oxalate synthesis (Sharma *et al.*, 1984). It has also been suggested that female rats produce more calcium oxalate and calcium phosphate inhibitors than male rats (Khan and Glenton, 1995). In humans, females have been shown to have an oestrogen-dependent increase in urinary citrate, a decrease in urinary calcium and enhanced glycosaminoglycan excretion, compared to males (Moore *et al.*, 1978; Othnes, 1983 (supplement); Hesse *et al.*, 1991). However, no research has yet been conducted comparing the urine composition of male and female dogs to establish any possible differences between them other than those that are anatomical in nature.

In contrast with other urolith analysis studies in cats, no differences in the prevalence of mineral type were seen between male and female cats. Previous studies in cats have shown that male cats were more likely to form calcium oxalate uroliths while female cats were more likely to present with MAP (Ling *et al.*, 1990; Thumchai *et al.*, 1996; Lekcharoensuk *et al.*, 2001). However, one recent study in cats concluded that the magnitude of risk that gender contributed to urolith formation was small (Lekcharoensuk *et al.*, 2000a), and a further study found no gender-associated risk of calcium oxalate formation (Kirk *et al.*, 1995). It is not known why apparent differences exist between this study and some previous studies, although it is possible that these data series may only reflect the gender distribution in the general cat populations of the countries concerned.

Age may be another factor contributing to the formation of uroliths of a specific mineral type. Cystine and ammonium urate/uric acid were removed at a younger age than other mineral types, probably because, as already discussed, these uroliths usually form as a result of a congenital abnormality. Dogs tended to present with calcium oxalate at an older age than with MAP, although MAP urolith formation

was found in all age groups. This finding is also consistent with other canine urolith analysis studies (Ling *et al.*, 1998c; Osborne *et al.*, 1999a; Lekcharoensuk *et al.*, 2000b). However, the results of this study and others did not suggest the reason why calcium oxalate uroliths tend to be found in middle-to-old age. In humans, urinary glycosaminoglycans , which may reduce the inhibitory capacity of the urine, have been shown to decrease with age (Hesse *et al.*, 1986), while another study showed that the inhibitory capacity of urine produced by children was higher than that in adults (Moore *et al.*, 1978). Unfortunately, variation in urine composition with age has not yet been studied in dogs.

The present study also highlighted differences in the age at which certain urolith types were removed from dogs between countries. In Hong Kong, dogs with MAP or calcium oxalate were younger in Hong Kong at the time of urolith removal than those in the Netherlands, Italy or Great Britain. In addition MAP occurred at an earlier age in the Netherlands than Great Britain. It is possible that the demographic risk factors discussed earlier in this chapter that may contribute to urolith formation were more exaggerated in Hong Kong than in other countries, leading to the development of stone disease at an earlier age than that found in other countries.

A similar trend in age profiles was not noted in the cat urolith analysis data series with both MAP and calcium oxalate having the same mean age of stone removal of 6.8 years. In previous studies within North America the mean age of cats with MAP was younger than cats with calcium oxalate uroliths (Thumchai *et al.*, 1996; Osborne *et al.*, 1997). The cause of the difference in age profiles between the present study and those previously reported is unclear.

Many previous studies of urolith analysis in dogs and cats concluded that certain breeds appear to be at increased risk of forming uroliths of a specific mineral type (Bovee and McGuire, 1984; Hesse, 1990; Wallerstrom and Wagberg, 1992; White, 1996; Ling *et al.*, 1998c; Houston *et al.*, 2000; Lekcharoensuk *et al.*, 2000b). In this study a large number of dog breeds were represented across all mineral types and countries. There was a large degree of variability in breed distributions for the same urolith type between countries, presumably due to the differing popularity of breeds between countries. It could be speculated, for example, that the Shih tzu is more popular in Hong Kong than in other countries since it appears in large numbers in all categories of stone types. Unfortunately, full details of breed distributions in Hong Kong, Italy and the Netherlands are not available.

As expected, the Dalmatian was the predominant breed forming ammonium urate/uric acid uroliths, because of its well-known genetically inherited abnormality that reduces the capacity of this breed to convert uric acid to allantoin (Porter, 1963). For this reason, the Dalmatian was greatly over-represented in the ammonium urate/uric acid group in Great Britain, compared with the popularity of the breed. The Dalmatian did not present with any ammonium urate/uric acid uroliths in Hong Kong, the most likely reason for this being that the Dalmatian is of low popularity in that country. In addition, a number of other breeds also formed this stone type including the Shih tzu, Miniature schnauzer, Cocker spaniel and Pug. Although in non-Dalmatian dogs portal-systemic shunts are the most common cause (Rothuizen *et al.*, 1982), the underlying reason for stone-formation in these dogs is unknown. Cystine uroliths also form as a result of a genetically inherited abnormality and occurred in relatively few breeds, the majority of which have been previously documented as

being at risk of cystine formation including the Bulldog, Bull terrier and Mastiff (Osborne *et al.*, 1999a).

A large number of dog breeds were represented within the calcium oxalate and MAP groups. When comparing the occurrence of MAP or calcium oxalate in particular breeds with their popularity in Great Britain, several breeds were highlighted as being over-represented (RF>1). The majority of studies assessing breed as a risk factor for formation of specific mineral types were conducted in North America, and it is likely that the popularity of breeds in the US differs from that in Great Britain. Although certain breeds appear to be at increased risk of MAP from this study, there is little evidence in the literature to suggest that MAP urolithiasis is inherited. One recent study excluded breed as a factor in MAP formation in dogs (Ling *et al.*, 1998a), while another found that both Labrador retrievers and German shepherds, breeds found to commonly present with MAP, were at increased risk of recurrent or persistent urinary tract infections (Norris *et al.*, 2000), a factor known to contribute to MAP formation in dogs. It is possible that susceptibility towards urinary tract infections is an important risk factor for MAP formation in dogs.

A number of breeds appear to be consistently identified as being at increased risk of forming calcium oxalate uroliths, including the Yorkshire terrier, Bichon frise, Miniature Schnauzer, Jack Russell terrier, West Highland white terrier and Lhasa apso (Ling *et al.*, 1998a; Osborne *et al.*, 1999a; Lekcharoensuk *et al.*, 2000b). Also in agreement with this study, Labrador retrievers, Golden retrievers and German shepherds have been identified as breeds at low risk of calcium oxalate formation (Ling *et al.*, 1998a; Lekcharoensuk *et al.*, 2000b). The high incidence in certain breeds has led to the suggestion that some of the factors promoting the formation of

calcium oxalate uroliths in dogs may be inherited (Lulich *et al.*, 1999b). The familial predisposition of humans to calcium oxalate kidney stones suggests that genetic factors may be involved in the pathogenesis (Resnick *et al.*, 1968), although within a given family, it is difficult to disentangle a true genetic predisposition to form stones from environmental factors such as a common diet and lifestyle. In support of the possible importance of genetic predisposition in the formation of calcium-containing stones, studies involving the selective breeding of hypercalciuric rats to increase the intensity and frequency of hypercalciuria in their offspring has provided evidence for the importance of hereditary hypercalciuria as a risk factor for calcium stone-formation (Bushinsky *et al.*, 2000). Although a genetic basis has not been established as a cause of calcium oxalate formation in dogs, inherited differences in mineral metabolism and urine composition may provide an explanation for the increased occurrence of calcium oxalate urolithiasis in certain breeds of dog. This possibility will be further explored in Chapter 5.

There is also evidence from this study and previously reported findings to suggest that in cats the Persian is at increased risk of urolith formation compared to other pure bred cats (Willeberg and Priester, 1976; Hesse and Sanders, 1985; Kirk *et al.*, 1995; Thumchai *et al.*, 1996; Lekcharoensuk *et al.*, 2000a; Lekcharoensuk *et al.*, 2001). However, in one study examining the risk factors associated with lower urinary tract diseases in cats the authors deduced that cats with long hair, rather than specific breeds, were at increased risk, possibly because owners of long-haired cats were more likely to house them indoors for longer periods of time (Jones *et al.*, 1997). The possibility that genetically inherited characteristics may increase the risk of urolith formation within the Persian breed have yet to be explored.

3.5 Conclusions and clinical relevance

MAP was the most common urolith mineral type found in cats and dogs, although when the data series were split according to country, in dogs calcium oxalate uroliths were more prevalent than MAP stones in Italy, while in cats within the Netherlands calcium oxalate was more prevalent than MAP. It is possible that differences in the pattern of urolith formation observed between countries are due to demographic factors such as environment and lifestyle.

In dogs, MAP stones were more common in females and occurred across all ages, while all other urolith types were more common in males. Calcium oxalate uroliths tended to be found in older dogs. No trends with age and sex were detected for cats. Dogs with MAP and calcium oxalate were detected at an earlier age in Hong Kong than in all other countries, although the reasons for this remain unclear.

Breed distributions indicate that certain breeds of dog are at increased risk of developing specific urolith types. The breeds commonly forming calcium oxalate tended to be small, and have been previously documented as being at high risk of calcium oxalate formation, indicating the possibility of a genetically inherited disorder.

The trends identified in this study will assist in clarifying those groups at increased risk of developing urolithiasis. This will allow researchers to work with "at risk" breeds to identify and reduce urinary risk factors and therefore the chances of initial stone-formation. The information will also assist the veterinarian in the accurate diagnosis of urolithiasis in cats and dogs, thereby allowing implementation of an appropriate management strategy for the prevention of stone recurrence.

Appendix 3.1 Percentage of pedigree dogs by country

Country	Percentage pure bred dogs in population
Hong Kong*	72
Italy*	20
Netherlands*	65
Great Britain+	60

* supplied by MARS inc.

+ supplied by GFK marketing survey, 1998

Appendix 3.2 The top 20 new kennel club breed registrations in Great Britain in 2000

Dog Breed	Percentage of new registrations in 2000
Labrador retriever	14
German shepherd	7
Cocker spaniel	5
West Highland white terrier	5
Golden retriever	5
English springer spaniel	5
Cavalier King Charles spaniel	5
Staffordshire bull terrier	5
Boxer	4
Yorkshire terrier	3
Rottweiler	2
Border terrier	2
Shih tzu	2
Lhasa apso	1
Doberman	1
Bull terrier	1
Dalmatian	1
Miniature schnauzer	1
Bichon frise	1

Appendix 3.3 Percentage of pedigree cats by country

Country	Percentage pure bred cats in population
Hong Kong	71
Italy	<1
Netherlands	10
Great Britain*	10

* supplied by The Governing Council of the Cat Fancy, Bridgewater, UK.

All other data sources as dog statistics

Appendix 3.4 New cat breed registrations in Great Britain in 2001 (supplied by The Governing Council of the Cat Fancy)

Cat breed	Percentage of pure bred population
Persian	19
British short hair	16
Siamese	13
Burmese	10
Birman	7
Bengal	6
Ragdoll	5
Maine coone	4
Oriental short hair	4

CHAPTER 4. DIFFERENCES IN NUTRIENT INTAKE AND URINE COMPOSITION BETWEEN CALCIUM OXALATE STONE-FORMING DOGS AND NORMAL HEALTHY DOGS

4.1 Introduction

As discussed in Chapter 1, the proportion of calcium oxalate uroliths surgically removed from dogs and submitted for analysis at one centre within the US has increased over time from 5.3% in 1981 to 35.1% in 1997 (Lulich *et al.*, 1999b). The reasons behind this increase are not known. Over the years, as the understanding of the pathophysiology of struvite urolithiasis has increased, the management and prevention of this urolith type has improved. As a result, both struvite formation and the submission of struvite uroliths for analysis may have decreased, resulting in a proportional increase in calcium oxalate uroliths (Stevenson *et al.*, 2000b). Other possible reasons include the fact that dogs are living longer and calcium oxalate formation is documented to occur more commonly in older dogs (Lulich *et al.*, 1999b; Stevenson *et al.*, 2000b). Thus, it is logical to postulate that the incidence of calcium oxalate would increase. As the lifestyle of humans has changed within the developed World, a relative increase in calcium oxalate urolith formation has also been documented (Robertson, 1993). Common links in environmental factors between humans and dogs have yet to be identified.

Although it appears that canine calcium oxalate urolithiasis is increasing in prevalence, very few studies have (a) investigated the factors that may lead to stone-

formation in dogs and (b) examined the possible role of diet in the prevention of recurrence of calcium oxalate uroliths. In humans, calcium oxalate uroliths are thought to form as a result of one or more metabolic abnormalities that alter the composition of the urine. It has been demonstrated that human stone-formers pass more crystals in their urine than healthy subjects (Robertson *et al.*, 1969; Robertson *et al.*, 1971), and that the crystals tend to be larger and more aggregated (Robertson *et al.*, 1969; Robertson *et al.*, 1971; Kok *et al.*, 1990b). It has also been shown that periods of abnormal crystalluria trigger off urolith formation in both humans and rats (Vermeulen *et al.*, 1966; Robertson *et al.*, 1969; Werness *et al.*, 1981). The risk of forming these abnormal particles is determined by the degree of supersaturation of the urine with calcium oxalate, and the balance between the concentration of relevant promotive and inhibitory factors (Robertson and Nordin, 1976). In general, the urine of human calcium oxalate stone-formers is more supersaturated with calcium oxalate than that of normal subjects, and there is a strong correlation between the degree of supersaturation and the severity of the disease (Robertson and Nordin, 1976). The degree of calcium oxalate supersaturation in urine from dogs at or close to the time of calcium oxalate urolith formation has never been determined, although one study reported urine and serum metabolites in six Miniature Schnauzers with calcium oxalate urolithiasis (Lulich *et al.*, 1991a). In that study, the stone-forming dogs were compared with normal healthy beagles and found to excrete higher concentrations of calcium and uric acid and lower concentrations of oxalate (Lulich *et al.*, 1991a).

Another technique that has been used to distinguish human calcium oxalate stone-formers from normal healthy individuals is "risk factor" analysis. As described in Chapter 1, Section 1.6.6, this method was first developed by Robertson *et al* in

1978 (Robertson *et al.*, 1978), and involves the use of overlapping frequency distributions of urinary factors, which have been found to be significantly different between human stone-formers and normal subjects. It ignores the excretions that are not different between the two groups. Initially the model included six factors: urinary volume, pH, and the excretions of calcium, oxalate, uric acid, and Alcian blue-precipitable polyanions (a measure comprising most of the macromolecular anions thought to influence calcium oxalate crystal growth and agglomeration at that time). The overlapping frequency distributions of the six factors were used to generate a set of risk curves, which define the contribution of each factor to the overall risk of forming uroliths. The risk curves operate over the entire normal and abnormal range of each particular factor in urine. Using Bayes's theorem, a method for calculating overall probability from a combination of individual probabilities, (Lee, 1997), the contribution of each risk factor can be combined to give an overall measure of the relative risk of calcium urolith formation in an individual. More recently the model has been adapted to incorporate some of the less important risk factors including citrate, pyrophosphate and magnesium (Robertson, 1993).

In humans, it is generally accepted that diet significantly influences the concentration of a number of substances in the urine that are thought to be involved in calcium oxalate urolithiasis. Thus, diet may play a role in both the formation and prevention of recurrence of this disease (Assimos and Holmes, 2000). Diet can affect the concentration of minerals in the urine as a result of the nutrient content or through the amount of moisture the diet provides. It is documented that increased fluid intake results in production of a higher volume of urine with a reduced degree of calcium oxalate supersaturation in humans (Borghi *et al.*, 1996; Borghi *et al.*, 1999). The

other main dietary components thought to impact on the risk of calcium oxalate urolith formation in humans include calcium, oxalate, sodium, potassium, and animal protein. Although many studies have been conducted comparing dietary intakes of calcium oxalate stone-formers and healthy individuals the influence of diet has so far proved a difficult factor to evaluate in human stone disease. There is little consistency between studies, with a few showing no difference between normal and stone-forming subjects (Rao *et al.*, 1982), and the majority finding a variety of significant differences with few consistent findings between them (Fellstrom *et al.*, 1989; Trinchieri *et al.*, 1991; Curhan *et al.*, 1993; Curhan *et al.*, 1997; Zahrani *et al.*, 2000). It appears that attempts to investigate diet, as a possible contributing factor in calcium oxalate formation in dogs, have not yet been made.

This aim of this study was to compare the urine composition of calcium oxalate stone-forming (SF) dogs with clinically normal age- breed- and sex-matched control dogs (N), while on a 'free choice' diet (FC) or a standardised diet (S), to establish the influence of diet on the risk of calcium oxalate stone-formation. In addition, the information on the urine composition of the two dog groups was used to identify the urinary risk factors contributing to calcium oxalate stone-formation, and from these to derive a measure of the overall probability of forming calcium oxalate stones.

4.2 Materials and methods

4.2.1 Dogs

4.2.1.1 Stone-formers (SF)

Nineteen dogs with confirmed calcium oxalate urolithiasis were recruited into

the study. All uroliths were surgically removed, sent for quantitative analysis by infrared spectroscopy, as described in Chapter 2 (Gould *et al.*, 1995), and confirmed to be >70% calcium oxalate in composition. Individual dog details are given in Table 4.1. In summary, the panel consisted of 14 males (10 castrated and 4 sexually intact) and 5 females (all neutered) with a mean age of 9.1±2.1 years. All dogs lived in a home environment in Great Britain.

4.2.1.2 Control group (N)

Seventeen normal healthy dogs (as determined by the owner, and through a physical examination) were age- (to within 1 year), breed-, and sex-matched against 17 of the SF group, and used as the control group. A control dog was unable to be located to match two of the SF group, as shown in Table 4.1. As with the SF group all dogs lived in a home environment in Great Britain.

4.2.2 Dietary history

At the start of the study all owners recorded a seven-day diet diary on a standardised form during which time dogs all received a "free choice" diet (FC), typical of that usually received by the dog, as decided by the owner. This method of collecting dietary information has been used in humans and shown to be more accurate than an interviewer asking an individual to recall food eaten over a single 24-hour period. (Young *et al.*, 1952) All types and amounts of human and pet foods, snacks and treats consumed by the dog were recorded, including brand details. The average daily amounts of dietary moisture, protein, fat, sodium, potassium, calcium, magnesium, phosphorus, oxalate and energy consumed were calculated using typical nutrient analyses of all consumed foods. The typical analysis information used for

human foods was supplied by Dr. WG. Robertson, and the analysis information for dog foods was supplied by the Central Nutrition Laboratory, Pedigree Petfoods, Melton Mowbray. Assuming energy intake stayed constant between the FC and S diets, the daily intake of nutrients was also calculated for both groups when receiving a standardised diet (S).

Table 4.1 Dog panel details

Dog number	**Breed**	**Age (years)**	**Sex**	**Status**	**Control**
1	Yorkshire terrier	8	M	SI	N
2	West Highland white terrier	11	M	N	Y
3	Flat coated retriever	7	M	N	Y
4	Miniature schnauzer	11	M	N	Y
5	Springer spaniel	12	M	SI	Y
6	Cavalier King Charles spaniel	11	M	N	Y
7	West Highland white terrier	10	M	N	Y
8	Lhasa apso	5	M	SI	Y
9	Cairn terrier	9	F	S	Y
10	Miniature poodle	12	M	N	N
11	Jack Russell terrier	10	M	N	Y
12	Shih tzu	5	M	SI	Y
13	Shih tzu	7	F	S	Y
14	Border collie	10	M	N	Y
15	Yorkshire terrier	11	M	N	Y
16	Scottish terrier	8	F	S	Y
17	Small cross breed	8	F	S	Y
18	Small cross breed	9	M	N	Y
19	Norfolk terrier	9	F	S	Y

M=male; F=female; S=spayed; N=neutered; SI sexually intact

Y=yes; N=no

4.2.3 Study design

Between days 3 and 7 a 24-hour sample of urine was collected while the dog was on the FC diet and the diet diary was being recorded. As far as possible, all urine naturally voided by the dogs was collected by the owner, into a sterile container and immediately transferred into a dry ice-chilled container, as described in Chapter 2. At the end of the seven days the dogs were then switched to the standardised Diet (WALTHAM™ Veterinary Diet: Canine Lower Urinary Tract Support (canned), manufactured in Bruck, Austria) (Appendix 4.1). Dogs received this diet for either 1 month (N) or 1 year (SF). Repeat urine samples were collected from all dogs after 1 month and in addition, from the SF group at 3 months, 6 months and 1 year.

4.2.4 Urinary measurements

Urine samples were defrosted overnight in a fridge. Each sample was titrated to pH2 with 37% hydrochloric acid, frozen and stored at –20°C. Samples were prepared and analysed by methods described in Chapter 2. Urinalysis data were then entered into a computer program, SUPERSAT for the calculation of calcium oxalate RSS, as described in Chapter 2.

4.2.5 Blood samples

A fasted blood sample was taken from the jugular vein of 12 dogs in the SF group only while on the FC diet, and plasma calcium concentrations were measured using a Cobas MIRA Plus Biochemistry Analyser (Roche Diagnostic Systems, 1080 US Hwy #202, Branchburg, NJ 08876-1760).

4.2.6 Statistical analysis

Results were expressed as means±standard deviations. The majority of the data generated within this study followed a non-normal distribution, and therefore a number of non-parametric methods were used for statistical analysis (Siegel, 1956; Zar, 1999).

1. To compare urine parameters between the N and SF groups a Mann-Whitney *U* test was used.

2. To compare urine parameters of the N group while on the FC and S diet, the Wilcoxon signed-rank test was used.

3. To investigate the effect of diet and time on the urine parameters of the SF group the Friedman analysis of variance test was used.

4. To compare the nutrient intakes of either the N or SF group on the FC and S diet a Mann-Whitney *U* test was used.

5. To compare the nutrient intakes of the N and SF groups the Wilcoxon signed- rank test was used.

In all cases, $P<0.05$ was considered significant.

4.2.7 Risk factor analysis

Using a method modified from Robertson *et al*, 1978 (Robertson *et al.*, 1978), the frequency distributions of three urine parameters that were seen to differ between SF and N were used to calculate the "risk" of being a calcium oxalate stone-former at each value of the parameters concerned. A set of "risk curves" for the variables was produced, and used to calculate the overall "relative probability" of being a stone-former.

4.3 Results

4.3.1 Dietary compliance and urine sample collection

4.3.1.1 SF group

Urine samples were collected from all 19 dogs within the SF group while on the FC diet and after 1 month on the S diet. However, 3 dogs (Dog numbers 1, 11 and 13) were removed from the study before the 3-month collection because the owners wanted to offer their dogs additional foodstuffs. A further 2 dogs within the SF group were removed from the trial between 6 and 12 months. Dog 5 formed gall bladder stones for reasons unknown, and Dog 10 started refusing to eat the diet. No dog suffered a recurrence of any urolith formation over the duration of the study.

4.3.1.2 N group

Urine samples were collected from 17 normal healthy dogs on the FC diet and 13 of these dogs on the S diet. The owners of dogs 8, 13, 15 and 16 would not feed the S diet *solus* for 1 month, and so these samples were not collected.

4.3.2 Nutrient intakes

4.3.2.1 Comparison between SF and N on FC

When comparing the SF and N groups on the FC diet, the SF group had a significantly lower daily intake of dietary sodium (P=0.025), calcium (P=0.03), potassium (P=0.012) and phosphorus (P=0.007), and a higher oxalate:calcium ratio

(P=0.006), (Table 4.2). Dietary moisture (P=0.08) and protein (P=0.07) also tended to be lower in the SF group, although these differences were not significant.

4.3.2.2 Comparison between SF and N on S diet

There were no differences in daily nutrient intakes between the SF and N groups on the S diet (Table 4.2).

4.3.2.3 Comparison of FC and S diets within the SF group

Dietary moisture (P=0.01), fat (P=0.0002) and sodium (P=0.0002) were significantly lower when the SF group received FC, compared with S (Table 4.2). In contrast, dietary potassium (P=0.006), calcium (P=0.015) and magnesium (P=0.01) and the calcium:phosphorus ratio (P=0.0002) were significantly higher when the SF group received the FC, compared with S. Dietary protein also tended to be higher on the FC compared to S, although this difference was not significant (P=0.053). Dietary oxalate and phosphorus intake were unaffected by the change of diet.

4.3.2.4 Comparison of FC and S diets within the N group

Dietary protein (P=0.001), potassium (P=0.0001), calcium (P=0.0001), magnesium (P=0.001), phosphorus (P=0.001) and the calcium:phosphorus ratio (P=0.001) were significantly higher in the N group when receiving the FC diet compared with S (Table 4.2). However, dietary fat (P=0.001), sodium (P=0.001), and the oxalate:calcium ratio (P=0.008) were significantly lower when the N group received the FC diet, compared to N. Daily oxalate and moisture intake were unaffected by the change of diet.

Table 4.2 Mean daily nutrient intake (±SD) per kg body weight of calcium oxalate stone-forming dogs (SF) and normal healthy control dogs (N) on a "free choice" diet (FC) or a standardised diet (S)

Nutrient (per kg body weight per day)	FC		S	
	SF	N	SF	N
Moisture (g)	15 ± 13^{a1}	29 ± 23^{a1}	32 ± 24^{b1}	32 ± 17^{a1}
Protein (g)	4 ± 2^{a1}	6 ± 3^{a1}	3 ± 2^{a1}	2 ± 1^{b1}
Fat (g)	2 ± 1^{a1}	3 ± 2^{a1}	5 ± 3^{b1}	5 ± 3^{b1}
Energy (kcal)	73 ± 53^{1}	71 ± 38^{1}		
Sodium (mg)	77 ± 40^{a1}	123 ± 61^{a2}	237 ± 174^{b1}	232 ± 122^{b1}
Potassium (mg)	103 ± 45^{a1}	168 ± 76^{a2}	78 ± 57^{b1}	77 ± 40^{b1}
Calcium (mg)	191 ± 127^{a1}	305 ± 156^{a2}	118 ± 87^{b1}	116 ± 61^{b1}
Magnesium (mg)	13 ± 7^{a1}	19 ± 13^{a1}	11 ± 8^{a1}	11 ± 6^{b1}
Phosphorus (mg)	114 ± 72^{a1}	208 ± 109^{a2}	113 ± 83^{a1}	111 ± 58^{b1}
Oxalate (mg)	6 ± 4^{a1}	6 ± 4^{a1}	5 ± 3^{a1}	5 ± 2^{a1}
Oxalate:Calcium	0.04 ± 0.04^{a1}	0.02 ± 0.01^{a2}	0.04 ± 0.00^{a1}	0.04 ± 0.00^{b1}
Calcium:Phosphorus	1.73 ± 0.67^{a1}	1.54 ± 0.48^{a1}	1.05 ± 0.00^{b1}	1.05 ± 0.00^{b1}

Different superscript letters in a row indicate a difference between diets within a dog group

Different superscript numbers in a row indicate a difference between dog groups on the same diet.

SF = stone-forming dog group; N = normal dog group

FC = free choice diet

4.3.3 Urinary measurements

4.3.3.1 Calcium oxalate RSS

Calcium oxalate RSS was significantly higher in the SF group than the N group irrespective of diet, although the difference between the groups was greater on the FC diet ($P<0.0001$) than on the S diet ($P=0.016$); (Table 4.3; Figure 4.1). Within the N group calcium oxalate RSS was not significantly affected by changing the diet from FC to N ($P=0.096$). In contrast, calcium oxalate RSS was significantly reduced ($P<0.0001$) when the SF group received the S diet at all timepoints compared to the FC diet. Calcium oxalate RSS was not significantly different between 1 month, 3 months, 6 months and 1 year of being fed the S diet.

4.3.3.2 Urine specific gravity

Urine specific gravity tended to be higher in the SF group, although when receiving the FC diet the SF and N groups were not significantly different ($P=0.12$); (Table 4.3). When the diet was changed to S, the SF group produced urine with a significantly higher specific gravity than the N group ($P=0.04$), although both groups produced urine with a lower specific gravity when on the S diet when compared to the FC diet.

4.3.3.3 Urinary concentrations

Urinary calcium concentration was significantly higher in the SF group when compared to the N group irrespective of diet, although the difference was greater on the FC diet ($P<0.0001$) than when receiving the S diet ($P=0.016$); (Table 4.3, Figure 4.2). Within the N group urinary calcium concentration was not significantly affected

by changing the diet from FC to S (P=0.18). In contrast, the change from FC to S resulted in a significant decrease in urinary calcium concentration in the SF group across all timepoints (P<0.0001). Urinary calcium concentration was not significantly different between 1 month, 3 months, 6 months and 1 year of being fed the S diet. Urinary oxalate concentration was significantly higher in the SF group compared to the N group on the FC diet only (P=0.049), (Table 4.3, Figure 4.3). When the groups changed to the S diet, oxalate concentration was not significantly different (P=0.17). Within the N (P=0.024) and SF (P=0.036) groups, oxalate concentration decreased when the dogs were moved from the FC diet to S, although within the SF group the difference was only significant from 3 months.

Urinary phosphate concentration was lower in the SF group when on the FC diet only (P=0.01). Although urinary sodium concentration was not significantly different between the SF and N groups on either diet, both groups demonstrated an increase in urinary sodium concentration when the diet changed from FC to N. However, the groups were not significantly different after 1 month on the S diet. Uric acid tended to be higher in the SF group than the N group on the FC diet, although the difference was not significant. When the diet was changed from FC to S urinary uric acid concentration decreased although this was only significant in the N group (P=0.035) due to the high variability within the SF group. Citrate, magnesium, ammonium, sulphate and potassium concentrations were not significantly different between the SF and N groups when fed the FC or S diet.

Table 4.3 Urine composition (±SD) of calcium oxalate stone-forming dogs (SF) on a free choice diet (FC) and after 1 month, 3 months, 6 months and one year of receiving a standardised diet, compared with normal healthy control dogs (N) receiving a FC diet or the standardised diet for 1 month

Parameter	SF					N	
	FC	1 mo	3 mo	6 mo	1 yr	FC	1 mo
CaOx RSS	21.08 ±15.26^{b1}	7.79 ±7.16^{a1}	7.05 ±4.56^{a}	6.94 ±4.12^{a}	5.10 ±2.86^{a}	4.09 ±2.02^{a2}	2.38 ±1.36^{a2}
Ca (mmol/l)	2.61 ±1.40^{b1}	1.27 ±0.86^{a1}	1.10 ±0.68^{a}	1.04 ±0.57^{a}	0.76 ±0.45^{a}	0.87 ±0.51^{a2}	0.64 ±0.24^{a2}
Oxalate (mmol/l)	1.19 ±0.54^{b1}	0.90 ±0.74^{b1}	0.82 ±0.44^{a}	0.83 ±0.35^{a}	0.87 ±0.38^{a}	0.86 ±0.39^{a2}	0.55 ±0.40^{b1}
Na (mmol/l)	100 ±60^{a1}	181 ±68^{b1}	165 ±55^{b}	151 ±68^{ab}	143 ±85^{ab}	106 ±61^{a1}	188 ±69^{b1}
Uric acid (mmol/l)	0.61 ±0.98^{a1}	0.36 ±0.13^{a1}	0.33 ±0.13^{a}	0.35 ±0.14^{a}	0.38 ±0.21^{a}	0.54 ±0.67^{a1}	0.31 ±0.16^{a1}
Citrate (mmol/l)	0.74 ±1.10^{a1}	0.51 ±0.54^{a1}	0.30 ±0.26^{a}	0.46 ±0.48^{a}	0.52 ±0.59^{a}	0.82 ±1.07^{a1}	0.24 ±0.27^{b1}
Mg (mmol/l)	3.22 ±1.93^{a1}	2.15 ±1.35^{a1}	1.92 ±0.91^{a}	2.00 ±1.32^{a}	1.98 ±1.89^{a}	2.13 ±1.43^{a1}	2.37 ±1.59^{a1}
PO_4 (mmol/l)	22.20 ±14.84^{a1}	29.96 ±14.00^{a1}	26.00 ±11.81^{a}	25.31 ±10.18^{a}	24.68 ±12.86^{a}	36.27 ±16.36^{a2}	30.86 ±19.22^{a1}
K (mmol/l)	63.42 ±31.41^{b1}	45.34 ±21.81^{b1}	38.11 ±22.24^{a}	41.64 ±21.17^{a}	45.28 ±23.33^{b}	89.51 ±51.82^{a1}	46.32 ±25.99^{b1}
Specific gravity	1.027 ±0.009^{b1}	1.021 ±0.009^{b1}	1.019 ±0.008^{a}	1.016 ±0.009^{a}	1.016 ±0.01^{a}	1.024 ±0.008^{b1}	1.014 ±0.006^{a2}

Key as Table 4.2; CaOx = calcium oxalate; mo = month; yr = year

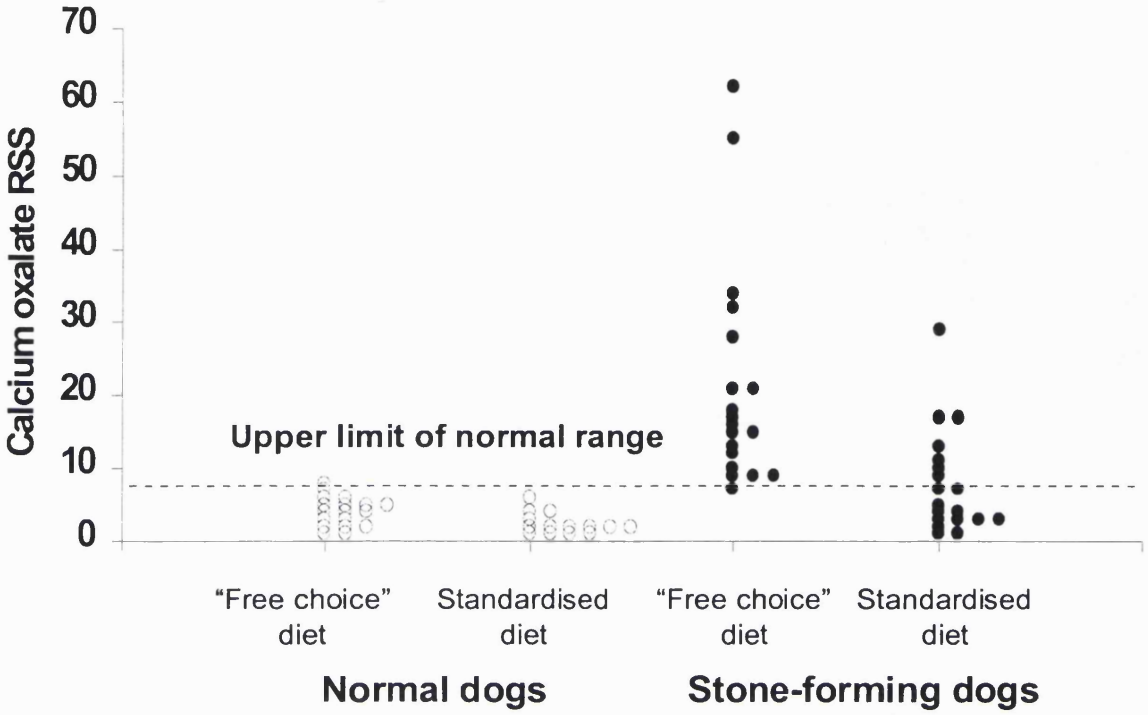


Figure 4.1 Calcium oxalate relative supersaturation (RSS) produced by normal and calcium oxalate stone-forming dogs fed either a "free choice" diet or a standardised diet

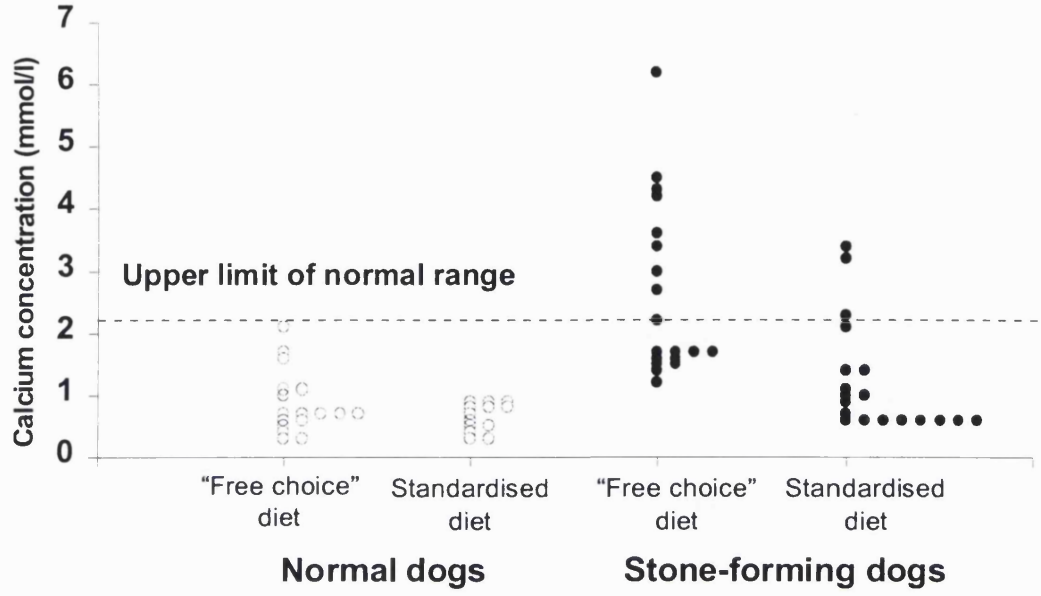


Figure 4.2 Urinary calcium concentration (mmol/l) produced by normal and calcium oxalate stone-forming dogs fed either a "free choice" diet or a standardised diet

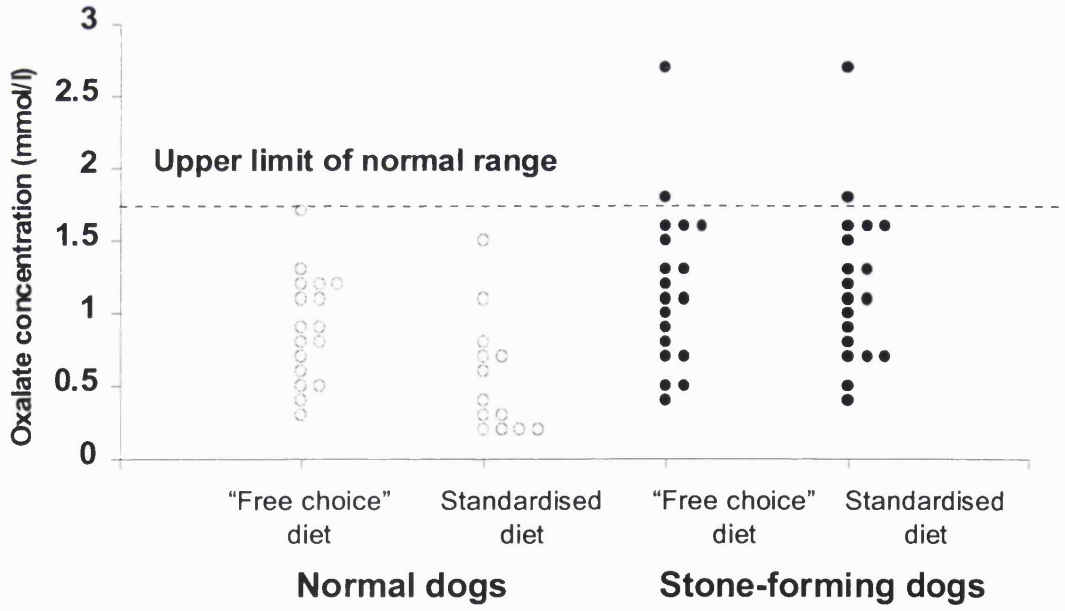


Figure 4.3 Urinary oxalate concentration (mmol/l) produced by normal and calcium oxalate stone-forming dogs fed either a "free choice" diet or a standardised diet

4.3.3.4 Urinary excretions (per mmol creatinine)

When converted into daily excretion ratios (mmol per mmol of creatinine), urinary calcium was significantly higher in the SF group when compared to the N group on the FC diet only (P=0.0003), (Table 4.4). In addition, the SF group showed a significant decrease in urinary calcium excretion when the diet changed from FC to S (P=0.028). Urinary calcium excretion of the N group was unaffected by the change in diet (P=0.11). Urinary oxalate and uric acid excretions were unaffected by group or diet. Urinary sodium excretion, although unaffected by group, increased significantly when the dogs changed from FC to S (N; P=0.004; SF, P=0.02).

4.3.5 Blood samples

The plasma calcium concentration of all dogs fell within the normal range of 2–3mmol/l identified by Bush, 1991 (Bush, 1991), with the exception of Dog 3 whose serum calcium concentration was 1.5mmol/l. The mean value (±SD) for the 12 dogs sampled was 2.56±0.41mmol/l.

4.3.6 Risk factor analysis

From a comparison of the urine composition of the N and SF groups on the FC diet the two parameters that were significantly different between the groups were calcium and oxalate concentrations. Although not significant, uric acid concentration also tended to be higher in the SF group, and therefore this parameter was also included in the risk factor analysis. The frequency distributions of these parameters in SF and N on the FC diet are shown in Figure 3.4a,b and c. Although the mean values for calcium and oxalate concentrations are significantly different between the two groups there is a large degree of overlap between them. However,

Figure 4.4 shows that the higher the urinary concentration of calcium, oxalate and uric acid, the greater the risk of being a calcium oxalate stone-former.

Table 4.4 Urinary excretions (±SD) of calcium, oxalate, uric acid and sodium (mmol/mmol creatinine [C]) produced by calcium oxalate stone-forming dogs (SF) on a free choice diet (FC) and after 1 month, 3 months, 6 months and one year of receiving a standardised diet, compared with normal healthy control dogs (N) receiving a FC diet or the standardised diet for 1 month

Parameter	**SF**					**N**	
	FC	**1 mo**	**3 mo**	**6 mo**	**1 yr**	**FC**	**1 mo**
Ca (mmol/mmol C)	0.40 ±0.30^{b1}	0.26 ±0.18^{a1}	0.24 ±0.14^{a}	0.25 ±0.12^{a}	0.18 ±0.12^{a}	0.14 ±0.11^{a2}	0.17 ±0.12^{a1}
Oxalate (mmol/mmol C)	0.19 ±0.17^{a1}	0.15 ±0.12^{a1}	0.18 ±0.10^{a}	0.20 ±0.10^{a}	0.20 ±0.12^{a}	0.13 ±0.08^{a1}	0.12 ±0.10^{a1}
Uric acid (mmol/mmol C)	0.10 ±0.17^{a1}	0.07 ±0.05^{a1}	0.07 ±0.03^{a}	0.08 ±0.03^{a}	0.08 ±0.06^{a}	0.09 ±0.15^{a1}	0.07 ±0.03^{a1}
Na (mmol/mmol C)	14.07 ±9.11^{a1}	34.21 ±12.5^{b1}	36.99 ±15.08^{b}	35.54 ±16.67^{b}	28.73 ±11.52^{b}	17.34 ±11.88^{a1}	43.51 ±22.15^{b1}

Key as Table 4.2, C = creatinine

Figure 4.5a,b and c show a possible way of quantifying this risk of calcium oxalate urolith formation as urinary calcium, oxalate or uric acid concentrations increase. A ratio (α) of the frequency of SF / frequency of N was calculated for each concentration of urinary calcium concentration. This ratio has been termed by Robertson *et al*, 1978, the "relative risk factor", and the curve of, for example, αCalcium (αCa) against urinary calcium is termed the "risk curve" for calcium.

These three parameters are shown together in Figure 4.6, in which the relative risk (α) attributable to each constituent is plotted in relation to the number of standard deviations above and below the mean value for each parameter in the N group. On this basis it is possible to compare the relative importance of each risk factor in the formation of calcium oxalate stones in dogs. Figure 4.6 shows that urinary oxalate and urinary calcium are the two most critical factors for calcium oxalate formation within this group of dogs. The relative risk factors ($\alpha_{Ca}\alpha_{Ox}\alpha_{UA}$) may be combined into an overall probability of being a calcium oxalate stone-former (P_{SF}) by using the equation derived from Bayes's theorem:

$$P_{SF} = \frac{\alpha_{Ca}\alpha_{Ox}\alpha_{UA}}{(1 + \alpha_{Ca}\alpha_{Ox}\alpha_{UA})}$$

The value of P_{SF} lies between 0 and 1. By measuring these three parameters in dog urine it is possible to obtain a measure of the risk of that dog forming calcium oxalate stones.

Figure 4.7 shows the relative probability of forming calcium oxalate stones (P_{SF}) within the N and SF groups of dogs on the FC and S diets used in this study. There is a good correlation between the P_{SF} and calcium oxalate RSS, with the urine sample (correlation coefficient=0.78; r^2=61.6%, P<0.0001 for the FC diet data), although, when compared to calcium oxalate RSS (Figure 3.1), the P_{SF} does not give as clear a distinction between the SF and N groups. Similar to the observations seen with calcium oxalate RSS, P_{SF} was significantly higher in the SF group than the N group on both the FC diet (P<0.0001) and S diet (P=0.004). The P_{SF} declined within both groups when the diet changed from FC to S, although the decrease was more marked in the SF group (P<0.0001) than in the N group (P=0.03).

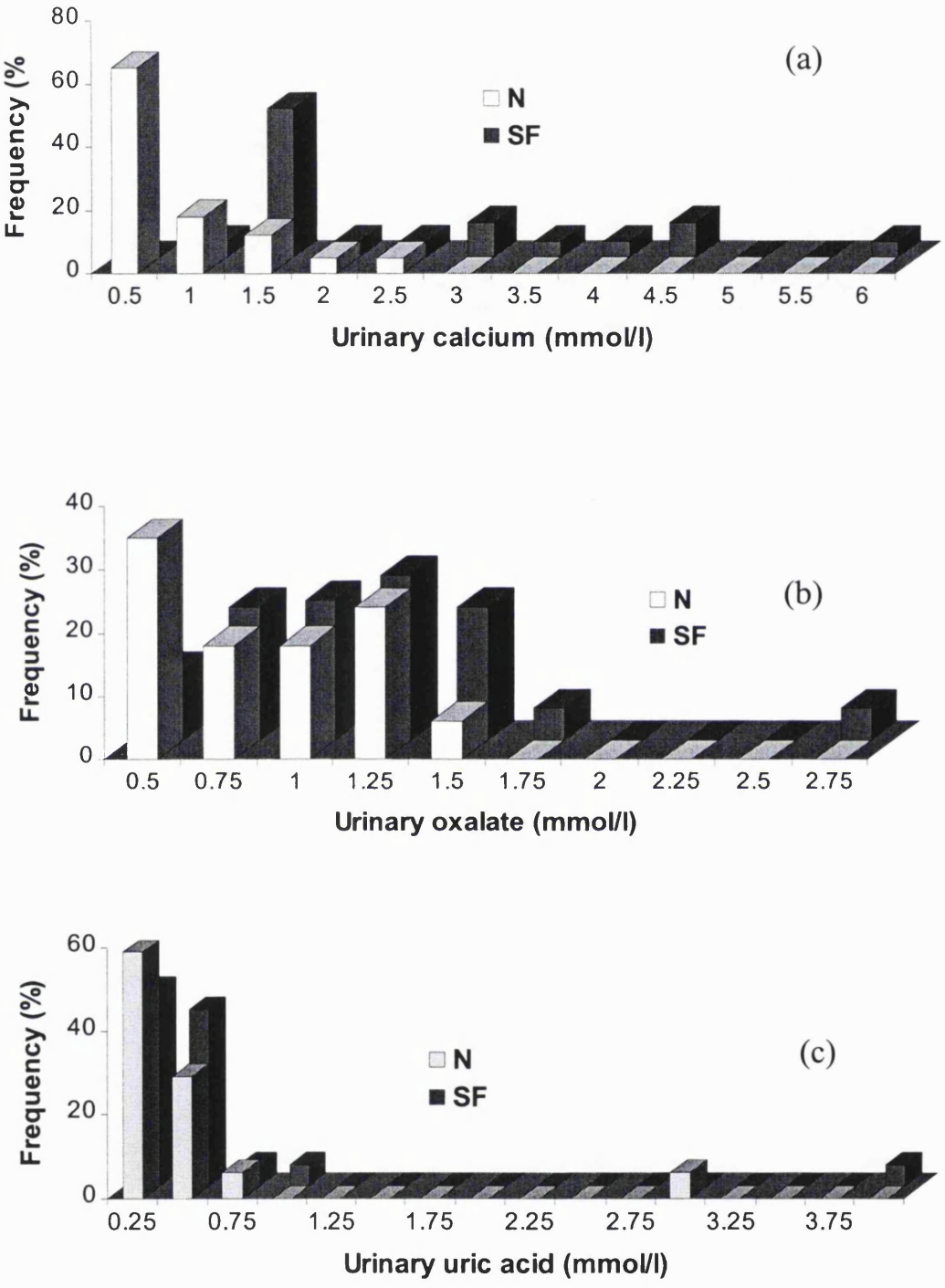


Figure 4.4 Frequency distributions for urinary concentrations (mmol/l) of calcium (a), oxalate (b) and uric acid in normal (N) and stone-forming dogs receiving a free choice diet

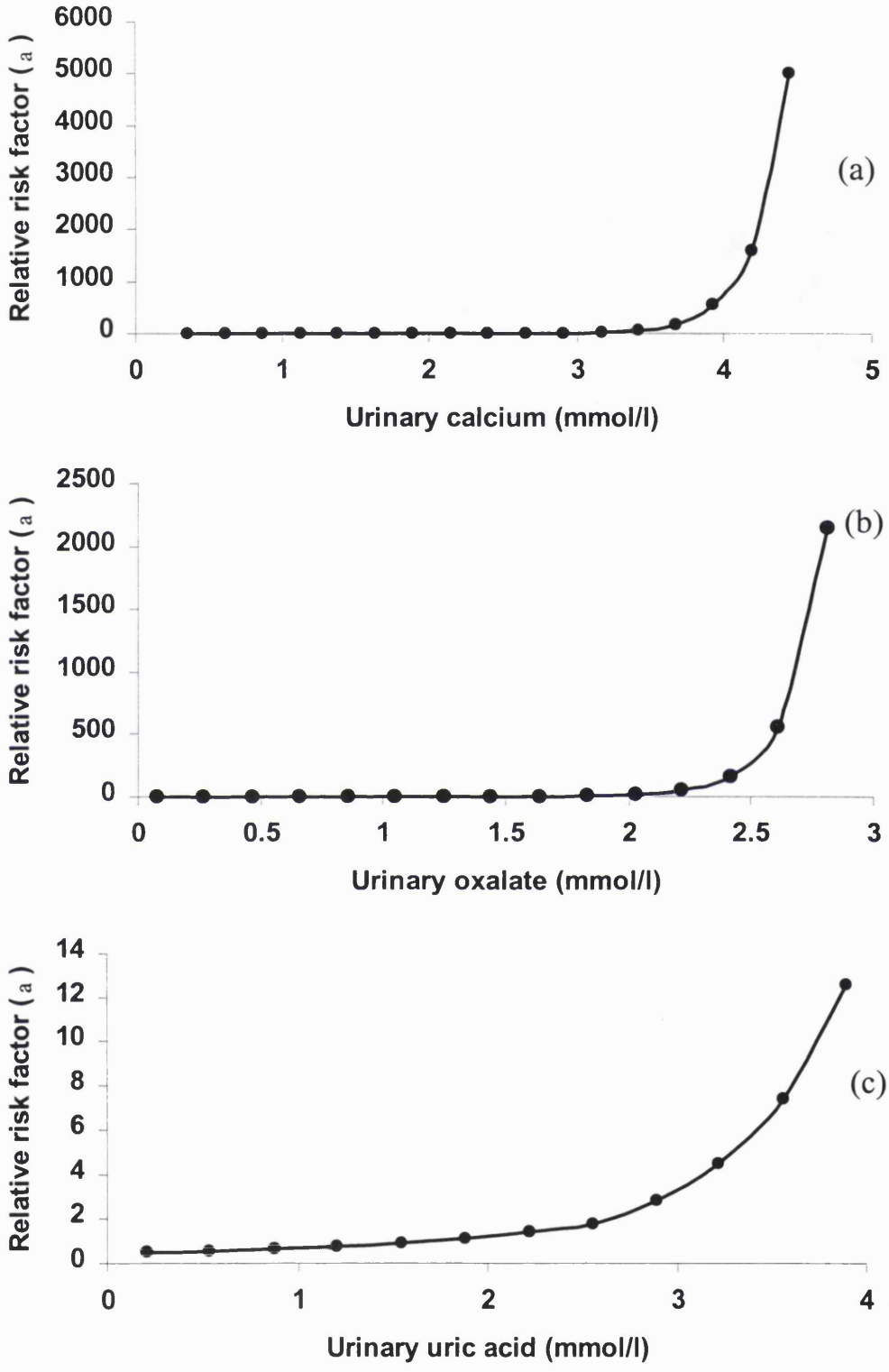


Figure 4.5 The relative risk factor (α) for calcium oxalate formation at various urinary concentrations of calcium (a), oxalate (b) and uric acid concentrations (c)

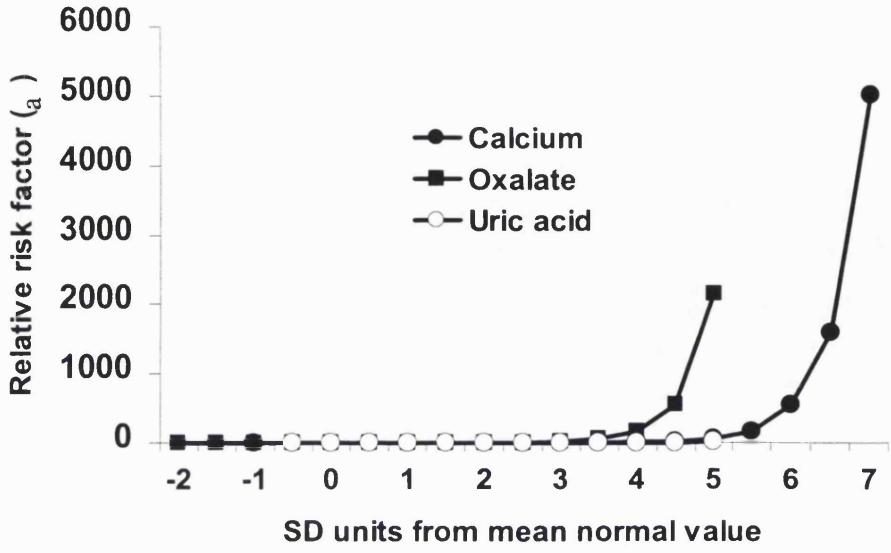


Figure 4.6 The risk curves for urinary calcium, oxalate and uric acid concentration in relation to the number of SD units around the mean value for the normal group of dogs

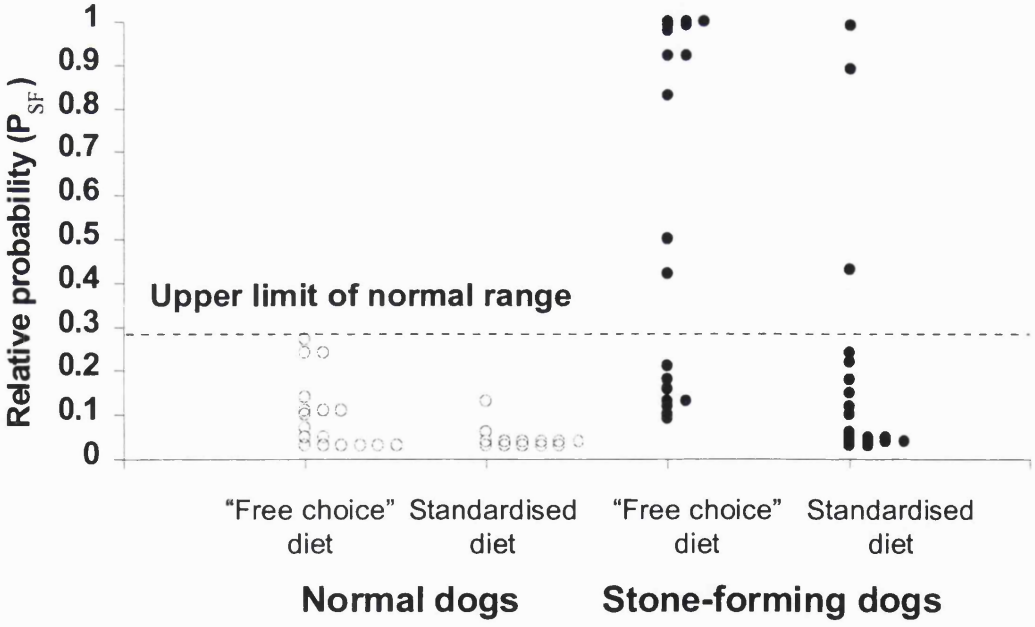


Figure 4.7 The relative probability (P_{SF}) of calcium oxalate stone-formation in normal and calcium oxalate stone-forming dogs on a "free choice" or standardised diet.

4.4 Discussion

A number of significant differences were observed between the dietary nutrient intakes of the SF and N groups while on the FC diet. Some of these differences may at least partially contribute to the calcium oxalate formation observed in the SF group. The dietary moisture intake of the SF group was significantly lower than that of the N group. It is well documented that a high moisture intake will result in the production of a more dilute urine with a lower concentration of minerals (Borghi *et al.*, 1996; Borghi *et al.*, 1999; Assimos and Holmes, 2000). Thus, a lower dietary moisture intake may increase the concentration of calculogenic minerals within the urine, thereby increasing the risk of stone-formation. Although specific gravity is a measure of urinary concentration, there was no significant difference between the two dog groups when the FC diet was fed, although the SF group had a tendency towards higher values. Twenty-four-hour urine volume would have been a better measure of daily water turnover; however, a reliable measure of this parameter was not possible within the home environment. Even so, it is unlikely that these factors alone fully explain why the SF group formed stones and the N group did not.

Dietary calcium intake was also lower in the SF group, when compared to the N group on the FC diet, while dietary oxalate intake remained the same between the groups. In humans, a diet low in calcium has been shown to increase the risk of calcium oxalate formation because of the passive intestinal absorption of oxalate in the colon followed by an increase in urinary oxalate (Zarembski and Hodgkinson, 1969; Hodgkinson, 1978). In some human studies a high dietary calcium actually decreased the risk of calcium oxalate formation (Marshall *et al.*, 1972; Curhan *et al.*, 1993; Curhan *et al.*, 1997). Nevertheless, urinary calcium is dependent upon dietary

calcium; this relationship is more pronounced in individuals who are hyperabsorbers of calcium (Lemann *et al.*, 1979; Robertson, 1993). In this study, the SF group were found to have a lower dietary calcium intake, yet produced urine with a significantly higher calcium concentration compared to that of the N group. Although decreased dietary moisture may partially explain the increase in urinary calcium concentration through production of a more concentrated urine, calcium excretion (mmol/mmol creatinine) was also increased indicating that the increase in urinary calcium concentration was not only the result of low urine volume. Although dietary intake of calcium was low, the dogs excreted more calcium, thereby providing evidence that dogs forming calcium oxalate stones are hyperabsorbers of calcium. However, the serum calcium concentrations of the stone-forming dogs were normal suggesting that the increased urinary calcium occurs in the absence of hypercalcaemia. Due to the limitations of this study, the precise mechanisms behind the hyperabsorption and resulting hypercalciuria could not be ascertained. Another study comparing six Miniature schnauzers that formed calcium oxalate stones with healthy beagles also found that the stone-forming dogs excreted more urinary calcium (Lulich *et al.*, 1991a), providing further evidence that hypercalciuria is a factor contributing towards calcium oxalate urolith formation in dogs.

Although dietary oxalate intake was not significantly different between the two groups of dogs on the FC diet, the urinary concentration of oxalate was higher in the SF group. It is thought that non-absorbed dietary calcium, complexes with anions such as phosphate, citrate, sulphate and oxalate, rendering them unavailable for absorption (Menon *et al.*, 1998). As a result of either (or both) the reduced calcium intake (and therefore the increased oxalate:calcium ratio), or the hyperabsorption of

calcium, the amount of oxalate bound to calcium within the colon may have decreased, and therefore the amount available for absorption by the SF increased, resulting in an increased concentration of oxalate in the urine. These findings contradict the results of the study conducted by Lulich *et al*, 1991, who observed that the six Miniature schnauzers that formed calcium oxalate stones excreted less urinary oxalate than healthy beagles. However, in the Lulich study all dogs received the same diet at the time of urine assessment. In the study reported here, when the N and SF dogs were all moved onto the S diet urinary oxalate was not different between groups.

Diet itself is likely to be a factor in calcium oxalate formation, and cannot be ignored when examining the causes of calcium oxalate formation in dogs. Dietary phosphate can also bind with calcium leaving both ions unavailable for absorption (Menon *et al.*, 1998). However, dietary phosphorus was lower in the SF group than the N group on the FC diet. Indirectly this may have contributed to the increase in dietary calcium absorption within the SF group. Urinary phosphate was also lower in this group than the N group when on the FC diet.

Uric acid concentrations also tended to be higher in the SF group than in the N group on the FC diet. This finding is consistent with the study by Lulich *et al*, 1991, in which the Miniature schnauzers that formed calcium oxalate stones excreted more uric acid than the normal healthy beagles. Hyperuricosuria is also a common finding in human calcium oxalate stone-formers (Coe and Raisen, 1973), although the role of uric acid in calcium oxalate stone-formation remains unclear. One possible mechanism that has been proposed is that increased uric acid may encourage heterogeneous nucleation on the surface of sodium or ammonium urate crystals (Coe *et al.*, 1975; Meyer, 1981). However, this seems unlikely since uric acid and urates

are rarely found as coprecipitates with calcium oxalate in canine stones. Another possible explanation is that uric acid binds macromolecular calcium oxalate inhibitors found in the urine, such as glycosaminoglycans or Tamm-Horsfall protein, thereby reducing the inhibitory capacity of the urine for prevention of calcium oxalate crystal formation (Robertson, 1993). Unfortunately, within the limits of the study reported here it was not possible to identify any differences in the content or composition of urinary macromolecular inhibitors in the dog groups. In humans, a diet high in animal protein (and therefore purines) contributes towards an increase in uric acid excretion (Coe *et al.*, 1976). Indeed, uric acid concentration decreased in both groups when the diet was changed from FC to S, which did contain a lower amount of protein. However, uric acid excretion (mmol/mmol creatinine) did not change, and thus the decrease in uric acid concentration is most likely to be as a result of decreased urinary concentration.

Together, the increased urinary calcium and oxalate concentrations resulted in an increased calcium oxalate RSS in the SF group when compared to the N group on the FC diet. At this time the mean calcium oxalate RSS was above the estimated formation product for this stone type (RSS~12) falling within the zone of oversaturation; comparable with data from human calcium oxalate stone-formers (Robertson *et al.*, 1971; Borghi *et al.*, 1996; Parks *et al.*, 1997; Milosevic *et al.*, 1998; Borghi *et al.*, 1999).

The S diet is designed to contain a relatively low concentration of protein, and a moderate concentration of fat and sodium. It is therefore logical to note these differences in intakes between the S and FC diets when fed to both groups of dogs. The format of the diet is also designed to deliver a high concentration of dietary

moisture with the objective of increasing urine volume. When the SF group received the S diet, dietary moisture intake increased and urine specific gravity decreased indicating the production of a more dilute urine. Due to the higher moisture intake of the N group on the FC diet, no difference in moisture intake was observed when these dogs received the S diet, although specific gravity was reduced to a concentration significantly lower than that of the SF group. The calcium intake of both groups was reduced on the S diet, compared with FC, although the urinary calcium concentration of the N group was not significantly affected by the dietary change. In contrast, the change of diet from FC to S significantly decreased urinary calcium (concentration and excretion). This effect was likely to be a combination of increased urinary dilution and reduced dietary calcium. Urinary oxalate concentration was also reduced in both groups by the change from FC to S. Since dietary oxalate intake remained similar between the FC and S diets, and the daily excretion of oxalate (mmol/mmol creatinine) was unchanged, the reduction in urinary oxalate concentration was probably due to urinary dilution.

Together these factors resulted in a significant decrease in the urinary calcium oxalate RSS of the SF group to within the zone of metastable supersaturation (RSS 1-12). Spontaneous homogeneous crystallisation will not occur at this concentration, indicating a reduction in the risk of recurrence of calcium oxalate uroliths in these dogs. This difference was maintained through the 12 months of feeding the S diet. Recurrence rates for urolithiasis in dogs are poorly documented. One study reported a recurrence rate of 25% (Brown *et al.*, 1977), although the time over which dogs were monitored is not clearly defined, and not all dogs were available for a follow-up evaluation. In the study by Lulich *et al*, 1991 (Lulich *et al.*, 1991a), 4 out of the 6

Miniature schnauzers with calcium oxalate stones had a recurrence within 1 year, and the remaining 2 dogs were unavailable for follow-up evaluation. It is therefore likely that the risk of recurrence of calcium oxalate urolithiasis is high in dogs if no form of intervention is attempted in order reduce the severity of risk factors contributing to the formation of this urolith type. This study demonstrates the effectiveness of a simple dietary change in reducing the risk of recurrence of calcium oxalate formation in the majority of affected dogs.

When assessing the risk factors associated with calcium oxalate formation this study attempted to define the most significant risk factors involved in calcium oxalate formation in dogs. The most widely used method for assessing the risk of calcium oxalate formation within the human field is RSS (Werness *et al.*, 1985; Milosevic *et al.*, 1998). However, this method involves the analysis of up to 12 different urinary components, which is not practical for the practising clinician or veterinarian. Therefore, from the comparison of urine from normal and stone-forming dogs, the three parameters that differed between these groups were combined into a measure of relative probability of stone-formation (P_{SF}), in an attempt to establish whether these would be sufficient to measure the risk of calcium oxalate stone-formation in dogs. Although the results show a good correlation between RSS and P_{SF}, RSS still more clearly defined the dogs in the N group compared to the SF group. At this time P_{SF} would not be considered a suitable substitute for RSS as a tool for assessing the risk of calcium oxalate formation in dogs. However, compared to studies in humans (n per group between 50 and 100) (Robertson *et al.*, 1978; Robertson *et al.*, 1981a), the numbers of dogs used in this study were small. It is possible that further factors may become apparent if numbers of dogs studied within each group increased, and this

would allow more accurate definition of the P_{SF}.

4.5 Conclusions and clinical relevance

Diet may at least partly contribute to the formation of calcium oxalate stones in dogs. Although affected dogs were receiving a diet lower in calcium around the time of calcium oxalate formation than normal dogs, this group appeared to hyperabsorb calcium, resulting in hypercalciuria. Indirectly this also led to hyperoxaluria and the increased risk of calcium oxalate stone-formation. Urinary calcium and oxalate concentrations are the major risk factors for calcium oxalate formation in dogs, although uric acid concentration was also elevated in some dogs. A measure of the relative probability of stone-formation from these three urinary parameters gave good distinction between the affected and normal dogs, although RSS still more clearly defined the two groups.

Appendix 4.1 Nutrient analysis of the standardised diet (S)

Nutrient	**Unit**	**Amount (per 100 kcal)**
Moisture	g	17.00
Protein	g	1.30
Fat	g	2.8
Ash	g	0.60
Calcium	g	0.06
Phosphorus	g	0.06
Ca:P ratio	g/g	1.00
Sodium	g	0.12
Potassium	g	0.08
Magnesium	g	0.005
Iron	mg	0.45
Copper	mg	0.04
Manganese	mg	0.21
Zinc	mg	1.50
Oxalate	mg	2.47

Published in American Journal of Veterinary Research, 62,11:1782-1786, 2001.

CHAPTER 5. THE EFFECT OF BREED ON URINE PARAMETERS IN DOGS

5.1 Introduction

Uroliths composed primarily of MAP or calcium oxalate are the mineral types found most commonly in dogs. MAP represented approximately 50% of canine uroliths submitted for analysis over a 17-year period at one centre (Osborne *et al.*, 2000b). The underlying cause of MAP formation in the majority of dogs is the presence of a urinary tract infection with urease producing bacteria such as *Staphylococcus intermedius* or *Proteus* spp. (Osborne and Lees, 1995). Calcium oxalate was the second most common mineral type found in uroliths submitted to this centre, making up 31% of the total (Osborne *et al.*, 2000b). Calcium oxalate uroliths can be pure, but more commonly present in combination with variable amounts of calcium phosphate, or less commonly, MAP or ammonium acid urate (Osborne *et al.*, 2000b). The proportion of calcium oxalate uroliths submitted has increased over time from 5.3% in 1981 to 35.1% in 1997 (Lulich *et al.*, 1999b). The reasons behind this increase are not known. Over the years, as the understanding of the pathophysiology of MAP urolithiasis has increased, so has effective management and prevention of this urolith type. As a result, both MAP formation and the submission of MAP uroliths for analysis may have decreased, resulting in a proportional increase in calcium oxalate uroliths (Stevenson *et al.*, 2000b). Other possible reasons include the fact that

dogs are living longer and calcium oxalate formation is documented to occur more commonly in older dogs (Lulich *et al.*, 1999b; Stevenson *et al.*, 2000b). Thus, it is logical to postulate that the incidence of calcium oxalate uroliths would increase. As the lifestyle of humans has changed within the developed World, a relative increase in calcium oxalate urolith formation has also been documented (Robertson, 1993), although common links in environmental factors between humans and dogs have yet to be identified.

Another predisposing factor for calcium oxalate formation is breed of dog. Although 120 breeds were affected in the data compiled by one analysis centre in North America, 58% of calcium oxalate uroliths occurred in only 6 breeds, with 25% occurring in Miniature Schnauzers (Lulich *et al.*, 2000; Osborne *et al.*, 2000b). Likewise, data from uroliths analysed by other centres within the USA (Ling *et al.*, 1998a), UK (Stevenson *et al.*, 2000b), Germany (Hesse, 1990) and Sweden and Norway (Wallerstrom and Wagberg, 1992), also found certain breeds of dog presented more commonly with calcium oxalate urolithiasis. When examining these breeds it becomes apparent that this condition occurs almost exclusively in small and toy breed dogs such as the Miniature Schnauzer, Cairn terrier, Yorkshire terrier, Bichon frise, Lhasa apsoa, Pekingese, Papillon, Maltese terrier and Cavalier King Charles spaniel. This high incidence in certain breeds has led to the suggestion that some of the factors promoting the formation of calcium oxalate uroliths in dogs may be inherited (Lulich *et al.*, 1999b). Genetic abnormalities have been identified as factors in the formation of other less common canine uroliths such as urate and cystine (Schaible, 1986; Casal *et al.*, 1995). Likewise, the familial predisposition of humans to calcium oxalate kidney stones suggests that genetic factors are involved in the pathogenesis (Resnick

et al., 1968). The transmission of nephrolithiasis, hypercalciuria and hyperoxaluria through generations of people indicates that they are likely to be inherited traits, although expression depends upon factors including, sex, age and diet (Baggio *et al.*, 1998). Additionally, the selective breeding of hypercalciuric rats increased the intensity and frequency of hypercalciuria in the offspring, and provided evidence for hereditary hypercalciuria (Bushinsky *et al.*, 2000). Although a genetic basis has not been established as a cause of calcium oxalate formation in dogs, inherited differences in mineral metabolism and urine composition may provide an explanation for the increased occurrence of calcium oxalate urolithiasis in certain breeds of dog.

The purpose of this study was initially to compare urine composition in healthy dogs of two breeds fed the same dogfood. Miniature schnauzers (MS) were selected as a breed predisposed towards calcium oxalate urolithiasis, and compared with a breed rarely found to form calcium oxalate (Labrador retriever [LR]). Parameters compared included the frequency of urination, urine pH, volume and specific gravity, and urinary concentrations of calcium, oxalate and phosphate. Urinary relative supersaturation (RSS) with calcium oxalate, brushite (calcium hydrogen phosphate, dihydrate) and MAP (defined as the activity product / solubility product) were also calculated. During the second phase of this study the urine composition of urine from LR was also compared with two other small breed dogs, Cairn terriers (CT), and Cocker spaniels (CS).

5.2 Materials and Methods

5.2.1 Dogs

5.2.1.1 Phase 1

Sixteen healthy adult dogs consisting of 8 LR (2 sexually intact females, 6 spayed females; mean age 3.1±1.7 years) and 8 MS, (4 sexually intact females, 3 spayed females, 1 castrated male; mean age 3.7±1.3 years) were used in the study. Two dogs from each breed were from the same litter; individuals from both breeds were sired from 4 different dogs.

5.2.1.2. Phase 2

A further 23 healthy adult dogs, consisting of 8 LR (3 sexually intact females, 1 castrated male, 4 spayed females; mean age 2.5±0.2 years), 7 CT (5 spayed females, 2 castrated males; mean age 7.0±1.7 years) and 8 CS (2 sexually intact females, 6 sexually intact males; 1.4±0.4 years) were used. Again, the dogs were selected to ensure that they were not closely related.

5.2.2 Study design

In both phases of the study dogs were individually fed a nutritionally complete dry dog food once daily from 10.30am for 24 days. Although the same brand of food was used in both phases, different batches were used. Hence the nutrient profile varies slightly between the two phases (Appendix 1). Food allowances were calculated according to adult maintenance energy requirements (110 $W^{0.75}$ kcal per day, where W is body weight expressed in kg) (Burger, 1995b), and adjusted during the study to ensure body weight maintenance within ±5% of original weight. Daily

food intake and faeces quality (Moxham, 2001) were recorded throughout the trial. All dietary nutrients were analysed as described in Chapter 2. Water was provided *ad libitum*.

5.2.3 Housing details

5.2.3.1 Phase 1

Dogs were housed as described in Chapter 2 for six 48-hour periods over days 3-4, 7-8, 11-12, 15-16, 19-20 and 23-24 to enable collection of naturally voided urine. During the remaining days the dogs were housed in pairs. Throughout this time all the dogs were walked once daily for approximately 15 minutes and group-exercised in grass paddock areas for 1 - 2 hours.

5.2.3.2 Phase 2

Dogs were housed as described in Chapter 2 for one 48-hour period in week 3. During the remaining days the dogs were housed in pairs. During this time all the dogs were walked once daily for approximately 15 minutes and group-exercised in grass paddock areas for 1 - 2 hours.

5.2.4 Urine measurements

5.2.4.1 Phase 1

While the dogs were individually housed, urine pH was continuously measured as described in Chapter 2. Immediately after each naturally voided urination the sample was collected into a glass bottle and individually processed. The time and frequency of urination was noted; urine volume and specific gravity were

recorded. Each sample was then titrated to pH2 with 37% hydrochloric acid (BDH Laboratory Supplies, Poole, Dorset), frozen and stored at –20°C. Samples were prepared and analysed as described in Chapter 2. Urinalysis data were then entered into a computer program, SUPERSAT (Robertson *et al.*, in press), which calculated RSS values for calcium oxalate, brushite and struvite.

5.2.4.2 Phase 2

One 48-hour urine sample was collected from each dog, immediately frozen, and analysed as described in Chapter 2. Urinary RSS values for calcium oxalate, brushite and MAP were calculated using SUPERSAT.

5.2.5 Blood samples

During phase 1 dogs were fasted overnight on day 24. A blood sample was collected from the jugular vein and analysed for haematology (Baker system 9000 automated cell counter, Serono Baker Diagnostics, Allentown, Pennsylvania), biochemistry (Cobas Mira Plus Biochemistry Analyser, Roche Diagnostics, Branchburg, New Jersey) and blood gas parameters (AVL Omni Blood Gas System, AVL Medical Instruments Ltd, Graz, Austria).

5.2.6 Statistical analyses

5.2.6.1 Phase 1

Data from each urination were grouped together for each dogs and expressed as mean values (±SD), so that n=8 for each breed. Unpaired two sided t-tests were used to assess the effect of breed on urine volume, specific gravity and pH, frequency of urination, urinary RSS for calcium oxalate, MAP and brushite, urinary

concentrations of calcium, phosphate, and oxalate, and blood parameters. Data were also compiled into mean (±SD) diurnal profiles with data from each breed grouped into two-hour blocks. The number of data points varied from 4 to 20 between blocks; data within each block were compared using unpaired two sided t-tests. For all statistical tests the concentration of significance was set at $P<0.05$.

5.2.6.2 Phase 2

Analysis of variance and multiple range tests (least significant difference) were used to assess the effect of breed on urinary RSS of calcium oxalate, MAP and brushite, and urinary concentrations of calcium, phosphate, and oxalate.

5.3 Results

5.3.1 Food intake and bodyweight maintenance

Through both phases all food offered to the dogs was consumed every day. Bodyweight remained stable throughout the trial with an overall weight change of 0.4% during phase 1 and 3% in phase 2.

5.3.2 Dietary mineral intakes

5.3.2.1 Phase 1

The average daily intakes of calcium, phosphorus, sodium, potassium, magnesium (g per kg body weight per day), oxalate (mg per kg body weight per day), and energy (kcal per kg body weight per day) were significantly higher ($P<0.05$) in the MS than in the LR (Table 5.1). When converted to intakes per kg metabolic body weight ($BWT^{0.75}$) no differences were found between the two breeds.

5.3.2.2 Phase 2

As in phase 1, the daily mineral intakes of the small breeds were significantly higher than the LR per kg body weight per day (Table 5.2). Again, when converted to intakes per kg metabolic body weight ($BWT^{0.75}$) no differences were found between the small breeds and the LR.

Table 5.1 Phase 1 mean (±SD) daily mineral and energy intakes per kg body weight

Breed	Mineral intake (g)					Intake (mg)	Energy intake
	Ca	P	Na	K	Mg	Oxalate	(kcal)
LR	0.24 ±0.02[a]	0.19 ±0.01[a]	0.04 ±0.01[a]	0.12 ±0.01[a]	0.02 ±0.00[a]	2.58 ±0.18[a]	62±4[a]
MS	0.54 ±0.02[b]	0.43 ±0.02[b]	0.09 ±0.01[b]	0.28 ±0.01[b]	0.04 ±0.00[a]	5.90 ±0.26[b]	81±10[b]

LR = Labrador Retriever. MS=Miniature Schnauzer

Ca = calcium, P = phosphorus, Na = sodium, K = Potassium, Mg = magnesium

Within a column values with different superscripts are significantly different ($P<0.05$)

Table 5.2 Phase 2 mean (±SD) daily mineral and energy intakes per kg body weight

Breed	Mineral intake (g)					Intake (mg)	Energy intake
	Ca	P	Na	K	Mg	Oxalate	(kcal)
LR	0.21 ±0.02[a]	0.19 ±0.01[a]	0.04 ±0.01[a]	0.09 ±0.01[a]	0.01 ±0.001[a]	4.10 ±0.29[a]	46±6[a]
CT	0.28 ±0.02[b]	0.25 ±0.02[b]	0.06 ±0.01[b]	0.12 ±0.01[b]	0.02 ±0.01[b]	5.43 ±0.49[b]	69±8[b]
CS	0.40 ±0.08[c]	0.36 ±0.07[c]	0.08 ±0.02[c]	0.17 ±0.03[c]	0.03 ±0.01[c]	7.85 ±1.57[c]	97±13[c]

LR = Labrador Retriever, CT=Cairn Terrier, CS=Cocker Spaniel; Key as Table 5.1

Within a column values with different superscripts are significantly different ($P<0.05$)

5.3.3 Urinary measurements

5.3.3.1 Phase 1

The MS urinated significantly less often over each 24 hour period (P=0.002), (MS ranged between 0.6 and 1.8 urinations per 24 hours; LR ranged between 1.5 and 4.5 urinations per 24 hours) producing a low volume of urine (P=0.04) with a significantly higher urine pH (P=0.007) than the LR (Table 5.3). The mean diurnal urine pH profiles indicated that the MS maintained a higher urine pH than the LR across a 24-hour period (Figure 5.2). Urine specific gravity was not significantly different between the breeds (P=0.06; Table 5.3). Nevertheless, the mean diurnal profile suggested that the urine from the MS tended to have a higher specific gravity than that of the LR for the majority of the 24-hour period (Figure 5.1).

5.3.3.2 Phase 2

Apart from urinary mineral concentrations and RSS measurements no other urine parameters were measured during Phase 2.

5.3.4 Urinary mineral concentrations

5.3.4.1 Phase 1

No significant differences in urinary concentrations of phosphate (P=0.06) or oxalate (P=0.09) were detected between breeds. Urinary calcium concentration was significantly higher in urine produced by the MS (P=0.009) than in that produced by the LR (Table 5.3).

5.3.4.2 Phase 2

Urinary calcium concentration was significantly higher in the urine of the CT and CS, compared to the LR (P=0.0001). The CT urine also contained a higher calcium concentration than the CS (Table 3). Urinary oxalate (P=0.045) and phosphate (P=0.0003) concentrations were significantly higher in the urine of the CS, when compared to the LR and CT.

5.3.5 Urinary RSS

5.3.5.1 Phase 1

The urine of both breeds was undersaturated with struvite (RSS<1.0). There was no significant difference in struvite RSS between the LR and MS. Calcium oxalate supersaturation was not significantly different between the LR and MS (Table 2). The MS produced urine with a significantly higher brushite RSS than the LR (P=0001) (Table 5.3).

5.3.5.2 Phase 2

The urine of all three breeds (CT, CS, LR) was undersaturated with respect to struvite. There was no difference in struvite RSS between the breeds. Calcium oxalate (P=0.00001) and brushite (P=0.003) RSS were significantly higher in the urine produced by the CT and CS when compared with the LR (Table 5.4).

5.3.6 Blood parameters

The majority of blood parameters measured in phase 1 remained within the reference ranges for normal healthy dogs, although two MS produced creatinine values above the WCPN reference range, and one MS produced a total calcium

concentration below the WCPN reference range. However, these values were normal compared with normal ranges for these parameters quoted by Bush, 1991. Total and ionised serum calcium concentrations were unaffected by breed (Table 5.5).

5.4 Discussion

The results from phase 1 of this study show that MS urinated significantly less often and had a lower urine volume than LR. Urine specific gravity was not significantly different between the breeds, although it tended to be higher in the MS (P=0.06). These data suggest that more concentrated urine was retained for a greater length of time in the bladder of the MS; factors that could increase the likelihood of crystal nucleation and subsequent crystal growth and aggregation. Within the human field many workers have examined the risk factors for calcium oxalate urolithiasis (Rao *et al.*, 1982; Fellstrom *et al.*, 1989; Hesse *et al.*, 1993). The risk factor model developed by Robertson (1993) identified low urine volume as the most important risk factor for stone-formation in humans (Robertson, 1993). Excretion rates of most of the constituents of calcium containing stones are independent of urinary flow, therefore, any decrease in urine volume would increase the concentrations of calcium, phosphate and oxalate in the urine. A decrease in urine volume may increase the concentration of crystallisation inhibitors, although this effect will largely be offset by a concurrent increase in promoters. The net balance of these effects is an increased risk of mineral crystallisation and stone-formation.

The main factors in humans leading to a low urine volume are a low fluid intake, percutaneous losses (minimal in dogs) or fluid losses through diarrhoea (Robertson, 1993). The dogs in this study all produced well-formed faeces, therefore, the likely cause of the lower urine volume of the MS was a lower water intake.

Table 5.3 Phase 1 mean (±SD) daily urine volume, urine specific gravity, urinary relative supersaturation (RSS) for calcium oxalate and brushite, and urinary concentrations of calcium, oxalate and phosphate

Breed	Mean urine pH	Urine volume (ml/kg BWT/day)	Specific gravity	Number of urinations per day	RSS		Urinary concentrations (mmol/l)		
					CaOx	B	Ca	Ox	PO_4
LR	6.14 ±0.34[a]	22 ±15[b]	1.023 ±0.010[a]	2.9±1.1[b]	4.60 ±1.66[a]	0.47 ±0.23[a]	0.61 ±0.23[a]	1.16 ±0.48[a]	63.03 ±22.27[a]
MS	6.52 ±0.18[b]	12 ±3[a]	1.030 ±0.008[a]	1.5±0.5[a]	5.31 ±1.62[a]	1.22 ±0.31[b]	0.93 ±0.25[b]	0.82 ±0.22[a]	79.25 ±15.24[a]

LR = Labrador Retriever, MS = Miniature Schnauzer

BWT = body weight, CaOx = calcium oxalate, B = brushite

Ca = calcium, Ox = oxalate, PO_4 = phosphate

Within a column values with different superscripts are significantly different ($P<0.05$)

Table 5.4 Phase 2 mean (±SD) urinary relative supersaturation (RSS) of calcium oxalate and brushite, and urinary concentrations of calcium, oxalate and phosphate

Breed	RSS		Urinary Concentrations (mmol/l)		
	CaOx	B	Ca	Ox	PO_4
LR	6.55±4.35[a]	0.38±0.31[a]	0.54±0.24[a]	1.34±0.99[a]	62.25±41.61[a]
CT	21.52±2.56[c]	1.06±0.46[b]	2.06±0.46[c]	1.54±0.42[a]	73.68±11.61[a]
CS	15.38±6.42[b]	1.52±0.75[b]	1.59±0.34[b]	2.19±0.34[b]	148.03±44.14[b]

LR = Labrador Retriever, CT=Cairn Terrier, CS=Cocker Spaniel

Key as Table 5.3

Within a column values with different superscripts are significantly different ($P<0.05$)

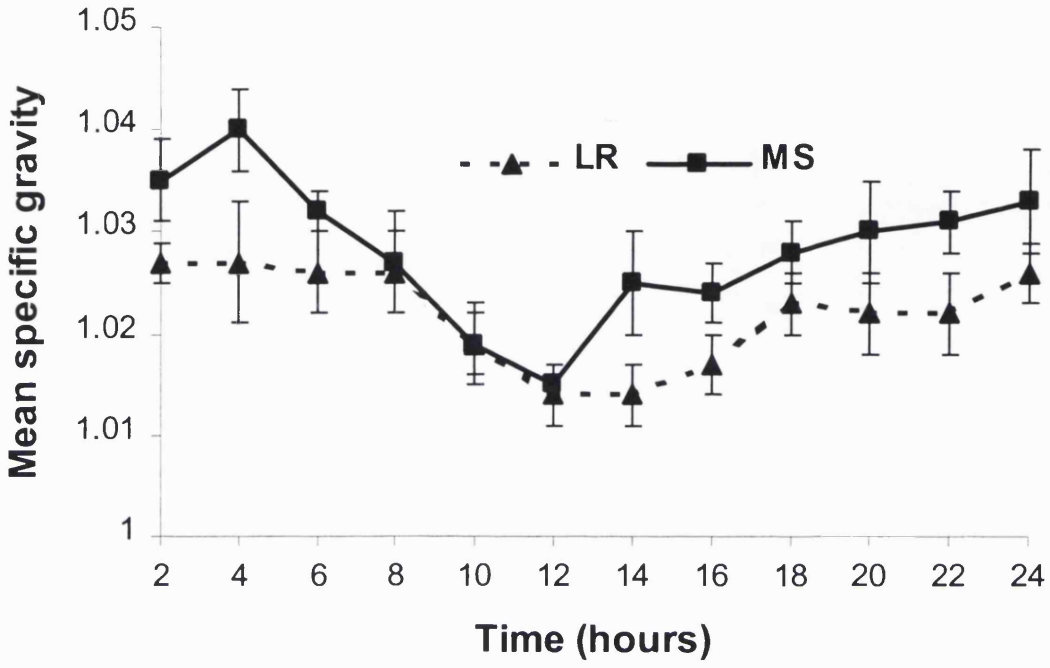


Figure 5.1 Mean diurnal specific gravity profile (±SD) for 8 Labrador retrievers and 8 Miniature Schnauzers fed a commercially prepared dogfood for 24 days

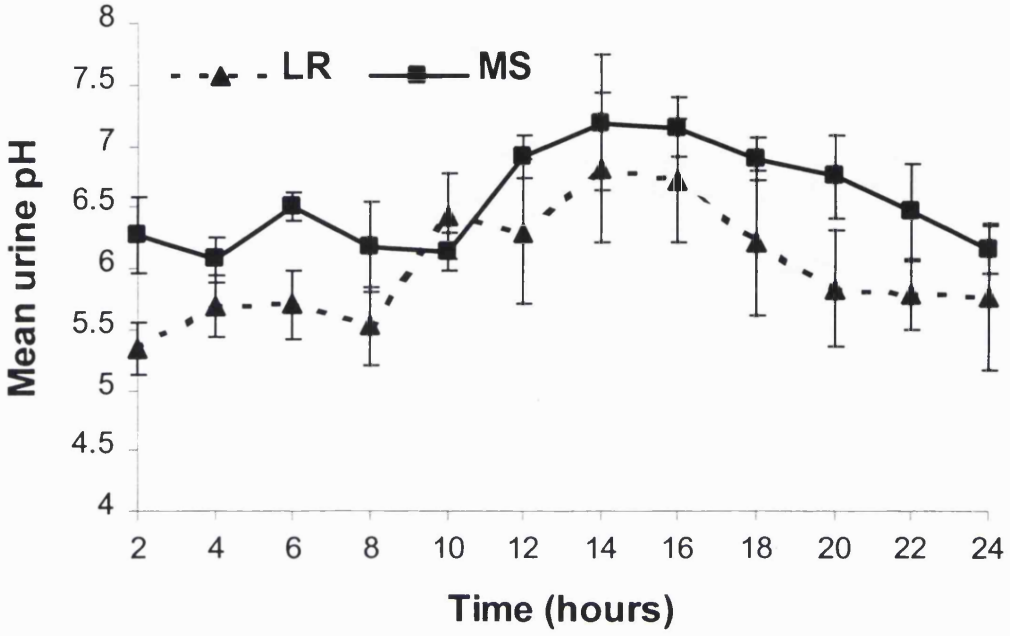


Figure 5.2 Mean diurnal urine pH profile (±SD) for 8 Labrador retrievers and 8 Miniature Schnauzers fed a commercially prepared dogfood for 24 days

Table 5.5 Phase 1 haematology, biochemistry and ionised calcium profiles

Parameter	Units	Labrador retrievers		Miniature Schnauzers		WCPN reference ranges (unless otherwise stated)
		Mean	SD	Mean	SD	
Alkaline phosphatase	**U/l**	49.21	10.76	47.32	12.57	21-146
Cholesterol	**mmol/l**	5.61	1.26	5.46	0.61	3.7-8.59
ALT	**U/l**	46.91	17.37	45.82	9.60	19-80
AST	**U/l**	19.79	9.11	27.42	12.54	14-41
Urea	**mmol/l**	5.62	1.04	4.71	0.98	3.31-6.75
Creatinine	**u/mol**	94.87	9.93	99.79	8.60	51-99 40-130 (Bush, 1991)
Phosphate	**mmol/l**	1.21	0.11	1.40	0.14	1.01-3.01
Total calcium	**mmol/l**	2.63	0.11	2.40	0.06	2.52-3.09 2.2-2.7 (Bush, 1991)
Protein	**g/l**	56.75	2.83	55.57	1.58	48.4-69.9
Albumin	**g/l**	33.58	1.69	34.56	2.14	27.1-36.2
White blood cells	**10^9/l**	9.54	3.35	10.48	3.26	6.1-14.5
Red blood cells	**10^{12}/l**	6.83	0.83	7.03	0.51	5.14-7.9
Haemoglobin	**g/l**	164.4	17.5	156.75	12.78	121-180
Haematocrit	**%**	41.5	2.8	44.7	4.9	35-53
Mean corpuscular volume	**fl**	68.11	2.15	62.79	1.64	63.5-71.6
pH	**pH**	7.34	0.03	7.37	0.02	7.31-7.42
Sodium	**mmol/l**	147.03	2.42	144.04	0.92	140-155
Potassium	**mmol/l**	4.42	0.14	3.99	0.15	3.7-5.8
Ionised calcium	**mmol/l**	1.42	0.03	1.38	0.04	-

However, it can also be postulated that the MS had an increased requirement for calories when compared to LR because of a higher activity concentration, leading to increased insensible water loss. Although no formal measure of energy expenditure was made, the increased calorie intake required by the MS (and also CT and CS in phase 2) to maintain body weight was more likely to be a function of an increased surface area to volume ratio, as with other small breed dogs. If these urinary characteristics are typical of MS and other small dog breeds, they may contribute to the increased risk of calcium oxalate urolithiasis recognised in this group of dogs.

Increased urinary calcium concentration may also be a factor leading to calcium stone-formation in dogs. The MS examined in phase 1 of this study had significantly higher urinary calcium concentrations that the LR, inferring an increased risk of calcium oxalate crystallisation. This observation was even more marked in the follow-up study (Phase 2) examining CS and CT, compared to LR, and contributed to the significantly higher calcium oxalate RSS observed in both small breeds compared with LR, in this phase. In a previous study, urinary calcium concentrations in a group of 16 calcium oxalate stone-forming dogs averaged 2.33±1.30 mmol/l, considerably higher than concentrations reported in healthy dogs (Markwell *et al.*, 2000; Stevenson *et al.*, 2000a). Urinary calcium oxalate RSS averaged 20.43±16.12 in the dogs around the time of stone-formation, indicating the presence of urine in a state of labile supersaturation (oversaturation), in which spontaneous nucleation of calcium oxalate is likely. Although the urinary calcium produced by the MS in this study was higher than that of the LR, the concentrations were lower than those seen in the stone-forming dogs. Hypercalciuria is a common disorder encountered in around 60% of human subjects with urolithiasis (Pak, 1991). This condition is an important risk

factor for the formation of calcium uroliths (oxalate and phosphate) since it will result in increased urinary supersaturation with calcium oxalate and calcium phosphate (Pak, 1991; Menon *et al.*, 1998). The small dogs (MS, CT and CS) had a significantly higher intake of dietary calcium per kg body weight, than the LR, which may have caused the increase in urinary calcium. However, there were no significant increases in the concentrations of the majority of other urinary minerals, despite equally high dietary intakes. Thus, although increased dietary calcium may contribute, it is likely that other mechanisms are facilitating the increase in urinary calcium seen in this breed. From phase 1 of this study, it is apparent that the increased urinary calcium occurred in the absence of hypercalcaemia, as indicated by the blood results. Further research is required to determine the nature of these mechanisms.

The MS in this study tended to have lower urinary oxalate concentrations (P=0.09) than the LR, despite a higher intake (Table 1). A high urinary oxalate excretion has been linked to calcium oxalate stone-formation, and hyperoxaluria can be detected in up to 50% of humans with calcium oxalate stones (Baker *et al.*, 1996). In one human risk factor assessment, hyperoxaluria was second only to low urine volume as a risk factor for stone-formation (Robertson, 1993). In contrast, there appear to be no reports of hyperoxaluria in calcium oxalate stone-forming dogs. Indeed, in one previous study reporting urine parameters in dogs, daily urinary oxalate excretion was found to be significantly lower in stone-forming MS compared with healthy beagles (Lulich *et al.*, 1991a). Thus, urinary oxalate may not be a major factor driving calcium oxalate stone-formation in dogs. However, in phase 2 the CS had a significantly higher urinary oxalate concentration than the LR and CT. Although the underlying mechanims for this remain unclear, this breed also had a

significantly higher dietary intake of oxalate, and this may contribute to the increased urinary oxalate concentration observed in this breed.

The specific gravity measured over 24 hours in phase 1 of this study showed a mild increase in the urine collected overnight (between 7.00pm and 7.00am), an effect that was more pronounced in the MS (Figure 2). Studies examining diurnal changes in humans found that the greatest risk of calcium oxalate formation occurred overnight (Ogawa, 1994). During that period urine volume decreases, while concentration increases and body temperature is also at its lowest, causing an increase in urine supersaturation, and hence, increase in the potential for crystallisation and stone growth in susceptible individuals (Ogawa *et al.*, 1983). It is likely that diurnal profiles in dogs are affected by similar factors.

Urinary pH is not constant and fluctuates during the course of a 24-hour period (Figure 1). In phase 1 of this study the same trends were noted in both breeds with increased urine pH during the day, peaking between two and five hours after feeding (Figure 1). This effect was thought to be partly attributable to a post-prandial alkaline tide and partly to increased activity. A similar pattern has been observed in humans. Factors including exercise, pulmonary ventilation, dietary habits and emotional status are all known to influence urine pH in humans and as a result of these diurnal variations, pH is usually lowest throughout the night and highest during the day (Murayama and Taguchi, 1993).

Calcium oxalate uroliths can form across the entire range of urine pH values (4.8-7.4) in humans, and urine pH is not considered to be a major risk factor for calcium oxalate formation (Robertson, 1993; Robertson and Markwell, 1999). Urine pH does, however, exert control on the minerals that co-precipitate with calcium

oxalate (Robertson, 1993). A urine pH above 6.2 increases the risk of calcium phosphate crystallisation through the deprotonation of phosphate ions, which then readily precipitate with calcium ions. The volume of calcium phosphate crystalluria increases sharply as urinary pH exceeds 6.2. Likewise, at urine pH <5.3 there is a strong likelihood of uric acid being included as a minor component of calcium oxalate stones (Robertson, 1993). The urine pH produced by the MS was significantly higher than that of the LR. In addition, the urinary concentration of phosphate produced by the MS tended to be higher than the LR although the difference was not significant (P=0.06). These factors together with a higher urinary calcium concentration resulted in production of urine with a significantly higher brushite RSS. Again, this observation was repeated when comparing the CT and CS with LR in phase 2.

Calcium phosphate crystals are known to trigger calcium oxalate crystallisation in humans as they allow heterogeneous crystallisation processes to take place, which occur at a lower concentration of urinary supersaturation than homogeneous crystallisation (Ashby *et al.*, 1999; Hojaard *et al.*, 1999). Although the most common form of calcium phosphate that precipitates with calcium oxalate is apatite, brushite is a thermodynamically metastable compound known to be a precursor for apatite formation (Hesse and Heimbach, 1999). Thus, the measurement of brushite RSS together with urine pH provides a method of assessment for the risk of calcium phosphate formation. Production of urine with a pH <6.2 increases the solubility of calcium phosphate crystals and has been shown to decrease brushite RSS in humans (Hesse and Heimbach, 1999). The MS maintained a urine pH above 6.2 throughout the 24-hour period, indicating an increased risk of calcium phosphate crystallisation. In contrast, the LR showed a marked decrease in urine pH (<6.0)

between 8.00 p.m. and 8.00 a.m. At this time any preformed calcium phosphate crystals would be expected to dissolve. In dogs, calcium oxalate co-precipitates with calcium phosphate more commonly than it occurs in the pure form or with other coprecipitates (Osborne *et al.*, 2000b). Thus, the risks associated with calcium phosphate formation also require consideration when examining the factors contributing to calcium oxalate formation in dogs.

Markedly acidic urine pH has been associated with an increased risk of calcium oxalate urolithiasis in epidemiological studies in cats (Kirk *et al.*, 1995). Recent data suggested, however, that urinary calcium concentrations may only be increased as urine pH approaches the concentration at which there is a risk of metabolic acidosis (Stevenson *et al.*, 2000d). Similar studies appear not to have been reported in dogs, although it has been suggested that urinary acidifiers that are associated with acidosis are risk factors for calcium oxalate urolithiasis in this species (Lulich *et al.*, 1999a). During metabolic acidosis acidifying metabolites are neutralised by phosphates and carbonates mobilised from bone. Bone calcium is released with the phosphorus resulting in hypercalciuria (Lulich *et al.*, 1999a). In this study, the MS produced urine with a higher pH than the LR despite receiving the same diet and therefore a similar acid load on a metabolic body weight basis ($BWT^{0.75}$), yet urinary calcium concentration was also higher. Further research is necessary to determine whether urine pH within a range that avoids metabolic acidosis has a significant effect on urinary calcium concentration and the risk of calcium oxalate stone-formation in dogs.

5.5 Conclusions and Clinical Relevance

Differences in urine composition exist between breeds fed the same diet. Some of these factors, e.g. the lower volume, higher urinary calcium concentration, higher calcium oxalate and/or brushite RSS in the small dog breeds may contribute to the increased prevalence of calcium oxalate uroliths observed within this group. Increased intake of dietary minerals as a result of increased calorie requirements may partially contribute to the increased urinary calcium concentration, although other urinary minerals were unaffected, despite increased dietary intakes. Differences between breeds should be considered when evaluating strategies for controlling calcium oxalate stone-formation.

Appendix 5.1 Nutrient content of the commercially prepared dogfood

Nutrient	**Unit**	**Nutrient analysis (per 100 kcal)**	
		Phase 1	**Phase 2**
Moisture	g	1.8	1.8
Protein	g	7.12	7.29
Fat	g	4.44	4.04
Ash	g	2.15	2.39
Calcium	g	0.38	0.42
Phosphorus	g	0.30	0.38
Ca:P ratio	g/g	1.27	1.11
Sodium	g	0.06	0.09
Magnesium	g	0.03	0.03
Iron	mg	7.92	6.71
Copper	mg	0.23	0.58
Manganese	mg	0.48	2.38
Zinc	mg	4.14	6.02
Oxalate	mg	4.14	8.36

CHAPTER 6. THE EFFECT OF DIET ON THE RISK OF CALCIUM OXALATE CRYSTALLISATION IN DOGS

"Whereas the motto of the 'cutting surgeon' mentality is 'a chance to cut is a chance to cure' the paradigm of the 'thinking surgeon' is to minimise the philosophy of 'chance' in terms of a cure" Carl.A.Osborne.

Section a: The effect of dietary moisture and sodium on urine parameters in two breeds of healthy dog

6a.1 Introduction

Urine supersaturation is the driving force for the formation of crystals within the urinary tract (Balaji and Menon, 1997). The simplest way of reducing the supersaturation is to increase urine volume (Borghi *et al.*, 1999). In humans, there is a great deal of evidence that shows low urine volume is a risk factor for calcium oxalate stone-formation. The prevalence of calcium oxalate stones is higher in areas with a hot climate and also in groups of people involved in work activities or sports which expose the body to severe, prolonged extra-urinary water loss (Blacklock, 1969; Embon *et al.*, 1990; Borghi *et al.*, 1993). One human study found that urine volume at the first episode of stone-formation was lower than the urine volume of healthy controls, and that a simple increase in water without any other associated dietary changes, could prevent recurrences in a large number of subjects (Borghi *et al.*, 1996). The importance of fluid intake and urine volume is not unique to humans as high incidences of stone disease have also been associated with periods of water shortage

and dehydration in ruminants (Udall and Chow, 1962; Bailey, 1973).

On the basis of these epidemiological studies in humans, an evaluation of the biochemical risk of forming calcium oxalate stones was made by assessing the imbalance between lithogenic and antilithogenic urinary factors. In this risk assessment model, low urine volume was the most important of all factors examined (Robertson *et al.*, 1981a; Robertson and Peacock, 1985; Robertson, 1993).

Enhancement of urine volume is, therefore, a very important part of managing and preventing calcium oxalate stone-formation in humans, and this can logically be extrapolated to dogs. Epidemiological data show that small and toy dog breeds present more commonly with calcium oxalate uroliths. Additionally, recent research has shown that certain factors including a lower urine volume, decreased frequency of urination and an increased urinary calcium concentration may contribute to the increased prevalence of calcium oxalate uroliths within small breeds (Stevenson and Markwell, 2001). Methods of increasing urine volume, through dietary manipulation, would thus be anticipated to be of value in helping to minimise the risk of calcium oxalate urolith formation in small breed dogs.

Two potential methods of increasing urine volume were investigated in the studies reported here. Many studies conducted in the cat show that when fed dry foods they take in less total moisture than when fed wet foods (Jackson, 1977; Burger *et al.*, 1980; Gaskell, 1985). In the study conducted by Burger *et al*, the total water intake for cats fed the dry food was only 46% of that when the cats received canned food (Burger *et al.*, 1980). Similar studies in Labrador retrievers (LR) suggested that total water intake did not differ even when foods with markedly different moisture contents were fed. However, this appears not to have been studied in small breeds of

dog. Thus, a study was conducted comparing the response of LR, a breed which rarely forms calcium oxalate uroliths and Miniature schnauzers (MS), a breed predisposed to calcium oxalate urolith formation, when fed a diet of uniform nutrient composition containing 7% or 73% moisture (Study 1).

A second study examined the effect of increasing dietary sodium (Na) on urine composition (Study 2). The link between dietary Na and water intake has been well documented in cats and dogs, as well as many other mammalian species, and it is know that sodium chloride stimulates thirst in these animals (Cizek, 1959; Hamar *et al.*, 1976). However, excess dietary Na has been shown to cause an increase in urinary calcium excretion in both dogs (Walser, 1961) and humans (Kleeman *et al.*, 1964; Zarkadas *et al.*, 1989; Siener *et al.*, 1991; Sakhaee *et al.*, 1993; Martini *et al.*, 2000), leading to recommendations that sodium should be restricted in individuals predisposed towards calcium oxalate stone-formation (Kok *et al.*, 1990a; Lulich *et al.*, 1999b). Increased dietary Na is thought to result in increased sodium excretion, which could encourage urolith formation by inhibiting renal tubular reabsorption of calcium, resulting in hypercalciuria (Sakhaee *et al.*, 1993). This is a controversial issue and other studies have shown that supplemental dietary Na given to dogs had no effect on urinary calcium (Allen *et al.*, 1989; Stevenson *et al.*, 2000c), or oxalate (Lulich *et al.*, 1992b; Stevenson *et al.*, 2000c). Thus, the aim of Study 2 was to establish the effect of dietary Na content on urine composition of healthy MS and LR.

6a.2 Materials and Methods

6a.2.1 Dogs

6a.2.1.1 Study 1

A panel of 16 healthy adult dogs consisting of 8 LR (6 spayed females, 1 sexually intact female, 1 castrated male; mean age 3.5±1.2 years) and 8 MS (5 spayed females, 2 sexually intact female, 1 castrated male; mean age 4.5±1.5 years) were recruited to the study.

6a.2.1.2 Study 2

A panel of 15 healthy adult dogs consisting of 7 LR (1 spayed female, 4 sexually intact females, 2 castrated males; mean age 3.4±0.5 years), and 8 MS (3 spayed females, 4 sexually intact females, 1 neutered male; mean age 4.9±1.3 years) were used in this study.

6a.2.2 Study design

Dogs were randomly allocated to feeding groups and fed the test diets for a period of 12 days, according to a crossover (Study 1) or Latin square (Study 2) design. During each study period the dogs were individually fed either a nutritionally complete dry dog food *solus* (Diet A; Appendix 6a.1) or in conjunction with supplemental water or with sodium. Each dog was fed twice daily from 8.30am and 3.30pm for 24 days (Study 1) or 36 days (Study 2). Food intake was measured for each meal. Food allowances were calculated according to adult maintenance energy requirements (110 W 0.75kcal/d where W is body weight expressed in kg) (Burger, 1995b) and adjusted during the studies to ensure body weight maintenance within

±5% of original weight. All dietary nutrients were analysed as described in Chapter 2. Faeces quality was monitored daily. Water was provided *ad libitum*, and daily voluntary water intake was measured in both studies for the MS only.

6a.2.2.1 Study 1

Diet A was fed *solus* or with the appropriate amount of supplemental deionised water to deliver 73% moisture (Diet B). Water was added the day before feeding to allow it to soak into the dry food. The food was stored in an airtight container in a refrigerator overnight to minimise evaporation and keep the diet fresh.

6a.2.2.2 Study 2

Diet A was fed *solus* or supplemented with the appropriate amount of sodium chloride to deliver 0.20g (Diet C) or 0.30g (Diet D) Na per 100 kcal. The sodium chloride was dissolved in a standardised amount of deionised water to make a solution, which was added onto the dry food just before feeding. When feeding Diets A and C, an appropriate amount of deionised water was added to the diet so that the total amount of supplementary water was kept constant between all treatments.

6a.2.3 Housing details

Dogs were housed separately, as described in Chapter 2, for three 48-hour periods (days 3-4, 7-8, 11-12) of each treatment phase. During the remaining days the dogs were housed in pairs. At this time all dogs were walked once daily for approximately 15 minutes and group exercised in grass paddock areas for 1 to 2 hours.

6a.2.4 Urinary measurements

Urine pH was continuously measured as described in Chapter 2. Specific gravity and urine volume were measured daily during days 3-4 and 7-8 of every period. During days 11-12, a 48-hour urine sample was collected from each dog and immediately frozen, as described in Chapter 2.

6a.2.5 Urinalysis

Samples were prepared and as described in Chapter 2. Urinalysis data were then entered into a computer program, SUPERSAT (Robertson *et al.*, in press), which calculated RSS values for calcium oxalate and brushite.

6a.2.6 Statistical analysis

Data were compiled into means±standard deviations (SD). In Study 1, breeds and treatments were compared using t-tests where unpaired two-sided tests were used between breeds and paired two-sided tests were used for treatments within a breed. In Study 2, the effects of breed and treatments were compared using analysis of variance and multiple range tests (least significant difference). The concentration of significance was taken as $P \leq 0.05$.

6a.3 Results

6a.3.1 Food intake and body weight maintenance

All food offered to the dogs was consumed every day, ensuring the dogs always received the correct supplementary treatments. Body weight maintenance remained constant with an overall weight change of 0.3% (Study 1) and 1% (Study 2).

Faeces quality was consistently between Grades 2 and 3 as scored using the WALTHAM™ Faeces scoring system (Moxham, 2001).

6a.3.2 Urine measurements

Urine pH was not significantly affected by breed or treatment (Tables 1 & 2).

6a.3.2.1 Study 1

Increasing dietary moisture significantly reduced the specific gravity of the urine produced by the MS (P=0.003) (Table 6a.1). The specific gravity of urine produced by the LR was not significantly changed by dietary moisture content. Urine volume (ml / kg body weight / d) of the LR was significantly higher throughout the study (P=0.03), although this parameter was not significantly affected by dietary moisture content.

6a.3.2.2 Study 2

Urine volume and specific gravity were not significantly affected by dietary sodium supplementation. The mean urine volume produced by the LR was significantly higher than that of the MS throughout the study (P=0.002).

6a.3.3 Urinary RSS

6a.3.3.1 Study 1

Increasing dietary moisture significantly reduced urinary calcium oxalate RSS in the MS (P=0.04). There was no significant effect of dietary moisture on the calcium oxalate RSS produced by the LR (Figure 6a.1). Calcium oxalate RSS was not significantly different between the LR and MS. Brushite RSS tended to be higher

in the MS and tended to decline with increasing dietary moisture although these effects were not significant.

6a.3.3.2 Study 2

Calcium oxalate and brushite RSS were significantly reduced in the urine produced by both the LR and MS when dietary sodium was increased (Figure 6a.2). Calcium oxalate RSS was not significantly different between breeds. Brushite RSS was significantly higher in the MS.

6a.3.4 Urinary concentrations

6a.3.4.1 Study 1

Dietary moisture content did not affect calcium or oxalate concentrations of urine produced by the LR. Urinary oxalate concentration in MS was significantly reduced by increasing dietary moisture (P=0.04), while urinary calcium was not significantly affected (P=0.07), (Table 6a.1). Urinary phosphate concentration was significantly reduced as dietary moisture increased for both breeds (P=0.03). Urinary calcium concentration of the MS tended to be significantly higher than that of the LR for Diet A only (P=0.05).

6a.3.4.2 Study 2

Urinary calcium and urinary oxalate concentrations were not significantly affected by Na supplementation. The urinary calcium concentrations of the MS were significantly higher than those of the LR while receiving Diet A (P=0.02) and Diet C (P=0.003), but were not significantly different from those of the LR when fed Diet D. Urinary Na concentration significantly increased in both breeds with increasing

dietary Na (Table 6a.2). Urinary phosphate tended to decline with increasing sodium although this effect was not significant.

6a.3.5 Water intake

This parameter was only measured for the MS.

6a.3.5.1 Study 1

The MS drank significantly more water when fed the dry food *solus* compared to the hydrated diet (P=0.001). However, the total moisture intake was still significantly greater when the MS were fed the hydrated diet (P=0.001), (Table 6a.1).

6a.3.5.2 Study 2

The MS tended to drink more water as dietary Na content increased (P=0.05), (Table 2a.2).

Table 6a.1 Study 1 mean urine volume, specific gravity, relative supersaturation (RSS), urinary concentrations of calcium, oxalate and phosphorus and water intake of Labrador Retrievers and Miniature Schnauzers fed a commercially prepared dogfood containing 7% (Diet A) and 73% moisture (Diet B)

Parameter	Labrador Retriever		Miniature Schnauzer	
	Diet A	Diet B	Diet A	Diet B
Urine pH	6.10±0.70	6.20±0.76	6.14±0.41	6.34±0.65
CaOx RSS	11.28±5.68[a]	9.83±5.74[a]	13.21±3.22[b]	8.50±4.00[a]
Brushite RSS	1.12±0.82	0.76±0.50	1.31±0.47	0.94±0.62
Ca (mmol/l)	1.05±0.38	0.90±0.47	1.43±0.22	1.13±0.28
Oxalate (mmol/l)	2.34±1.07	1.40±0.68	1.72±0.64[a]	0.90±0.45[b]
PO_4 (mmol/l)	105.35±33.77[a]	63.83±35.55[b]	114.39±45.87[a]	62.23±23.41[b]
Urine volume (ml/kg BWT/d)	42±32	54±26	21±10	28±5
Urine specific gravity	1.033±0.015	1.023±0.010	1.034±0.006[a]	1.024±0.003[b]
Water drunk (ml/d)			361±58[b]	116±28[a]
Total water intake[#] (ml/d)			372±61[a]	482±86[b]

BWT = bodyweight, CaOx -= calcium oxalate

Within a column and breed, values with different superscripts are significantly different ($P<0.05$)

#Total water intake = moisture from food + water drunk

Table 6a.2 Study 2 mean urine volume, specific gravity, urine pH, relative supersaturation (RSS) and mean urinary concentrations of calcium, oxalate and sodium produced 7 Labrador retrievers and 8 Miniature Schnauzers fed a commercially prepared dogfood containing 0.05g (Diet A), 0.20g (Diet C), and 0.30g (Diet D) sodium per 100 kcal

Parameter	Labrador Retriever			Miniature Schnauzer		
	Diet A	Diet C	Diet D	Diet A	Diet C	Diet D
Urine pH	5.91 ±0.29	5.94 ±0.35	5.76 ±0.36	6.12 ±0.26	6.23 ±0.38	6.10 ±0.44
CaOx RSS	8.97 ±4.00^{b}	5.42 ±2.82^{ab}	3.62 ±2.15^{a}	13.87 ±8.78^{b}	9.13 ±6.52^{ab}	5.73 ±2.92^{a}
Brushite RSS	0.34 ±0.17^{b}	0.21 ±0.11^{ab}	0.11 ±0.10^{a}	1.10 ±0.69^{b}	0.83 ±0.51^{ab}	0.43 ±0.27^{a}
Ca (mmol/l)	0.71 ±0.37	0.56 ±0.18	0.50 ±0.34	1.40 ±0.58	1.27 ±0.50	0.93 ±0.49
Oxalate (mmol/l)	1.39 ±0.92	1.11 ±0.54	0.62 ±0.26	1.45 ±0.75	0.99 ±0.60	0.92 ±0.53
Na (mmol/l)	135.71 ±13.39^{a}	137.02 ±26.27^{b}	147.05 ±49.42^{b}	69.24 ±50.05^{a}	143.35 ±35.71^{b}	156.66 ±64.76^{b}
PO_4 (mmol/l)	38.73 ±30.31	27.09 ±9.53	17.97 ±6.76	62.08 ±47.60	35.45 ±13.34	39.57 ±26.62
Urine volume (ml/kg BWT /d)	49±23	55±34	71±22	19±15	28±15	31±23
Urine specific gravity	1.024 ±0.006	1.025 ±0.006	1.020 ±0.005	1.029 ±0.006	1.027 ±0.004	1.025 ±0.005
Water drunk (ml/d)				284±72^{a}	355±95^{ab}	368±67^{b}

Key as Table 6a.1

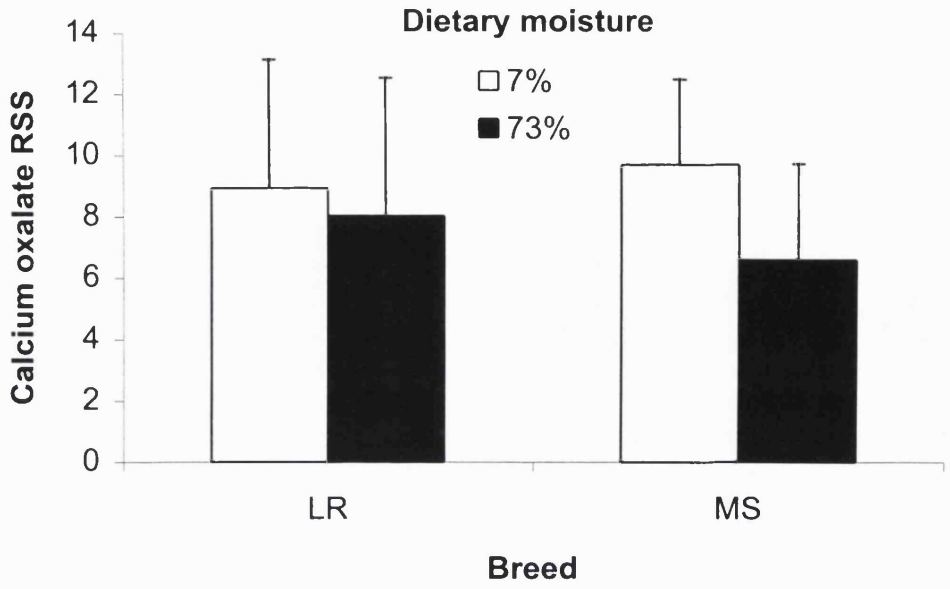


Figure 6a.1 The effect of dietary moisture (7% and 73%) on urinary calcium oxalate relative supersaturation (RSS) produced by 8 Labrador Retrievers (LR) and 8 Miniature Schnauzers (MS).

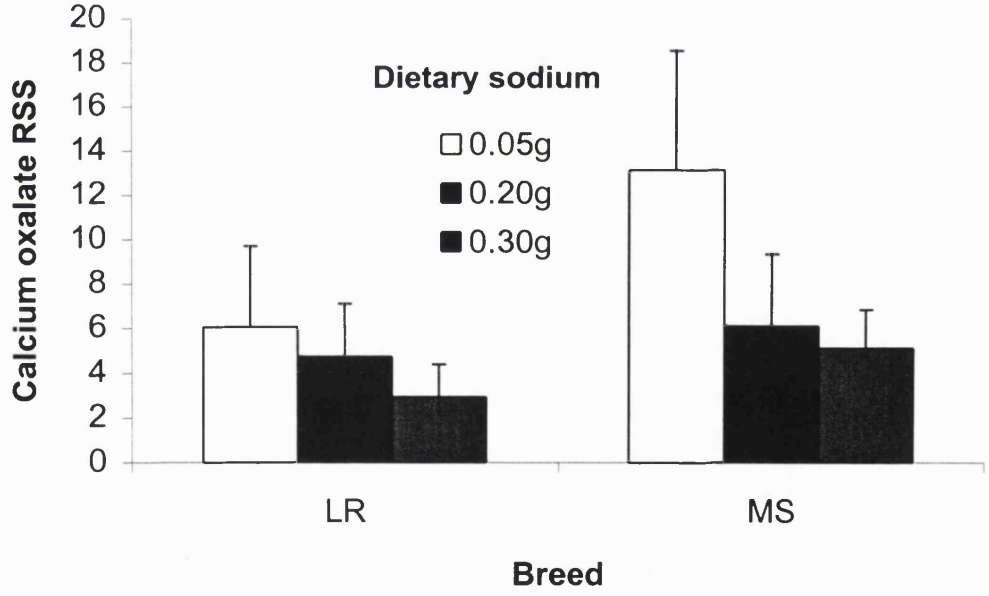


Figure 6a.2 The effect of increasing dietary sodium (0.05, 0.2 and 0.3g/100kcal) on urinary calcium oxalate relative supersaturation (RSS) produced by 7 Labrador Retrievers (LR) and 8 Miniature Schnauzers (MS).

6a.4 Discussion

The studies reported here show that when fed a low moisture, low sodium diet (Diet A) normal, healthy MS had a tendency towards increased risk of calcium oxalate crystal formation, compared with LR, a breed which rarely forms calcium oxalate uroliths. It is widely accepted that small breed dogs, including the MS, present more commonly with calcium oxalate uroliths, than larger breeds (Hesse, 1990; Wallerstrom and Wagberg, 1992; Ling *et al.*, 1998a; Lulich *et al.*, 2000; Osborne *et al.*, 2000b; Stevenson *et al.*, 2000b). Recent data have also demonstrated that a number of factors including a lower urine volume, decreased frequency of urination and increased urinary calcium concentration contribute to the increased risk of calcium oxalate formation within the MS breed (Stevenson and Markwell, 2001). These risk factors are also demonstrated in the studies reported here. In humans, a low urine volume has been identified as the most important risk factor contributing to calcium oxalate urolith formation (Robertson *et al.*, 1981a). Thus, it is customary to advise patients to increase fluid intake to ensure an adequate urine volume in order to prevent the recurrence of stones (Smith *et al.*, 1978). Theoretically, a high fluid intake should inhibit calcium oxalate stone-formation since it dilutes the urine, thereby lowering the urinary concentrations of calcium and oxalate and in turn the supersaturation with respect to calcium oxalate. These benefits have been observed both *in vitro* (Pak *et al.*, 1980), and *in vivo* in human calcium oxalate stone-formers (Borghi *et al.*, 1996). Methods proven to increase urine volume and to decrease the concentration of calculogenic minerals within the urine should, therefore, be of benefit for the management of calcium oxalate urolithiasis in dogs.

Increasing dietary moisture content (Study 1) significantly reduced the

calcium oxalate RSS, of the MS. Similar effects were not observed in LR. It is well documented that that dietary moisture content directly affected total water intake in cats (Jackson, 1977; Burger *et al.*, 1980; Gaskell, 1985). It has also been shown that dogs maintain a constant water intake, irrespective of dietary moisture content, through adjustment of voluntary water intake (Cizek, 1959; Burger *et al.*, 1980). However, these studies were only conducted in LR or beagles, breeds known to be at low risk of calcium oxalate urolith formation (Stevenson and Markwell, 2001). The results of the study reported here indicate that the response to dietary moisture content differs between breeds of dog. In contrast to LR, the total water intake of the MS, a breed at increased risk of calcium oxalate urolith formation, was significantly reduced on the low moisture diet. Consequently, MS produced a lower volume of more concentrated urine. Increasing dietary moisture resulted in a significant increase in total moisture intake. This in turn resulted in production of a more dilute urine with a lower risk of calcium oxalate crystal formation. Thus, it would seem logical to feed canned diets known to contain a higher concentration of dietary moisture to small breeds of dog identified as being at increased risk of calcium oxalate urolith formation.

An alternative strategy to increase water intake, and therefore urine volume, would be to increase sodium intake. However, previous studies in humans and dogs have shown that a high sodium intake increases urinary calcium excretion. Although the mechanism for this is still unclear it has been proposed that there is a linked or common reabsorption pathway for both ions in the nephron (Shortt and Flynn, 1990). Humans in developed countries take in an average of 60-75mg/kg bodyweight sodium per day (Edwards *et al.*, 1989). When compared with a low sodium intake of around

20mg/kg/d (60mmol/d), intakes of approximately 100mg/kg/d (300 mmol/d) have been demonstrated to increase urinary calcium excretion (Meyer *et al.*, 1976; Kok *et al.*, 1990a; Sakhaee *et al.*, 1993). Sakhaee *et al* also found that calcium oxalate supersaturation was unaffected, while urine pH, calcium phosphate supersaturation and citrate excretion increased in healthy volunteers receiving 100mg/kg/d sodium, deducing that the overall risk of calcium stone-formation increased (Sakhaee *et al.*, 1993). In contrast, one study examining the crystallisation rate of calcium and oxalate in both stone-forming and normal human patients found no relationship between crystallisation rate and sodium intake (Singh *et al.*, 1987).

The upper concentration of sodium supplementation reported here was similar to that shown to create hypercalciuria in humans. However, the maximum sodium intake of 1.2g/400 kcal (170-230mg/kg/d; 7-10mmol/kg/d) did not result in increased urinary calcium or citrate concentration, for reasons yet to be determined. Consequently, calcium oxalate supersaturation and therefore the risk of calcium oxalate crystallisation, declined significantly in both breeds. Brushite RSS also declined reducing the risk of heterogeneous nucleation. Even when urinary concentrations were converted to total daily excretions (or mmol/mol creatinine) no significant increases in daily excretion of calcium were observed although urinary sodium excretion increased significantly in both breeds. A previous study conducted in dogs found that dietary supplementation with sodium up to 1% did not increase urinary calcium, while urine volume increased by 21% (Allen *et al.*, 1989). This concentration of sodium supplementation is similar to that used in Diet 3 (1.14%), and urine volume increased by an average of 40% in the LR and 60% in the MS while receiving this diet. Increased dietary sodium stimulated thirst, as demonstrated by the

increased water intake shown by the MS (but not monitored for the LR), resulting in the tendency towards a more dilute urine with a significantly lower calcium and oxalate concentration. A reduction in the formation of uroliths has been observed in calves during sodium chloride supplementation because of increased water consumption and a reduction in the urinary concentration of calculogenic minerals (Bailey, 1973).

The effective osmotic pressure of the plasma is one of the major determinants of thirst, which stimulates the desire to drink (McKinley *et al.*, 1992). Serum sodium concentration is the major determinant of plasma osmolarity (Blumenfeld and Vaughan, 1998). When systemically infused with a concentrated solution of sodium chloride (or sucrose, fructose, sorbitol or mannitol) dogs showed a considerable increase in water intake (McKinley *et al.*, 1978). These solutes penetrate cell wall membranes slowly gradually establishing an osmotic gradient. Water then leaves the cells by osmosis resulting in cellular dehydration (McKinley *et al.*, 1978). In humans, extracellular Na has to increase approximately 2 mEq /l above normal to activate the thirst mechanism, and stimulate drinking (Guyton and Hall, 1996). In contrast, with solutions of urea or glycerol, less drinking results because these molecules quickly move across cell membranes; the osmotic potential is not maintained, little water leaves the cells and dehydration is minimal. Thus, the osmotic stimuli that are the most effective drivers of thirst are also those that cause cellular dehydration. Because of this it has been proposed that thirst results from the cellular dehydration of specific sensor cells (osmoreceptors), located within the third ventricle of the hypothalamus, commonly referred to as the thirst centre (Wolf, 1986; Guyton and Hall, 1996). The hypothalamus also contains large neurones that

synthesise the antidiuretic hormone (ADH). Secretion of ADH in response to osmotic stimuli is very rapid, so that plasma ADH concentrations can increase severalfold within minutes, providing rapid means for altering renal excretion of water. Osmoreceptors rapidly respond to changes in the osmolarity of extracellular fluid, exerting powerful control over the secretion of ADH and over thirst (Cowley *et al.*, 1986; Guyton and Hall, 1996; Dunn, 1998). The normal physiological response to a decrease in plasma osmolarity is a decrease in ADH secretion so that the urine becomes more dilute. Water reabsorption by the collecting ducts decreases, water is lost in excess of solute and a larger volume of more dilute urine is produced until osmolarity returns to normal (Dunn, 1998). Thus, increased dietary sodium should increase plasma osmolarity, which will initially increase ADH production. However, the thirst mechanism should also be activated by the osmoreceptors, which will drive up water intake and ultimately result in reduced plasma osmolarity, reduced ADH synthesis and the production of more dilute urine. In normal dogs with free access to water, elevated plasma osmolarity and extracellular Na concentration as a result of high salt intake have been shown to be controlled by increased drinking rather than enhanced ADH secretion and water conservation (Cowley *et al.*, 1986). ADH only becomes a significant regulatory mechanism when water availability is restricted. Under these conditions ADH stimulates water conservation, slowing the rise of extracellular Na, and the rate of cellular dehydration (Dunn, 1998). In Study 2, the stepwise increase in dietary sodium promoted an increased water intake though drinking, which resulted in the production of a progressively more dilute urine with a lower calcium, phosphate and oxalate concentration. Although these factors in isolation were not statistically significant, together they resulted in a significant

reduction in calcium oxalate and brushite supersaturation, and therefore a reduced risk of calcium oxalate urolith formation in both breeds. This second method of increasing urine volume in dogs may be beneficial in managing calcium oxalate stone disease in dogs fed dry foods.

6a.5 Conclusions and clinical relevance

Small breed dogs, such as the MS appear predisposed to calcium oxalate urolith formation. Increased dietary moisture content or dietary Na content significantly reduced the calcium oxalate RSS, and thus the risk of calcium oxalate crystal formation in this breed. These factors should be considered when evaluating feeding strategies for preventing calcium oxalate urolith formation within high-risk groups.

Appendix 6a.1 - Dietary analysis

Nutrient	Unit	Amount (per 100 kcal)
Moisture	g	1.86
Protein	g	7.22
Fat	g	3.90
Ash	g	2.26
Calcium	g	0.43
Phosphorus	g	0.32
Ca:P ratio	g/g	1.36
Sodium	g	0.06
Potassium	g	0.20
Magnesium	g	0.03
Iron	mg	6.88
Copper	mg	0.37
Manganese	mg	2.44

Zinc	mg	6.17
Oxalate	mg	3.53

Section b: The relative importance of dietary calcium and dietary oxalate as risk factors for calcium oxalate formation in dogs

6b.1 Introduction

Calcium oxalate urolithiasis appears to be a growing problem in dogs (Lulich *et al.*, 1999b). The proportion of this urolith type submitted for analysis at one centre in the USA increased from 5.3% in 1981 to 35.1% in 1997 (Lulich *et al.*, 1999b). Recent research has also shown that small dogs have a higher risk of calcium oxalate crystallisation than do large dogs fed the same diet (Stevenson and Markwell, 2001). Many articles have been written evaluating aspects of diet and specific nutrients, including calcium, oxalate, protein, sodium, phosphorus and potassium, in the formation and subsequent prevention of calcium oxalate uroliths in dogs (Lulich *et al.*, 1992a; Osborne *et al.*, 1995b; Lulich *et al.*, 1999b). However, information has often been extrapolated from studies in humans.

Much research has been conducted in humans to evaluate the relative importance of dietary calcium and oxalate on the risk of calcium oxalate urolith formation. Urinary calcium is largely dependent upon dietary calcium (Marshall *et al.*, 1976; Lemann *et al.*, 1979), although hypercalciuria as a consequence of dietary intake alone is unusual in humans. Urine calcium excretion rises sharply up to intakes of around 10mg / kg body weight / d, after which the concentration flattens off, unless individuals are hyperabsorbers of calcium (Lemann *et al.*, 1979; Robertson, 1993). Calcium is absorbed in the ionic state, and therefore substances that complex with calcium, such as phosphate, citrate, sulphate, oxalate and fatty acids decrease the availability of calcium for absorption (Menon *et al.*, 1998). If dietary calcium is

restricted without a concomitant decrease in dietary oxalate, passive intestinal absorption of oxalate in the colon followed by an increase in urinary oxalate may occur (Zarembski and Hodgkinson, 1969; Hodgkinson, 1978). Thus, in human calcium oxalate stone-formers severe calcium restriction is not recommended (Bataille *et al.*, 1983; Jaeger *et al.*, 1985). In some studies a high dietary calcium intake actually decreased the risk of human calcium oxalate crytal formation (Marshall *et al.*, 1972; Curhan *et al.*, 1993; Curhan *et al.*, 1997). The mechanism for this is thought to be the binding of calcium with oxalate in the intestinal lumen leading to excretion in the stools rather than the urine (Zarembski and Hodgkinson, 1969; Marshall *et al.*, 1972).

Oxalate is a simple organic dicarboxylic acid present in many foods, particularly cereal grains and leafy plants (Holmes and Kennedy, 2000). Humans cannot metabolise oxalate directly, and thus with the possible exception of bacterial action in the gut, renal excretion becomes the sole source of oxalate elimination (Asplin *et al.*, 2000). Although it was originally thought that a large amount of the oxalate found in human urine (up to 80%) was derived from endogenous production (Menon *et al.*, 1998; Lulich *et al.*, 1999b), recent studies have shown that the contribution of dietary oxalate to urinary oxalate may have been underestimated (Holmes *et al.*, 1995; Holmes and Kennedy, 2000). In humans, primary hyperoxaluria can occur as a result of metabolic errors, inherited through an autosomal recessive pattern (Williams and Wilson, 1990). However, a number of workers within the human field have proposed that mild hyperoxaluria, often as a result of a high dietary oxalate to calcium ratio, is secondary in importance only to urine volume as a risk

factor for human calcium oxalate stone-formation in humans (Robertson *et al.*, 1978; Robertson and Peacock, 1980; Bataille *et al.*, 1983; Larsson and Tiselius, 1987).

The relative importance of dietary calcium and oxalate on the risk of calcium oxalate crystallisation in dogs does not appear to have been examined. In addition, there appears to be very little information available concerning the amount of oxalate commonly encountered in dry commercially prepared dog foods. Thus, the purpose of the study reported here was to compare the relative importance of dietary calcium and oxalate when fed to dogs at concentrations commonly encountered within commercially prepared dog foods.

6b.2 Materials and Methods

6b.2.1 Dogs

Eight healthy adult dogs consisting of four Cairn Terriers and three Miniature schnauzers (5 spayed females, 2 castrated male; mean age 5.2±1.0 years) were fed a nutritionally complete dry dog food (Appendix 1) twice daily at 8.30 am and 3.30 pm. Food allowances were calculated according to adult maintenance energy requirements (110 $W^{0.75}$kcal/d where W is body weight expressed in kg), (Burger, 1995b), and adjusted during the studies to ensure body weight maintenance within ±5% of original weight. All dietary nutrients were analysed by previously described methods (Stevenson *et al.*, 2000e). Faeces quality was monitored daily. Water was provided *ad libitum,* and voluntary water intake was measured daily when dogs were individually housed.

6b.2.2 Study design

Seven different combinations of dietary calcium and oxalate were examined (Table 6b.1). The maximum concentrations were selected from the analysis of 30 different dry

Table 6b.1 Dietary supplementation of calcium and oxalate

Diet	Calcium (g/100 kcal)	Oxalate (mg / 100 kcal)	Calcium : Oxalate	
			mg/mg	mmol/mmol
LCa-LOx	0.18	10	18	40
LCa-MOx	0.18	17.5	10	22
LCa-HOx	0.18	25	7	16
MCa-MOx	0.45	17.5	26	58
HCa-HOx	0.75	25	30	68
MCa-LOx	0.45	10	45	101
HCa-LOx	0.75	10	75	169

dog foods from 13 different manufacturers in eight different countries including USA (n=8), Australia (n=3), Japan (n=3), Italy (n=4), Germany (n=2), Netherlands (n=3), UK (n=4) and France (n=3), designed for adult small breed dogs), although calcium content was always higher than oxalate content. Thus the maximum concentrations selected for this study were 0.75g calcium/100kcal and 25mg oxalate/100kcal. Each dog received each combination for a 12-day period in a Latin square design. Each study period was separated by an 8-day interval. During each study period the dogs either received a nutritionally complete dry dogfood containing a low concentration of

both dietary calcium (0.18g/100kcal) and oxalate (10mg/100kcal), (LCa-LOx); or the complete diet plus one of six combinations of supplementary calcium and oxalate; low calcium-medium oxalate (LCa-MOx), low calcium-high oxalate (LCa-HOx), medium calcium-medium oxalate (MCa-MOx), high calcium-high oxalate (HCa-HOx), medium calcium-low oxalate (MCa-LOx), and high calcium-low oxalate (HCa-LOx) (Table 1). Supplements were made into stock solutions with deionised water once weekly and added to the food directly before feeding. HCa-HOx delivered the maximum amounts of both calcium and oxalate. Dogs receiving all other diets were given deionised water up to the total volume supplied by HCa-HOx. The calcium supplement consisted of a combination of calcium sulphate and calcium carbonate, dosed at a concentration to deliver equal amounts of sulphate and carbonate ions. Oxalate was given in the form of oxalic acid. All supplements were obtained from BDH Laboratory Supplies, Poole, UK

6b.2.3 Housing details

Dogs were housed separately, as described by Stevenson *et al* (Stevenson *et al.*, 1998b), for three 48 hour periods (days 3-4, 7-8, 11-12) of each treatment phase. During the remaining days the dogs were housed in pairs. Whilst in pairs all dogs were walked once daily for approximately 15 minutes and group exercised in grass paddock areas for one to two hours.

6b.2.4 Urinary measurements

Urine pH was continuously measured using the non-invasive automated urine pH monitoring system (Stevenson *et al.*, 1996; Stevenson *et al.*, 1998b), and specific gravity and urine volume were measured daily during days 3-4 and 7-8 of

every period. Over days 11-12, a 48 hour urine sample was collected from each dog and immediately frozen, as described by Stevenson *et al.* (Stevenson *et al.*, 1998b).

6b.2.5 Urinalysis

Samples were prepared and analysed by previously described methods (Markwell *et al.*, 1999). Urinalysis data were then entered into a computer program, SUPERSAT, which calculated RSS values for calcium oxalate, struvite and brushite (Robertson *et al.*, in press).

6b.2.6 Statistical analysis

Data were compiled into means±standard deviations (SD). Analysis of variance and multiple range tests (least significant difference) were used to test the significance of calcium and oxalate supplementation on calcium oxalate RSS, urine pH, and urinary concentrations of calcium, oxalate, sulphate, phosphate and ammonia. The concentration of significance was taken as $P<0.05$.

6b.3 Results

6b.3.1 Food intake and body weight maintenance

All food offered to the dogs was consumed every day, ensuring the dogs always received the correct treatment. Body weight maintenance remained constant with an overall weight change of 1 Faeces quality was consistently between Grades 2 and 3 as scored using the WALTHAM™ Faeces scoring system (Moxham, 2001).

6b.3.2 Urine measurements

Urine volume and specific gravity were not affected by dietary calcium or

oxalate supplementation. Urine pH was not affected by supplementary oxalate alone, but was significantly reduced during calcium supplementation (Table 6b.2).

6b.3.3 Urinary RSS

All diets produced urine that was undersaturated with respect to struvite and brushite (RSS<1). Urine produced when LCa-LOx was fed tended to have the lowest calcium oxalate RSS (Table 6b.2, Figure 6b.1). HCa-LOx produced the highest calcium oxalate RSS. Over the range used in this study dietary calcium tended to have a greater influence over the calcium oxalate RSS than dietary oxalate.

6b.3.4 Urinary concentrations

Urinary calcium was significantly increased by dietary calcium supplementation only; dietary oxalate had no effect on urinary calcium (Table 6b.2, Figure 6b.1). Variability in urinary calcium between dogs became high as dietary calcium increased (Figure 6b.1). Urinary oxalate concentration increased significantly with LCa-MOx and tended to increase with LCa-HOx, although the difference was not significant. No change in urinary oxalate concentration occurred with oxalate supplementation when calcium was also increased. Due to variability in response between dogs, urinary oxalate concentration was significantly higher on the LCa-MOx only. When dietary oxalate was supplemented with dietary calcium, urinary oxalate, and the variability between dogs, remained low (MCa-MOx and HCa-HOx). Urinary ammonium concentration increased with calcium supplementation so that HCa-HOx and HCa-LOx were significantly higher than LCa-LOx (Table 6b.2). Urinary phosphate concentration significantly increased when dietary oxalate only was supplemented, but decreased when dietary calcium was added (Table 6b.2).

Supplementary oxalate had no effect on urinary sulphate, while calcium supplementation significantly increased urinary sulphate. Urinary citrate, potassium, magnesium, sodium and creatinine concentrations were unaffected by dietary calcium or oxalate supplementation.

6b.3.5 Water intake

Water intake was unaffected by dietary calcium or oxalate supplementation.

Table 6b.2 Mean relative supersaturations (RSS) and mean urinary concentrations (mmol/l) of calcium, oxalate, ammonium, phosphate and sulphate produced by seven dogs fed a commercially prepared dogfood containing various combinations of calcium and oxalate

(for diet details see Table 6b.1; values shown as mean±standard deviation)

Parameter	Diet						
	LCa-LOx	LCa-MOx	LCa-HOx	MCa-MOx	HCa-HOx	MCa-LOx	HCa-LOx
CaOx RSS	3.70 ±0.97^{a}	7.72 ±6.11^{bc}	4.98 ±1.60^{ab}	5.76 ±3.07^{abc}	8.70 ±4.40^{bc}	6.29 ±3.20^{abc}	9.07 ±7.79^{c}
Urine pH	6.34 ±0.42^{d}	6.17 ±0.65^{cd}	6.15 ±0.54^{cd}	5.99 ±0.71^{bcd}	5.70 ±0.44^{ab}	5.86 ±0.43^{abc}	5.55 ±0.34^{a}
Ca	1.13 ±0.28^{a}	1.26 ±0.66^{a}	1.04 ±0.28^{a}	2.57 ±0.91^{ab}	5.14 ±2.73^{c}	3.07 ±1.15^{b}	6.28 ±3.46^{c}
Oxalate	0.36 ±0.17^{ab}	0.59 ±0.45^{c}	0.52 ±0.21^{bc}	0.22 ±0.08^{a}	0.23 ±0.10^{a}	0.21 ±0.07^{a}	0.22 ±0.17^{a}
NH_4	31.76 ±5.62^{a}	36.24 ±16.84^{ab}	37.75 ±18.83^{ab}	43.10 ±15.85^{abc}	53.69 ±21.64^{bc}	40.70 ±28.38^{abc}	56.91 ±27.32^{c}
SO_4	11.80 ±4.48^{a}	11.63 ±5.03^{a}	11.77 ±4.19^{a}	29.56 ±10.09^{b}	43.52 ±17.32^{cd}	33.20 ±16.71^{bc}	44.59 ±19.66^{d}
PO_4	11.16 ±4.45^{b}	15.65 ±8.92^{bc}	18.82 ±10.29^{c}	4.04 ±3.22^{a}	2.07 ±2.44^{a}	2.52 ±3.98^{a}	1.36 ±2.22^{a}

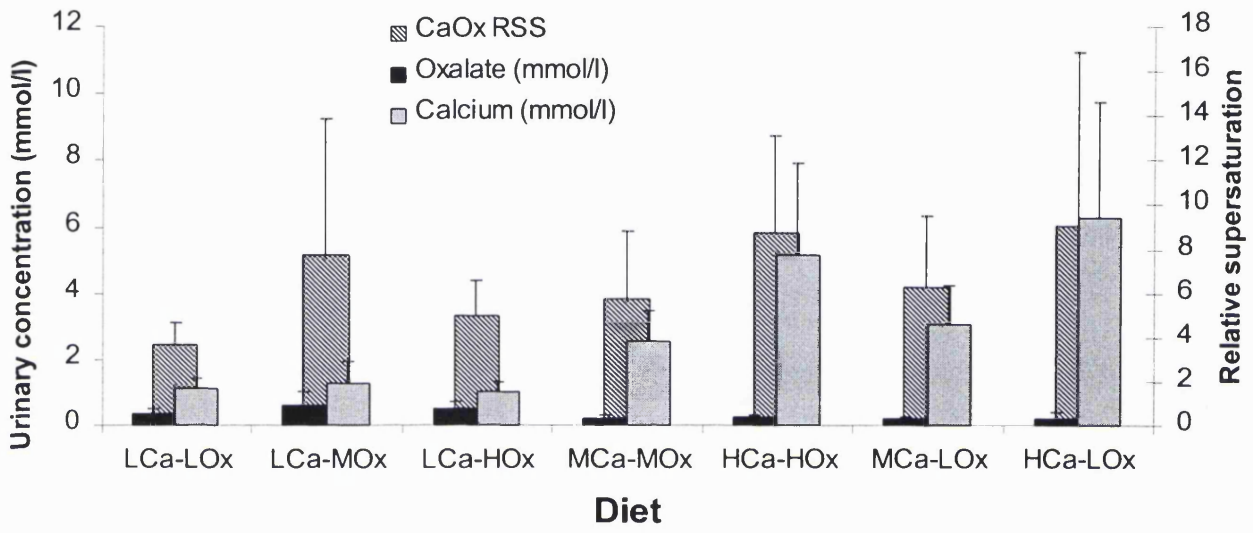
CaOx = calcium oxalate

^{a,b}Within a row values with different superscripts are significantly different ($P<0.05$)

Figure 6b.1 Mean relative supersaturations (RSS) and mean urinary concentrations (mmol/l) of calcium and oxalate of seven dogs fed a commercially prepared dogfood containing various combinations of calcium and oxalate

(for diet details see Table 6b.1; values shown as mean±standard deviation)

For Key see Table 6b.2



6b.4 Discussion

The concentrations of dietary calcium and oxalate used within this study were based upon the analysis of 30 different dry dogfoods designed for feeding to adult small breed dogs. Thus, in this study, dietary calcium always exceeded dietary oxalate although dietary calcium to oxalate ratio ranged between 7 and 75 on a weight basis, or from 16 to 169 on a molar basis. Calcium usually considerably exceeds oxalate in the diet of humans in most developed countries (Marshall *et al.*, 1972). Average daily intakes are 2-3mg oxalate/kg/d and 15-20mg calcium/kg/d with a molar calcium to oxalate ratio of around 18 (Marshall *et al.*, 1972; Holmes *et al.*, 1995). At the lowest concentrations within this study the dogs consumed 130mg/kg/d calcium

and 7mg/kg/d oxalate (molar calcium:oxalate ratio = 40). Most prepared petfoods contain concentrations well above these values and thus dogs are relatively loaded with both these minerals, and particularly calcium, compared to humans.

The LCa-LOx diet used in this study is designed to produce urine with a low risk of calcium oxalate crystal formation. Manipulation of dietary calcium and oxalate did not influence water turnover since water consumption, urine volume and specific gravity remained constant throughout the study. One study conducted in rats demonstrated an increased urine volume, and a reduction in calcium oxalate supersaturation with increased dietary oxalate (supplied as sodium oxalate) supplementation (Bushinsky *et al.*, 1999). Miniature schnauzers supplemented with sodium chloride were also observed to increase urine output (Stevenson *et al*, submitted for publication). Thus, the use of oxalic acid allowed the assessment of the influence of dietary oxalate concentrations in the absence of any changes in urine output.

At the concentrations investigated in this study, dietary calcium had a far greater effect on the calcium oxalate supersaturation, and therefore the risk of calcium oxalate crystallisation, than did changes in dietary oxalate. However, when dietary calcium was kept low, an increase in dietary oxalate, as shown during the feeding of LCa-Mox, resulted in an increase in urinary oxalate concentration and calcium oxalate RSS, implying that dietary oxalate may become a more important risk factor for calcium oxalate formation when dietary calcium is low. The relative importance of dietary calcium and dietary oxalate has been studied extensively in humans (Zarembski and Hodgkinson, 1969; Marshall *et al.*, 1972; Bataille *et al.*, 1983; Bataille *et al.*, 1985; Nakada *et al.*, 1988; Holmes *et al.*, 1995; Masai *et al.*, 1995;

Curhan *et al.*, 1997; Liebman and Chai, 1997; Messa *et al.*, 1997; Hess *et al.*, 1998; Liebman and Costa, 2000; Holmes *et al.*, 2001). It was common practice during the second half of last century for clinicians to recommend restriction of dietary calcium in human calcium oxalate stone-formers based upon the fact that a large proportion have elevated urinary calcium concentrations (Pak *et al.*, 1974), and dietary restriction reduces urinary calcium excretion (Messa *et al.*, 1992; Curhan, 1997; Messa *et al.*, 1997). However, it has been demonstrated in both humans (Marshall *et al.*, 1972; Messa *et al.*, 1997; Morozumi and Ogawa, 2000), and rats (Bataille *et al.*, 1983) that restricting dietary calcium actually caused an increase in urinary oxalate because the amount of oxalate available for absorption within the colon increased. Hyperabsorption of calcium, a possible mechanism leading to the hypercalciuria observed in a small number of Miniature schnauzers with calcium oxalate stones (Lulich *et al.*, 1991a), may also indirectly induce greater absorption of oxalate leading to subsequent hyperoxaluria (Morozumi and Ogawa, 2000). In a study comparing the relative influence of mild hyperoxaluria and hypercalciuria on a number of parameters involved in stone-formation in humans, mild hyperoxaluria had a far more marked effect on urinary supersaturation with respect to calcium oxalate, and on the maximum amount of crystalluria produced, than hypercalciuria (Robertson and Peacock, 1980). No such relationships were seen with hypercalciuria, except at very low urinary oxalate concentrations (Hallson, 1988). The relatively greater importance of changes in urinary oxalate compared with calcium in humans is probably a reflection of the fact that the molar ratio of calcium to oxalate concentrations in human urine is high (Brown and Purich, 1992).

In human urine, calcium ions tend to outnumber oxalate ions by a factor of

10 or more, on a molar basis (Brown and Purich, 1992), which appears to correlate well with the ratio of these ions in the diet. Urinary oxalate is thought to be the limiting factor in the crystallisation process, and small increases in urinary oxalate will increase the urinary crystallisation potential (Robertson and Peacock, 1980). The molar urinary calcium to oxalate ratio produced by the dogs in this study was largely influenced by the dietary concentrations of the two minerals, ranging from 2 on the LCa-MOx and LCa-HOx diets up to 28 on the HCa-LOx diet. A high degree of variability in the response of individuals to oxalate loading was observed in this study. This has also been observed in humans during oxalate loading, but not on a low oxalate diet (Holmes *et al.*, 2001). The reasons for the variability in oxalate absorption during oxalate loading on otherwise controlled diets remain unclear although it could be related to differences in gastrointestinal absorption of calcium and oxalate, different activities of oxalate-degrading organisms, differences in transport processes, or renal handling of oxalate. Nevertheless, an increase in dietary oxalate resulted in an increase in urinary oxalate concentration when calcium was kept low and, therefore, a potentially increased risk of calcium oxalate crystallisation in the majority of dogs, when calcium intake was kept low.

Urinary oxalate concentration did not increase when oxalate was supplemented in the presence of moderate or high calcium. It is likely that the supplementary oxalate given under these circumstances was bound by calcium within the intestine causing much of dietary oxalate to become unavailable for absorption. Thus, at any concentration of calcium supplementation, urinary oxalate concentrations were low and consistently between 0.21 to 0.23 mmol/l, equating to a daily excretion of between 0.6 and 0.7mg/kg/d (0.01 mmol/kg/d). It is suggested that this oxalate was

derived predominantly from endogenous production, as the concentration remained low and constant irrespective of dietary oxalate content. One study in humans estimated that endogenous production was between 0.15 and 0.2 mg/kg/d (Holmes *et al.*, 1995). However, endogenous oxalate production itself is not fixed and varies with dietary protein consumption (Hess *et al.*, 1998), since glycollate, a product of protein metabolism, is a precursor for endogenous oxalate production (Assimos and Holmes, 2000). Other possible influences include vitamins B_6 and C (Assimos and Holmes, 2000). However, consumption of dietary protein and vitamins B_6 and C remained constant throughout this study, and thus endogenous production also remained unchanged.

If it is assumed that approximately 0.6mg/kg/d of urinary oxalate resulted from endogenous production, the contribution of dietary oxalate to urinary excretion was 0.3mg/kg/d rising to 1.04mg/kg/d on the high oxalate diet, when dietary calcium was low. These values equate to approximately 4 to 5.5% of the dietary oxalate load. These estimates are very close to those found in humans where oxalate absorption has been estimated at between 2 and 5% of the administered oxalate load (Archer *et al.*, 1957; Zarembski and Hodgkinson, 1969), and one recent study found urinary oxalate to be 5.8% of dietary oxalate when fed at 4mg/kg/d (Holmes *et al.*, 2001). Urinary calcium concentration was not reduced as a result of increased dietary oxalate, indicating that the same amount of calcium was being absorbed. In contrast, supplementary dietary calcium resulted in a significant rise in urinary calcium concentration, and an increase in calcium oxalate RSS, and thus the risk of calcium oxalate crystallisation.

Urinary calcium and oxalate were not the only parameters to be affected by

the dietary supplementation. Urinary phosphate concentration increased as a result of oxalate supplementation. In humans intestinal calcium binds with phosphate preventing its absorption (Slatopolsky *et al.*, 1989). In addition, the preferential binding of calcium with the increasing amounts of dietary oxalate was shown to decrease the calcium available for phosphate binding in rats (Bushinsky *et al.*, 1999). Thus, in this study, as dietary oxalate increased more phosphate became available for intestinal absorption, resulting in a significant increase in urinary phosphate. When increased dietary oxalate was given with additional calcium these effects were abolished, as calcium was present in amounts large enough to meet requirements for both oxalate and phosphate binding.

Although the study reported here was designed so that the sulphate and carbonate ions contributed equal amounts to the diet, calcium supplementation resulted in a reduced urine pH, and consequently an increased ammonia concentration (r^2=57%, correlation coefficient –0.75). However, calcium carbonate is known to be poorly absorbed in both rats and humans (Harvey *et al.*, 1988; Classen *et al.*, 1995). When administered to rats and cats this salt did not significantly affect urine pH (Pastoor *et al.*, 1994b; Classen *et al.*, 1995). The acidifying effect observed in this study is likely to have been caused by calcium sulphate supplementation. The increased urinary excretion of sulphate will lower the excretion of bicarbonate, lower the urine pH and increase ammonium excretion. These measures are a normal response, ensuring the maintenance of a constant acid-base balance within the body. The lower urine pH will stimulate ammonium production within the kidney to buffer H^+ ions as ammonium ions (Pitts, 1948; Good, 1989). A similar effect was found in a study replacing dietary calcium carbonate with calcium chloride in cats (Pastoor *et*

al., 1994a).

During metabolic acidosis acidifying metabolites are neutralised by phosphates and carbonates mobilised from bone. Bone calcium is released with the phosphorus resulting in hypercalciuria (Lulich *et al.*, 1999a), thus it could be suggested that the reduction in urine pH observed in this study contributed to the increased urinary calcium concentration. Recent data from studies in cats suggested, however, that urinary calcium concentrations may only be increased as urine pH approaches the concentration at which there is a risk of metabolic acidosis (Stevenson *et al.*, 2000d). Whilst similar studies appear not to have been reported in dogs, urine pH in this study remained within the normal range for healthy dogs (Bush, 1991), and the dogs would not be considered at risk of metabolic acidosis. In addition, both in this study and those of Pastoor *et al* (1994) in cats there was no correlation between urinary calcium concentration and urine pH, suggesting that within the normal urine pH range this was not the major factor driving the increase in urinary calcium concentration.

6b.5 Conclusions and clinical relevance

Measures to reduce both calcium and oxalate should be considered when implementing dietary changes to reduce the risk of calcium oxalate formation in dogs. A reduction in dietary calcium without a concomitant decrease in dietary oxalate may increase the risk of calcium oxalate crystallisation in susceptible dogs.

Appendix 6b.1 Dietary analysis

Nutrient	Unit	Amount (per 100 kcal)
Moisture	g	2.15
Protein	g	3.94
Fat	g	3.42
Ash	g	1.74
Calcium	g	0.18
Phosphorus	g	0.15
Ca:P ratio	g/g	1.20
Sodium	g	0.28
Potassium	g	0.21
Magnesium	g	0.02
Iron	mg	3.78
Copper	mg	0.17
Manganese	mg	0.28
Zinc	mg	5.57
Oxalate	mg	9.76

CHAPTER 7. THE EFFECT OF TWO DIFFERENT TREATMENT STRATEGIES ON THE RISK OF UROLITH FORMATION IN CATS (Section a) AND DOGS (Section b)

"Too often we think about what drug to use rather than whether or not to prescribe it" H.I. Wright

Section a: The effect of urine pH on urinary relative supersaturation in healthy adult cats

7a.1 Introduction

Clinical conditions that lead to precipitation of minerals are among the numerically important identifiable causes of lower urinary tract diseases in cats. In two studies, urolithiasis was present in approximately 11% of obstructed cats and 15 to 30% of non-obstructed cats (Osborne *et al.*, 1989a). Magnesium ammonium phosphate (MAP) has been the mineral found most commonly in feline uroliths but recent data suggest that calcium oxalate may be of increasing importance (Osborne *et al.*, 1995c; Buffington *et al.*, 1997). Data from one urolith analysis centre within the USA found that uroliths composed of calcium oxalate increased from 2% in 1984 to 40% in 1995 (Osborne *et al.*, 1996b).

Dietary manipulation has been the mainstay for the management and prevention of MAP in cats for some years, primarily because of the influence of dietary ingredients on urine pH. Urine pH is a much more important determinant of

MAP formation than is the magnesium content of the diet (Marshall and Robertson, 1976; Taton *et al.*, 1984; Buffington, 1988), because changes in pH have a proportionately greater effect on changing MAP activity product than can be achieved by changes in the concentrations of one or more crystalloid components of MAP. Reduction of urinary pH through dietary manipulation is the most reliable means of creating urine that is undersaturated with respect to MAP.

The effect of urine pH on the risk of calcium oxalate crystal formation is more controversial. Although by no means confirmed, some researchers consider that dietary measures to reduce the risk of MAP formation in cats in recent years, including urinary acidification and reduced dietary magnesium, may have exposed a population of cats already predisposed to calcium oxalate urolith formation (Buffington and Chew, 1999). However, this dietary modification has not been applied to dog foods yet a similar increase in the occurrence of calcium oxalate stones has been observed (Lulich *et al.*, 1999b). This study was conducted to assess the effect of dietary acid load on urinary pH and relative supersaturation (RSS) of calcium oxalate and MAP in healthy adult cats.

7a.2 Materials and Methods

7a.2.1 Cats

Six healthy adult cats, (3 spayed females, 3 neutered males; mean age 3.5±1.9 years) were randomly allocated to three feeding groups and fed a nutritionally complete canned cat food (Control diet, CON)[a], (Appendix 1). The cats were fed twice daily at 8.30am and 3.30pm and food allowance was calculated based upon an energy requirement of 50kcal/kg body weight/d. Food adjustments were made during

the study to ensure body weight maintenance within ±5% of original weight. All dietary nutrients were analysed by previously described methods (Stevenson *et al.*, 2000e). Faeces quality was monitored daily, and water was provided *ad libitum.*

7a.2.2 Study design

The cats received Diet CON *solus* or supplemented with ammonium chloride (NH_4Cl), a urinary acidifier, (200 mg / kg BWT / day) or sodium bicarbonate ($NaHCO_3$), a urinary alkaliniser, (640 mg / kg BWT / day) for two weeks each in a Latin square design. Each study period was separated by an 8-day interval. The supplements were obtained from BDH Chemical Supplies, Poole, Dorset, UK.

7a.2.3 Housing details

The cats were housed individually as described in Chapter 2, for each two week feeding period. During the 8-day intervals the cats were group housed and fed Diet C twice daily.

7a.2.4. Urinary measurements

The cats were trained to urinate into angled litter trays from which urine drained rapidly into glass U-tubes for assessment of urine pH, as described in Chapter 2. For the last 2 days of each period the U-tubes were substituted by enclosed glass, dry ice-chilled containers, to ensure rapid freezing of collected urine.

7a.2.5 Urinalysis

Samples were prepared and analysed by previously described methods (Markwell *et al.*, 1999). Urinalysis data were then entered into a computer program,

SUPERSAT, which calculated RSS values for calcium oxalate and MAP (Robertson *et al.*, in press).

7a.2.6 Statistical analysis

Data were compiled into means±standard deviations (SD). Analysis of variance and multiple range tests (least significant difference) were used to test the effect of dietary manipulation on calcium oxalate RSS, MAP RSS, and urinary concentrations of calcium, oxalate, citrate, sodium and ammonium. $P<0.05$ was considered significant.

7a.3 Results

7a.3.1 Food intake and body weight maintenance

All food offered to the cats was consumed every day, ensuring the cats always received the correct treatment. Body weight maintenance remained constant with an overall weight change of 1%. Faeces quality was consistently between Grades 2 and 3 as scored using the WALTHAM™ Faeces scoring system (Moxham, 2001).

7a.3.2. Urine measurements

Urine volume and specific gravity were not affected by the addition of NH_4Cl or $NaHCO_3$. Urine pH was significantly increased when feeding the $NaHCO_3$ diet, compared with feeding Diet CON *solus* (Table 7a.1). NH_4Cl tended to reduce urine pH although the difference was not significant.

7a.3.3 Urinary RSS

Supplementation with NH_4Cl resulted in production of a urine with a

significantly higher calcium oxalate RSS than when fed Diet CON *solus* (Table 7a.1, Figure 7a.1). Addition of $NaHCO_3$ did not affect calcium oxalate RSS but resulted in a significant increase in MAP RSS.

7a.3.4 Urinary concentrations

Urinary calcium (P=0.02) and ammonium (P<0.001) concentrations were significantly increased when cats were fed NH_4Cl (Table 7a.2). Supplementation with $NaHCO_3$ resulted in a significant increase in oxalate (P=0.006), citrate (P=0.008), sodium (P<0.001) and potassium (P=0.001) concentrations and a significant decrease in ammonium concentration compared to those observed during Diet CON (Table 7a.2). Urinary creatinine (P=0.24), magnesium (P=0.39) and phosphate (P=0.07) concentrations remained unchanged throughout the study.

Table 7a.1 Mean urine pH, calcium oxalate and magnesium ammonium phosphate (MAP) relative supersaturations (RSS) produced by six cats fed a control diet (CON) or the control diet plus NH_4Cl or $NaHCO_3$

Diet	Urine pH	CaOx RSS	MAP RSS
NH_4Cl	5.81 $\pm$0.14^a	1.54 $\pm$0.67^b	0.17 $\pm$0.04^a
CON	6.18 $\pm$0.26^a	0.55 $\pm$0.24^a	0.27 $\pm$0.20^a
$NaHCO_3$	6.81 $\pm$0.33^b	0.65 $\pm$0.40^a	1.52 $\pm$0.81^b

Different superscript letters within a column indicate significant differences (P<0.05)

Table 7a.2 Mean urinary excretions (mmol/l) of calcium (Ca), oxalate (Ox), citrate (Cit), potassium (K), sodium (Na), phosphate (PO_4) and ammonium (NH_4) produced by six cats fed a control diet (CON) or the control diet plus NH_4Cl or $NaHCO_3$

Diet	Ca	Ox	Cit	K	Na	PO_4	NH_4
NH_4Cl	0.62 $\pm$0.29^{b}	0.43 $\pm$0.07^{ab}	0.05 $\pm$0.04^{a}	112.83 $\pm$16.41^{a}	49.13 $\pm$6.95^{a}	35.92 $\pm$4.55^{a}	335.24 $\pm$35.00^{c}
CON	0.35 $\pm$0.19^{a}	0.34 $\pm$0.12^{a}	0.71 $\pm$0.72^{a}	141.36 $\pm$17.17^{ab}	57.02 $\pm$8.25^{a}	38.43 $\pm$7.11^{a}	225.74 $\pm$25.53^{b}
$NaHCO_3$	0.35 $\pm$0.15^{a}	0.47 $\pm$0.12^{b}	1.83 $\pm$1.36^{b}	161.30 $\pm$35.71^{b}	139.55 $\pm$34.24^{b}	43.74 $\pm$11.14^{a}	139.37 $\pm$45.03^{a}

Different superscript letters within a column indicate significant differences ($P<0.05$)

7a.4 Discussion

The moderately acidic urine produced by cats fed Diet CON resulted in production of urine undersaturated with respect to MAP (RSS<1.0). Increasing the dietary acid load through supplementation with NH_4Cl, a salt of a strong acid and a weak base (Lloyd and Sullivan, 1984), did not significantly change MAP supersaturation, although urinary ammonium excretion increased. An increase in urine pH towards 7.0 during $NaHCO_3$ supplementation resulted in a significant increase in MAP supersaturation, and thus an increased risk of MAP formation. Acidification of urine has been recommended for the management and prevention of MAP urolithiasis in cats (Buffington, 1988). Manipulation of urine pH has a major effect on the activity product of struvite, primarily because pH influences the concentrations of the different ionic forms of phosphate, although it also influences the proportion of ammonia present as ammonium ions.

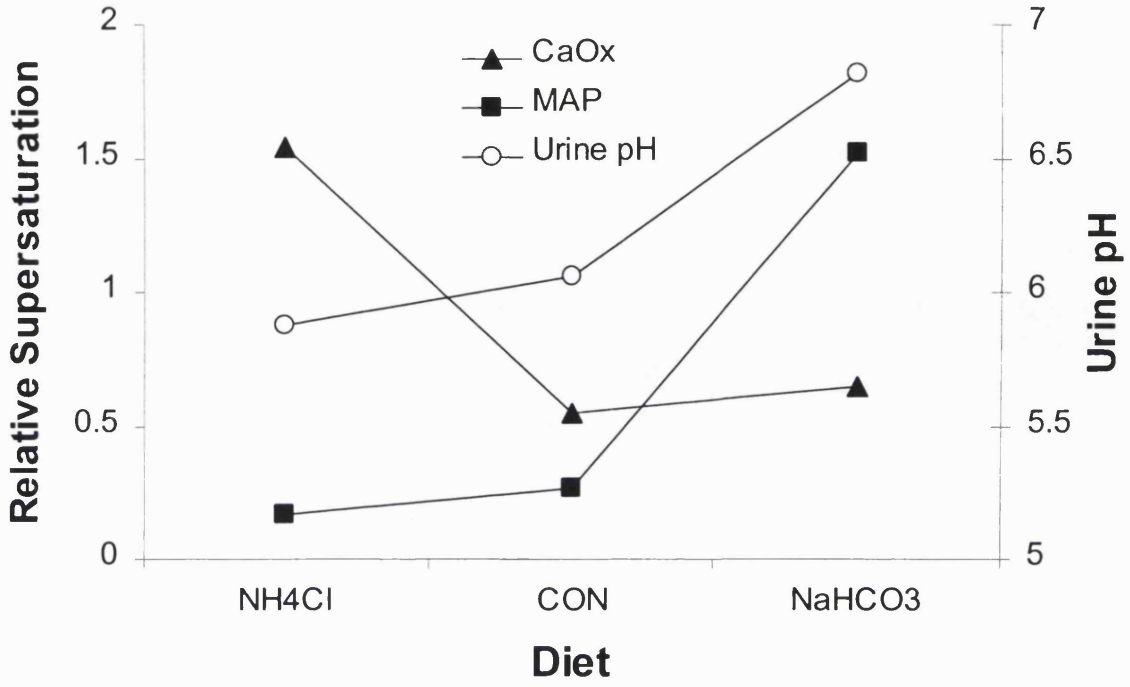


Figure 7a.1 Mean urine pH, magnesium ammonium phosphate (MAP) and calcium oxalate (CaOx) relative supersaturation (RSS) from cats fed a canned Diet *solus* (CON), or supplemented with ammonium chloride (NH_4Cl) or sodium bicarbonate ($NaHCO_3$)

Urinary acidification has been implicated as a risk factor for calcium oxalate urolithiasis in epidemiological studies of cats (Kirk *et al.*, 1995). One mechanism may involve the increase in urinary calcium excretion produced by the increased dietary acid load. During metabolic acidosis, acidifying metabolites are neutralised by phosphates and carbonates mobilised from bone. Bone calcium is released with the phosphorus resulting in hypercalciuria (Lulich *et al.*, 1999a). Increased urinary calcium excretion has been reported as a consequence of dietary supplementation with a urinary acidifier, which produced a urine pH of less than 6. This is thought to be

due to decreased tubular reabsorption of calcium in the kidney (Ching *et al.*, 1989). When the cats in the study reported here were fed the supplemented diet resulting in a urine pH below 6, the urinary calcium concentration significantly increased, compared with those during the other two diets, resulting in a significantly higher concentration of calcium oxalate supersaturation. No differences in urinary calcium concentration or calcium oxalate supersaturation, were seen between the moderately acidic urine and when the urine pH was close to neutral. It is likely that effects of dietary acid load on urinary calcium excretion in cats may not become marked until the acid load is sufficient to result in a risk of metabolic acidosis (Ching *et al.*, 1989).

Supersaturation of urine with respect to calcium oxalate is a prerequisite for crystal formation, either by heterogeneous or homogeneous nucleation; crystals will not form in undersaturated urine. Diet CON resulted in production of moderately acidic urine undersaturated with respect to calcium oxalate. This diet could therefore, be fed with the expectation of preventing calcium oxalate stone-formation. This concentration of acidification would also be appropriate for the prevention of MAP stone-formation especially when compared with urine of near neutral pH, as resulted from feeding the $NaHCO_3$ supplemented diet.

Dietary manipulation of urine pH significantly affected the concentrations of a number of other parameters within the urine of the cat. Urinary citrate may act as an inhibitor of calcium oxalate crystallisation, primarily through complex formation with calcium ions, and the potency of this inhibitor may be increased at a more alkaline urine pH (Pak, 1987). Urinary concentrations of citrate significantly increased when the cats received the $NaHCO_3$ diet. Urinary oxalate concentration also increased during $NaHCO_3$ supplementation. Although this has not been previously reported

during urinary alkalinisation it is possible that calcium absorption was reduced in the intestinal tract due to calcium phosphate precipitation as a result of less acidic conditions, even though urinary phosphate remained unaffected by changes in urine pH. This may have reduced 'free' calcium concentrations within the colon and thus reduced the capacity for calcium and oxalate binding. This would also explain the reduced concentrations of urinary calcium found as a result of feeding Diets CON and the $NaHCO_3$ supplemented diet. This, in turn, may have lead to increased availability of oxalate for absorption resulting in increased amounts of oxalate within the urine. However, regardless of changes in the urine composition during $NaHCO_3$ supplementation, this did not result in a concentration of calcium oxalate supersaturation that differed from that on Diet CON.

Increasing the acidification of the urine significantly increased urinary ammonia concentration. This is a normal response to an increased acid load, ensuring the maintenance of a constant acid-base balance within the body (Pitts, 1948; Good, 1989). Increased urinary acidification also decreased urinary potassium concentration. This effect has been previously observed in rats (Malnic *et al.*, 1971), humans (Barker *et al.*, 1957; Berliner, 1961) and dogs (Toussaint and Vereerstraeten, 1962). It was suggested that potassium ions compete with hydrogen ions so that during acidosis more hydrogen ions and less potassium ions are available for secretion (Toussaint and Vereerstraeten, 1962). Thus, the urinary potassium concentration decreases. Finally, supplementing the diet with $NaHCO_3$ resulted in a direct increase in urinary sodium concentration, because dietary sodium is excreted directly into the urine.

7a.5 Conclusions and clinical relevance

In conclusion, this study, therefore, indicates that it is possible to design one diet to aid in the control of both calcium oxalate and MAP urolithiasis. This study showed that, when compared to a diet designed to produce a moderately acidic urine pH within the range of 6.0-6.5:

1. the addition of a urinary acidifier, (resultant urine pH < 6.0), increased urinary calcium concentration, indicating that overacidification of the urine may be a risk factor for calcium oxalate stone-formation,
2. the addition of a urinary alkaliniser (resultant urine pH around 6.8), did not reduce calcium oxalate RSS, and resulted in production of a urine oversaturated with respect to MAP.

[a] WALTHAM™ Feline Lower Urinary Tract Support Diet, Masterfoods, Bruck, Austria.

Appendix 7a.1 Dietary analysis

Nutrient	Unit	Amount (per 100 kcal)
Moisture	g	3.40
Protein	g	14.37
Fat	g	2.75
Ash	g	3.08
Calcium	g	0.48
Phosphorus	g	0.38
Ca:P ratio	g/g	1.27
Sodium	g	0.26
Potassium	g	0.35
Magnesium	g	0.03
Iron	mg	13.7
Copper	mg	0.38
Manganese	mg	0.62
Zinc	mg	2.22

Published in American Journal of Veterinary Research, 61,4:430-435, 2000.

Section b: Dietary potassium citrate supplementation in normal healthy dogs

7b.1 Introduction

Currently, a recognised treatment for the medical dissolution of calcium oxalate uroliths *in situ* does not exist. However, dietary potassium citrate supplementation ($K_3C_6H_5O_7 \cdot H_20$) has been used for the past decade, in humans, to help prevent recurrence of calcium oxalate uroliths within the kidney (Pak, 1987; Robertson, 1993).

The beneficial effects are thought to be caused primarily by the alkalinising properties of citrate, although they may be partially attributable to concurrent advice for the patient to increase fluid intake (Robertson, 1993). During normal conditions of acid-base balance there is a steady removal of citrate from the tubular and peritubular cells of the nephron (Simpson, 1983). Citrate entering cells by means of a carrier accumulates within the cytoplasm to concentrations considerably in excess of those in the plasma. Cytoplasmic citrate is transported into the mitochondrion by a tricarboxylate carrier where it is disposed of by oxidative metabolism. The rate of citrate metabolism by these pathways is sufficient to cause net renal citrate utilisation that exceeds citrate reabsorption, and also results in consumption of the peritubular citrate. Some filtered citrate escapes reabsorption and appears in urine to an extent that varies between mammalian species (Simpson, 1983).

In humans, oral intake of potassium citrate does not *directly* affect the amount of citrate excreted in the urine because most citrate that is absorbed from the

gastrointestinal tract is metabolised to bicarbonate (Parivar *et al.*, 1996). However, the increase in bicarbonate creates an alkaline tide, and increases the mitochondrial pH gradient, causing citrate entry into the mitochondria to slow down. Citrate accumulates within the cytoplasm, reducing uptake on both sides of the cell. Net renal utilisation and reabsorption of citrate decreases and urinary citrate excretion increases (Simpson, 1983; Butz, 1986). The reverse effect is also found in metabolic acidosis (Simpson, 1983). Metabolic alkalosis results in an increase in urine pH and an increase in reabsorption of calcium in the distal tubule (Butz, 1986). As much as 60% of filtered citrate may also appear in human urine during alkalosis (Simpson, 1983).

Together, these factors help to decrease urinary calcium oxalate crystallisation in humans in a number of ways. Total urinary calcium excretion decreases (Edwards and Hodgkinson, 1965), and the availability of ionised calcium decreases further in alkalised urine by an increase in the amount and strength of binding to form soluble citrate and phosphate salts (Butz, 1986; Robertson, 1993). Certain inhibitors of the growth and precipitation of calcium oxalate crystals found in the urine of humans, such as citrate and pyrophosphate, are also activated by an increase in urine pH (Robertson, 1993). Indirectly, the amount of citrate excreted in the urine may also increase (Simpson, 1983). The cumulative effect is one of a decrease in urinary calcium oxalate supersaturation (Butz, 1986). Thus, the overall beneficial effect of dietary potassium citrate supplementation is far more complex and controversial than a simple increase in urinary excretion of citrate.

Dietary potassium citrate supplementation as a wax matrix tablet, launched in the United States in 1996, is commonly prescribed in humans for calcium oxalate

urolith formation (Pak *et al.*, 1985; Pak, 1987). The manufacturers claim that this form of potassium citrate will reduce the rate of stone recurrence rate in more than 90% of human patients by decreasing urinary supersaturation of calcium oxalate and inhibiting crystallisation. The wax matrix also permits delayed absorption and excretion of citrate throughout the day (Lulich *et al.*, 1992a).

Dietary potassium citrate supplementation has also been advocated in the prevention of recurrence of calcium oxalate uroliths in dogs (Lulich *et al.*, 1992a; Lulich and Osborne, 1995; Osborne *et al.*, 1995b; Lulich *et al.*, 2000; Osborne *et al.*, 2000b). Potassium citrate has been permitted as a food preservative for all species, including the dog, since 1991 and has been authorised for use as a urinary modifying substance in pet food since 1994 within Europe (Petfood Manufacturers Association, 1995). Within the United States, potassium citrate is listed as an acceptable mineral supplement in the American Association of Feed Control Officials Handbook, 1998, although no similar directives exist. Inclusion amounts of 0.2 to 0.5% in canned food and 1 to 2% in dry food have been recommended for modifying urine pH (Petfood Manufacturers Association, 1995). This amount of potassium citrate corresponds to approximately 150 mg/kg/d and, in dogs, has been reported to cause an increase in urine pH, although no consistent increase in citrate excretion was found (Osborne *et al.*, 1986b; Lulich and Osborne, 1995; Osborne *et al.*, 1995b). On the basis of this information, potassium citrate has been included in a commercially available diet designed to help prevent recurrence of calcium oxalate uroliths in dogs (Osborne *et al.*, 1995b).

Nevertheless, evidence to support the inclusion of potassium citrate in such diets is inconclusive. The effect of dietary potassium citrate supplementation on

urinary calcium oxalate supersaturation in dogs has not been established to date. Furthermore, dietary use of potassium citrate in the form of wax matrix tablets has not been studied in dogs, although recommendations for their use do exist in the literature (Osborne *et al.*, 1986b; Lulich *et al.*, 1992a).

The purposes of the study reported here were to assess the effects of dietary potassium citrate supplementation on the urine pH, urinary relative supersaturation (RSS) of calcium oxalate and MAP, and urinary concentrations of magnesium, ammonium, phosphate, citrate, calcium, and oxalate in healthy adult dogs.

7b.2 Materials and Methods

7b.2.1 Dogs and diet

Twelve healthy adult dogs consisting of 6 Miniature schnauzers, (1 sexually intact male, 2 castrated males, 3 sexually intact females; mean age 5.5±1.3 yrs), 4 Beagles, (3 spayed females, 1 sexually intact female; mean age 4.5±0.6 yrs), 2 Labrador retrievers, (sexually intact females, mean age 4.9±2.3 yrs) were fed a commercially prepared canned dog food (Cesar; [chicken variety], Pedigree Petfoods, WALTHAM™ on the Wolds, Leics, UK) (Appendix 1) twice daily at 8:30 am and 3.30 pm for 37 days. Food allowances were calculated according to adult maintenance energy requirements (110 $W^{0.75}$ kcal/d, where W is body weight expressed in kg) (Burger, 1995b), and adjusted during the study to ensure body weight maintenance within ±0.5% of original weight. All dietary nutrients were analysed as detailed in Chapter 2. Water was provided *ad libitum*.

7b.2.2 Study design

The dogs were randomly allocated to 3 feeding groups and fed the test diets for a period of 8 days according to a Latin Square design. Each study period was separated by a 6-day interval. During each study period the dogs were fed either the standard diet *solus* (control) or the standard diet plus 1 of 2 types of potassium citrate supplement mixed with the food twice daily. Supplements were either tri-potassium citrate (BDH Laboratory Supplies, Poole, Dorset), or wax matrix tablets (Urocit-K, Mission Pharmacal, San Antonio, Texas, USA) and were administered at a dosage of 150 mg of potassium citrate/kg/d. Each dog was weighed once weekly to ensure an accurate dosage was administered throughout the study.

7b.2.4 Housing details

Dogs were housed as described in Chapter 2 for two 48-hour periods (days 3 to 4 and 7 to 8). During the remaining 4 days, and during the interval phases, the dogs were housed in pairs. During this time all the dogs were walked once daily for approximately 15 minutes and group exercised in grass paddock areas for 1 to 2 hours.

7b.2.5 Urine measurements

Urine pH was continuously measured and specific gravity and urine volume were measured, as described in Chapter 2, during days 3 and 4 of every treatment. During days 7 and 8, a 48-hour urine sample was collected from each dog, immediately frozen, and analysed as described in Chapter 2. Urinary RSS concentrations for calcium oxalate and MAP were calculated using SUPERSAT.

7b.2.6 Statistical analysis

Analysis of variance and multiple range tests (least significant difference) were used to test the significance of dietary potassium citrate supplementation on urine volume, specific gravity and pH, urinary RSS of calcium oxalate and MAP, and urinary concentrations of magnesium, ammonium, phosphate, calcium, oxalate, and citrate. Concentration of significance was set at $P < 0.05$.

7b.3 Results

7b.3.1 Food intake and bodyweight maintenance

All food offered to the dogs was consumed every day ensuring that the dogs always received the correct amount of potassium citrate. Bodyweight remained constant throughout the trial with an overall weight change of 0.4%. Faeces quality was consistently between Grades 2 and 3 as scored using the WALTHAM™ Faeces scoring system (Moxham, 2001).

7b.3.2 Urine measurements

Dietary potassium citrate supplementation (in either form) did not have an effect on urine volume or specific gravity (Table 7b.1). Results of mean diurnal urine pH profiles (Figure 7b.1) indicate that all 3 diets resulted in an increase in urine pH between 8:00 am and 12 noon. Diets containing potassium citrate maintained a higher urine pH than the control diet from 3.00 pm to 9.00 pm. Consequently compared with control diet, the mean urine pH was higher in diets supplemented with potassium citrate by approximately 0.2 pH units (control diet, pH 6.75±0.34; diet with wax matrix tablet, pH 6.97±0.56), although this difference was not significant (P=0.34).

7b.3.3 Urinary RSS

Dietary potassium citrate supplementation in either form did not significantly influence the mean urinary RSS of calcium oxalate or MAP (Table 7b.1). On examination of individual RSS data, a decrease in calcium oxalate RSS was found in 3 dogs (1 sexually intact male and 2 sexually intact female Miniature schnauzers) fed diets supplemented with potassium citrate, compared with control diet (P=0.04). Both forms of potassium citrate supplementation produced the same effect in these three dogs (Table 3).

7b.3.4 Urinary concentrations

Dietary potassium citrate supplementation did not have an effect on the urinary concentrations of magnesium, calcium, oxalate and citrate (Table 7b.2). However, when examining data from each dog, the 3 Miniature schnauzers with decreased calcium oxalate RSS, also tended to excrete greater amounts of citrate. The reduction in calcium oxalate RSS was also driven by a decrease in urinary oxalate for both supplemented diets compared to the Control (P=0.02). Urinary calcium (P=0.79) and concentrations of all other minerals remained unchanged with potassium citrate supplementation.

Table 7b.1 Mean (±SD) daily urine volume, specific gravity, urine pH, and urinary RSS of calcium oxalate and MAP in 12 healthy dogs fed a commercially prepared dog food *solus* (control diet) or the same diet supplemented with two forms of potassium citrate (powder or tablet) at a dosage of 150 mg/kg of body weight/d.

Diet	Urine volume (ml/d)	Specific gravity	Relative supersaturation	
			Calcium oxalate	MAP
Control diet	436±312	1.027±0.008	1.12±0.43	0.53±0.34
Diet + TCP	463±408	1.026±0.007	1.32±1.44	0.83±0.87
Diet + WMT	479±347	1.028±0.006	0.98±0.41	0.76±0.62

TCP = potassium citrate supplemented as tri-potassium citrate powder.
WMT = potassium citrate supplemented as wax matrix tablets.

Table 7b.2 Mean (±SD) urinary concentration of magnesium (Mg), ammonium, phosphate, calcium (Ca), oxalate, and citrate in 12 healthy dogs fed a commercially prepared dog food *solus* (control diet) or the same treated with 2 forms of potassium citrate supplement (powder or tablet) at a dosage of 150 mg/kg of body weight/d.

Diet	Urinary concentration (mmol/l)					
	Mg	NH_4	PO_4	Ca	Oxalate	Citrate
Control diet	1.25 ±0.84	72.70 ±18.42	52.28 ±16.47	0.51 ±0.16	0.33 ±0.06	0.10 ±0.06
Diet + TCP	0.95 ±0.35	59.37 ±15.10	48.13 ±15.81	0.56 ±0.43	0.31 ±0.11	2.81 ±8.97
Diet + WMT	1.13 ±0.70	72.28 ±37.87	49.11 ±12.88	0.48 ±0.18	0.31 ±0.08	0.23 ±0.20

See Table 1 for key.

Table 7b.3 Calcium oxalate RSS, and urinary concentrations of calcium, oxalate, and citrate of the three individual dogs affected by potassium citrate supplementation

Diet	Individual dog	CaOx RSS	Urinary concentration (mmol/l)		
			Ca	Oxalate	Citrate
Control diet	MS 1	1.84	0.65	0.32	0.15
	MS 2	1.16	0.5	0.33	0.19
	MS 3	1.84	0.6	0.36	0.09
Diet + TCP	MS 1	0.70	0.55	0.28	0.26
	MS 2	0.72	0.42	0.37	0.24
	MS 3	1.11	0.77	0.12	0.31
Diet + WMT	MS 1	0.58	0.37	0.24	0.22
	MS 2	0.80	0.45	0.30	0.28
	MS 3	1.32	0.75	0.75	0.28

See Table 1 for key

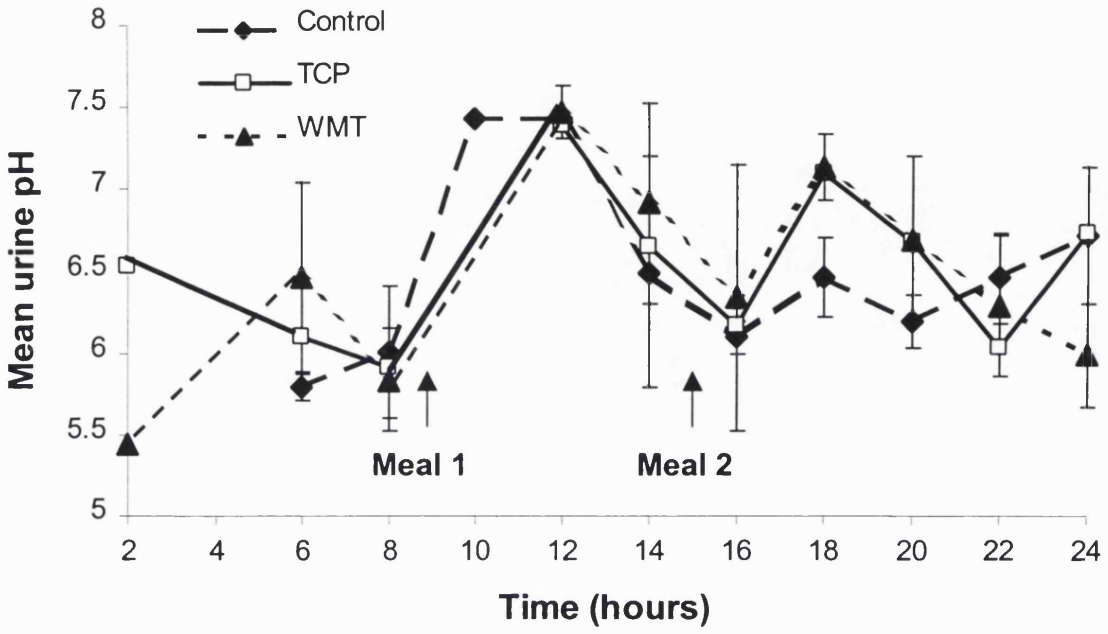


Figure 7b.1 Mean diurnal urine pH profiles for 12 healthy dogs fed a commercially prepared dog food *solus* (control) or treated with 2 forms of potassium citrate supplement (powder or tablet) at 150 mg/kg of body weight/d.

7b.4 Discussion

Results of many studies within the field of human medicine indicate that dietary potassium citrate supplementation significantly increases urine pH and citrate excretion, while decreasing urinary calcium oxalate supersaturation (Sakhaee *et al.*, 1983; Pak *et al.*, 1985; Pak, 1987; Berg *et al.*, 1992; Caudarella *et al.*, 1996; Lindberg *et al.*, 1996). Hypocitraturia may be a risk factor for calcium oxalate urolith formation in humans, with reports of 15 to 50% of affected individuals having low amounts of citrate excretion (Parivar *et al.*, 1996). In healthy humans, citrate chelates

calcium in the urine, helping to prevent precipitation of calcium salts, particularly in alkaline urine (Simpson, 1983). Under usual conditions, as much as 70% of urinary calcium may be bound to citrate urine (Simpson, 1983). When citrate excretion decreases, less calcium is chelated and calcium urolithiasis is promoted within the urine (Simpson, 1983). The reference range of urinary citrate concentration in humans is 2 to 5 mmol/l and hypocitraturia is defined as <1.5 mmol/l (Butz, 1986). In a study conducted by Whalley *et al*, 1996, giving hypocitraturic human subjects 31 mmol of potassium citrate/d (approx. 100mg of potassium citrate/kg) resulted in an increase in urinary citrate concentration to within reference range (Whalley *et al.*, 1996). Consequently, the rate of stone-formation was decreased by more than 80%. There are many other published examples of dietary potassium citrate supplementation increasing urinary citrate concentrations in humans (Pak *et al.*, 1985; Pak, 1987; Parivar *et al.*, 1996; Fuselier *et al.*, 1998).

Despite a tendency to hypocitraturia, there is no relationship between the mean excretion of citrate and the severity of the disorder in people that form calcium oxalate uroliths within the UK. However, hypocitraturia is far more common in countries such as the US and Saudi Arabia where there is a greater intake of animal protein than in the UK (Robertson *et al.*, 1989). As discussed in Section 1.7.2.5.1 a diet high in animal protein results in a more acidic urine. This in turn, inhibits the renal production of citrate and leads to the development of hypocitraturia (Robertson, 1993). In contrast, studies have also been conducted in which dietary citrate supplementation has had no effect on citrate excretion in humans (Thomas *et al.*, 1994). It is possible that dietary potassium citrate supplementation is of benefit in people with hypocitraturia, but may be of limited use when the amount of citrate

excretion is within reference range prior to treatment. In view of the complex nature of the biochemical response to dietary potassium citrate supplementation, conclusions have not been drawn as to the importance of high citrate excretion in the prevention of calcium oxalate urolith formation in humans (Robertson, 1993).

It is not known whether hypocitraturia is a risk factor for calcium oxalate urolith formation in dogs. Results of a study comparing urine excretion of citrate in 6 Miniature schnauzers, that form calcium oxalate uroliths with that of healthy beagles revealed that there was no difference in citrate excretion, indicating that hypocitraturia may not be an important contributory factor for calcium oxalate formation in dogs (Lulich *et al.*, 1991a). Results of another study on healthy dogs indicated that administration of up to 150 mg of potassium citrate/kg/d was not associated with a consistent increase in urinary citrate excretion (as it is in humans), although there was a dose-dependent increase in urine pH (Lulich *et al.*, 1992a). One explanation for this difference between people and dogs may be that although 10 to 35% of filtered citrate is excreted in urine by humans, only 1 to 3% of filtered citrate is excreted by dogs (Simpson, 1983). This is further clarified by comparing urinary citrate concentration in healthy dogs in our study with those in healthy humans (Kok *et al.*, 1990a). Our dogs excreted far less citrate (0.1 mmol/l of urine) than reported for humans (2.5 mmol/l of urine) (Kok *et al.*, 1990a).

In this study, a consistent increase in citrate excretion was not seen with dietary potassium citrate supplementation. However, three miniature schnauzers did excrete larger amounts of citrate when fed the supplemented diets. This finding is consistent with that found from the same dogs in a separate dose-response study using potassium citrate powder (Stevenson *et al.*, 1998a). In another study, involving 5

healthy dogs, supplementation of the diet with 100 mg of potassium citrate /kg/d, resulted in a mean increase in urine pH of 0.2 pH units from 7.34 to 7.55 (increase not significant) (Petfood Manufacturers Association, 1995). This increase in urine pH is comparable with the increase in mean urine pH in our study. Results of studies on people that form calcium oxalate uroliths indicate a far more pronounced effect of citrate treatment on daily urine pH (increases in pH from 5.92 to 6.22 when potassium citrate dosage is approximately 30 mEq/d (approx. 100 mg of potassium citrate/kg), and from 5.62 to 6.55 when potassium citrate dosage ranges from 30 to 100 mEq of potassium citrate/d with a mean of 60 mEq/d (approx. 200 mg of potassium citrate/kg). (Pak *et al.*, 1985)

Urinary pH is not a constant and fluctuates markedly during a 24-hour period. Factors including exercise, pulmonary ventilation, dietary habits, and emotional status are all known to influence urine pH in humans and as a result of these diurnal variations, pH is usually lowest throughout the night and highest during the day (Murayama and Taguchi, 1993). Humans were found to be at greater risk of calcium oxalate crystallisation overnight when urine pH is at its lowest (Ogawa *et al.*, 1983). At this time of day the urine volume decreases and body temperature is also at its lowest, facilitating an increase in urine supersaturation, and hence, increasing the potential for crystallisation and stone-formation in susceptible people (Ogawa, 1994). It is likely that the diurnal urine pH profile in dogs is affected by similar factors. In the dogs of this study, urine pH peaked between 1 and 4 hours after the first meal and the amount of response was similar in all feeding groups (Figure 7.1). This effect was thought to be attributable partly to a postprandial alkaline tide effect and partly to an increase in activity. A second smaller peak in urine pH was observed between 1 and 4

hours after the second meal, and this effect was greater in the potassium citrate supplemented dogs. A higher urine pH was maintained during a longer period when the diet was supplemented with potassium citrate. This may shorten the period of greatest risk for calcium oxalate formation in susceptible dogs.

Compared with our study, a substantially higher postprandial urine pH has been described for dogs fed a potassium citrate supplemented diet (Petfood Manufacturers Association, 1995), but dogs in that study were fed only once daily, in the morning. The apparent benefit of this postprandial pH response is debatable. Results of our study indicate that a postprandial increase in urine pH develops after feeding in the morning even without potassium citrate supplementation, and a further increase in urine pH may not be of any additional clinical benefit. Furthermore, the response time is limited when dietary supplementation is given only once daily, and may not coincide with the period of greatest risk for stone-formation. For dogs fed twice daily, however, a single dose of potassium citrate may be more beneficial if given with the evening meal because this would add to the initial postprandial pH effect and may increase the magnitude of the second increase in urine pH at a time when the potential for crystallisation may be highest. Results of a study conducted in healthy humans reveal that administration of potassium citrate at approximately 62 mg/ kg/d in a single evening dose increased urine pH to a peak at 2 hours after administration and this increase in urine pH was maintained until the next morning, although excretion of calcium and citrate were unaffected (Berg *et al.*, 1992). In that study the response to potassium citrate administration in healthy humans was compared with that of humans that form calcium oxalate uroliths. These results indicate that in patients a single dose of potassium citrate in the evening had a more

favourable effect, decreasing urinary calcium oxalate supersaturation in addition to increasing urine pH, during the overnight risk period (Berg *et al.*, 1992). It can also be postulated that dogs that form uroliths may have a more favourable response than healthy dogs when supplemented with potassium citrate in a single evening dose.

The clinical importance of increasing urine pH for the management calcium oxalate urolithiasis in dogs has not been investigated. Determination of calcium oxalate RSS is a well established method for assessing the potential efficacy of prophylactic measures on the calcium oxalate forming potential of urine in humans (Borghi *et al.*, 1996; Parks *et al.*, 1997; Milosevic *et al.*, 1998), although similar studies in dogs that form uroliths do not appear to have been published. In this study the increase in urine pH had no effect on urinary calcium oxalate RSS in most healthy dogs, although the increase in urine pH was minimal. Nevertheless, dietary potassium citrate supplementation significantly reduced urinary calcium oxalate RSS in three dogs, all miniature schnauzers, and in these dogs there was also an increase urinary citrate excretion, although this increase was not significant. Miniature schnauzers are known to be susceptible to calcium oxalate formation (Lulich *et al.*, 1991b). Results of one study indicate that miniature schnauzers that form calcium oxalate uroliths differ from control dogs (healthy beagles) in terms of urinary variables (Lulich *et al.*, 1991a). Compared with healthy dogs, affected Miniature schnauzers had a higher calcium excretion and a lower oxalate excretion than the control dogs, whereas citrate excretion was unchanged (Lulich *et al.*, 1991a).

To compare urinary excretion values from healthy dogs in this study with those of dogs that form stones in other studies, the urinary data were converted from mmol/l of urine to mg/kg/d. Calcium and oxalate excretion remained unchanged

throughout our study, hence the overall mean values were used for comparison with published data (Lulich *et al.*, 1991a). Calcium excretions in the dogs from this study (0.65±0.29 mg/kg/d) were comparable with values from healthy Beagles (0.51±0.28 mg/kg/d) (Lulich *et al.*, 1991a), and were lower than values from Miniature schnauzers that form stones (2.54±1.20 mg/kg/d) (Lulich *et al.*, 1991a). Oxalate excretions in this study (0.90±0.35 mg/kg/d) were comparable with those of Miniature schnauzers (0.89±0.85 mg/kg/d) (Lulich *et al.*, 1991a), and lower than those of healthy Beagles (1.74±0.90 mg/kg/d). (Lulich *et al.*, 1991a) Citrate excretions in the dogs of this study (0.57±0.32 mg/kg/d; control diet to 1.55±1.00 mg/kg/d; diet with wax matrix tablet) were lower than those reported for Miniature schnauzers that form stones (6.68±7.7 mg/kg/d) (Lulich *et al.*, 1991a), or healthy Beagles (2.57±2.31 mg/kg/d) (Lulich *et al.*, 1991a). Differences between studies in citrate and oxalate excretion values may be the result of different procedures for analysis, or differences in dietary content of oxalate and citrate.

Another proposed benefit of dietary potassium citrate supplementation is that the induced urinary alkalinisation increases the renal tubular reabsorption of calcium, thereby, decreasing its excretion in urine. In a study in humans, intake of 10 g (approx. 142 mg/kg) of potassium citrate caused a 30% mean decrease in urinary calcium excretion (Butz, 1986). However, there are also the results of many other studies in humans that indicate that citrate treatment had no effect on calcium excretion (Pak *et al.*, 1985; Thomas *et al.*, 1994; Whalley *et al.*, 1996; Fuselier *et al.*, 1998). It has been suggested that this effect may also be beneficial in dogs even without an associated increase in urinary citrate (Lulich *et al.*, 1992a; Lulich and Osborne, 1995; Osborne *et al.*, 1995b; Lulich *et al.*, 2000). In our study, a reduction

in calcium excretion was not seen when dogs were given a diet supplemented with potassium citrate (P=0.67). However, in the three dogs in which potassium citrate supplementation reduced calcium oxalate RSS urinary oxalate concentration was reduced. This effect has not been observed in any of the studies in which human subjects received potassium citrate supplementation (Pak *et al.*, 1985; Berg *et al.*, 1992; Whalley *et al.*, 1996; Fuselier *et al.*, 1998), and the underlying mechanisms responsible for this effect remain unclear.

Although dietary potassium citrate supplementation in the form of wax matrix tablets has been recommended for management of canine calcium oxalate urolithiasis (Lulich *et al.*, 1992a; Osborne *et al.*, 1995b), there are no published data to support this recommendation. Results of a study in humans, in which people that form uroliths were treated with potassium citrate as a wax matrix tablet, indicate that potassium citrate supplementation significantly increases urine pH and citrate excretion (Lindberg *et al.*, 1996). Although it has been suggested that the wax matrix formulation may delay gastrointestinal tract absorption and subsequent urinary excretion of citrate, there is no evidence currently available to support this claim in dogs. In our study of healthy dogs, no differences in citrate excretion or urine pH response were found between wax matrix and powder supplements.

7b.5 Conclusions and clinical relevance

Dietary potassium citrate supplementation had limited effects on urine variables in most healthy dogs although supplementation resulted in maintenance of a higher urine pH later in the day. Consequently, if supplementation is introduced dogs should be fed twice daily and potassium citrate should be given with both meals or with the evening meal only.

Appendix 7b.1 Dietary analysis

Nutrient	Unit	Amount/100 kcal
Moisture	g	109.3
Protein	g	10.41
Fat	g	7.16
Ash	g	2.47
Calcium	g	0.36
Phosphorus	g	0.41
Ca:P	g/g	0.87
Sodium	g	0.16
Magnesium	g	0.02
Iron	mg	5.46
Copper	mg	0.61
Manganese	mg	0.39
Zinc	mg	2.98

CHAPTER 8. GENERAL DISCUSSION

8.1 Principal findings

1. MAP and calcium oxalate were the two most common uroliths found in cats and dogs across the four countries studied.

- Differences in the relative occurrence of the two mineral types were found between countries, possibly due to demographic factors such as environment and lifestyle.

- Other factors associated with specific mineral types in dogs were sex, age and breed. In cats, the Persian was over-represented compared to other breeds.

2. Calcium oxalate stone-forming dogs showed hyperabsorption of calcium leading to hypercalciuria. Indirectly, this lead to hyperoxaluria and the increased risk of calcium oxalate urolith formation. Urinary calcium and oxalate were the major risk factors for calcium oxalate urolith formation in dogs.

3. Differences in urine composition exist between breeds fed the same diet, which may increase the risk of certain breeds, such as the Miniature schnauzer and Cairn terrier, forming calcium oxalate uroliths.

4. Increased dietary moisture or dietary sodium reduced calcium oxalate RSS, and thus the risk of calcium oxalate urolith formation in the Miniature schnauzer, a breed susceptible to formation of this mineral type.

5. A reduction in dietary calcium without a concomitant decrease in dietary oxalate may increase the risk of calcium oxalate crystallisation in susceptible breeds.

6. It is possible to design a diet that will aid in the control of both calcium oxalate and MAP crystal formation in cats. An optimum pH range that minimises the risk of both urolith types was identified as being between 6 and 6.5

8.2 General discussion

The accurate, quantitative analysis of over 1000 uroliths removed from dogs and cats demonstrated that MAP and calcium oxalate were the two most common urolith types found in both species, across the four countries participating in the study (Chapter 3). There were some differences in the relative prevalence of the two mineral types between countries. MAP uroliths were also more prevalent in female dogs, while all other mineral types were more prevalent in males. Calcium oxalate, in particular, appeared to form in dogs at an older age for reasons yet to be explored. The age and sex trends are not new observations noted in dogs (Hesse, 1990; Ling *et al.*, 1998c; Osborne *et al.*, 1999a), yet they remain highly intriguing, and this is an area that warrants further investigation. Contrary to the findings in some other studies, no trends in age- or sex-predisposition were noted in cats.

That certain breeds of dog were at increased risk of ammonium urate/uric acid and cystine uroliths is of no surprise given the nature of the genesis of these metabolic uroliths. Although MAP was associated with certain breeds, many of these were also the more popular breeds. In addition, because urinary tract infections play a major role in MAP urolith formation in dogs, the relevance of breed as a factor in MAP urolith formation remains unclear. Calcium oxalate uroliths, in particular, appeared to

be strongly associated with certain breeds both in this study and in those previously reported (Hesse, 1990; Hesse *et al.*, 1997; Ling *et al.*, 1998a; Osborne *et al.*, 1999a), the majority of which were small in size, and genetically inherited traits are a possibility. From urolith analysis data, both in this thesis and previous studies (Thumchai *et al.*, 1996), it appears that the Persian is at increased risk of both MAP and calcium oxalate urolith formation, compared to other pure breeds. However, it is not clear whether this is a true breed predisposition or whether demographic factors are more responsible.

When examining the risk factors associated with calcium oxalate urolith formation in dogs, a comparison of normal and stone-forming dogs indicated that diet might play a role (Chapter 4). However, because urinary calcium excretion remained high in affected dogs while on a relatively low calcium diet, this group appeared to demonstrate calcium hyperabsorption, resulting in hypercalciuria. Increased absorption of calcium led to the increased availability of oxalate for absorption, and subsequent hyperoxaluria. Consequently, urinary calcium and oxalate were identified as the major risk factors for calcium oxalate formation in dogs, although uric acid was elevated in some dogs. An attempt was made to quantify the relative probability (P_{SF}) of calcium oxalate stone-formation from these three parameters, and a good distinction between stone-forming and normal dogs was obtained, although calcium oxalate RSS still more clearly defined the two groups.

Since, through urolith analysis, certain breeds have been identified as being at increased risk of calcium oxalate urolith formation in both this thesis and in previous studies, a study was designed to compare the urine composition of susceptible breeds with that of the Labrador retriever, a breed at low risk of calcium oxalate urolith

formation (Chapter 5). A number of parameters were consistently different between the urine of susceptible breeds and the Labrador retriever, including a lower urine volume (per kg body weight), higher urinary calcium concentration, higher calcium oxalate and/or brushite RSS. Although this study also showed that susceptible breeds have an increased intake of dietary minerals, which may partly contribute to the increased urinary calcium, other urinary minerals were unaffected. The underlying mechanisms behind the differences identified in urinary composition were not determined during these studies, and warrant further investigation.

The calcium oxalate RSS observed in the susceptible breeds, and in particular the Cairn terriers, was as high as those observed in some of the calcium oxalate stone-forming dogs, yet the dogs used in Chapter 5 were healthy. The healthy dogs were only maintained on this study for three weeks and it is possible that they would have progressed on to urolith formation if kept on the study for a longer period of time. The diet fed in Chapter 5 was a low moisture dry diet low in sodium and relatively high in calcium. These factors have all been shown to increase the calcium oxalate RSS in susceptible breeds, as reported in this thesis. However, it is also possible that there are other, as yet unidentified, differences that exist between normal dogs and those that form calcium oxalate uroliths, acting as either inhibitors or promoters of this stone type.

Through dietary manipulation, a number of studies were conducted in an attempt to establish the effect of diet on the urine composition of the Miniature schnauzer, a breed susceptible to calcium oxalate urolith formation, compared with the Labrador retriever. Since the Miniature schnauzer was identified as producing a low urine volume with a high calcium concentration, the effect of dietary moisture

and dietary sodium were investigated in the hope that these interventions would increase urine volume, reduce urinary calcium concentration and thereby reduce the risk of calcium oxalate crystallisation (Chapter 6a). In the Miniature schnauzer both methods were successful. In contrast, dietary moisture had no effect on the urine volume and calcium concentration produced by the Labrador retriever. Again, further investigation into the mechanisms behind these observations and in particular the difference between breeds, is required. That increased urinary calcium has been observed when humans are supplemented with sodium (Kleeman *et al.*, 1964; Zarkadas *et al.*, 1989; Siener *et al.*, 1991; Sakhaee *et al.*, 1993; Martini *et al.*, 2000), but not in the dogs of this study is also intriguing.

The relative importance of dietary calcium and oxalate as risk factors for calcium oxalate urolith formation has been extensively studied in humans, and was studied in Miniature schnauzers, at concentrations encountered in commercially available dog foods (Chapter 6b). Dietary calcium was found in far larger amounts than oxalate in the majority of foods. However, the results demonstrated that both minerals should be controlled in order to minimise the risk of calcium oxalate crystallisation in susceptible dogs. As found in humans, if dietary calcium is reduced without a concomitant decrease in oxalate, the risk of calcium oxalate crystallisation is increased.

In the final section of this thesis (Chapter 7), two forms of treatment therapy were investigated. From the study reported here, there is little evidence to suggest that potassium citrate therapy will be beneficial in the majority of dogs, whether fed as a powder, or as a wax matrix tablet, although this study was conducted in healthy dogs rather than those affected by calcium oxalate urolithiasis. Potassium citrate

appears to be most beneficial in humans suffering from hypocitraturia (Pak *et al.*, 1985; Pak, 1987; Whalley *et al.*, 1996). At the time of this study it was not known whether the dogs with calcium oxalate uroliths were also hypocitraturic. However, since the completion of the clinical study in Chapter 4, it is now clear that these calcium oxalate stone-forming dogs were not hypocitraturic since there were no differences between the stone-forming and normal dogs, in terms of urinary citrate concentration. Thus, even in calcium oxalate stone-forming dogs, potassium citrate may not be an effective treatment method for prevention of recurrence.

An acid urine pH is commonly cited as a factor contributing towards the formation of calcium oxalate uroliths in cats (Buffington and Chew, 1999; Bartges *et al.*, 2000; Hesse *et al.*, 2000). The introduction of acidifying diets to minimise the risk of MAP formation has been held responsible for the increase in prevalence of calcium oxalate seen by stone analysis centres in Germany (Hesse *et al.*, 2000), and North America (Buffington and Chew, 1999; Bartges *et al.*, 2000). Evidence from this study does suggest that overacidification (urine pH<6) increases urinary calcium concentration which may increase the risk of calcium oxalate stone-formation in cats. When urine pH was increased towards neutral there were no benefits in terms of a reduced risk of calcium oxalate, and in addition, the urine became oversaturated with MAP. In contrast moderately acidic urine (pH~6.3) was found to reduce the risk of both calcium oxalate and MAP crystal formation, offering an opportunity to manage the two most common types of urolith found in cats with one dietary approach.

8.3 Conclusions

Quantitative urolith analysis techniques allowed accurate identification of predominant urolith types, and trends towards age, sex and breed for each mineral

type. The major urinary risk factors for calcium oxalate urolith formation were established as calcium and oxalate, although uric acid was also increased in some dogs. Although together these three parameters were found to differentiate stone-formers from normal dogs, RSS more clearly defined the two groups. Differences between the urine composition of susceptible breeds, compared with that of a breed at low risk of calcium oxalate urolith formation, were identified. Dietary moisture and increased dietary sodium were shown to reduce the severity of some of these differences, thereby reducing the risk of calcium oxalate urolith formation. It was also demonstrated that both dietary calcium and oxalate have to be controlled in order to reduce the risk of calcium oxalate crystallisation in susceptible dogs. Dietary potassium citrate therapy had limited effects on urine variables in the majority of dogs. Finally, a moderately acidic urine pH was shown to be optimal for the control of both calcium oxalate and MAP formation in cats.

"We must not cease from exploration and the end of our exploring will be to arrive where we began and to know the place for the first time" *T.S. Eliot*

REFERENCES

Agnew, L.R.C. (1951) Renal lesions in pyridoxin-deficient rats. *Journal of Pathology and Bacteriology* 63: 699.

Allen, T.A., Kruger, J.M. (2000) Feline lower urinary tract disease. In: *Small animal clinical nutrition.* M. S. Hand, C. D. Thatcher, R. L. Remillard and P. Roudebush (eds). USA, Walsworth Publishing Company : pp 689-723.

Allen, T.A., Magerkurth, J., Hand, M.S., *et al* (1989) The effect of dietary sodium on urinary excretion in normal dogs [Abstract]. In: *7th ACVIM Forum.* G. Pidgeon, (ed) Wisconsin, Omnipress.: 128 (only).

Allison, M.G., Cook, H.M. (1981) Oxalate degradation by microbes of the large bowel of herbivores: the effect of dietary oxalate. *Science* 212: 675-676.

Allison, M.G., Cook, H.M., Milne, D.B., *et al.* (1986) Oxalate degradation by gastrointestinal bacteria from humans. *Journal of Nutrition* 116: 455-460.

Andersen, D.A. (1973) Environmental factors in the aetiology of urolithiasis. In: *Urinary calculi.* L. Cifuentes Delatte, A. Rapado and A. Hodgkinson (eds). Basel, Karger : pp 130-144.

Archer, H.E., Dormer, A.E., Scowen, E.F., Watts, R.F.E. (1957) Studies on the urinary excretion of oxalate by normal subjects. *Clinical Science* 16: 405-411.

Argenzio, R.A., Liacos, J.A., Allison, M.J. (1988) Intestinal oxalate-degrading bacteria reduce oxalate absorption and toxicity in guinea pigs. *Nutrition* 118: 787-792.

Ashby, R.A., Byrne, J.P., Gyory, A.Z. (1999) Urine is a saturated equilibrium and not a metastable supersaturated solution: evidence from crystalluria and the general composition of calcium salt and uric acid calculi. *Urological Research* 27: 297-305.

Ashmont, C. (1891) *Dogs: Their management and treatment in disease.* Boston, J. Loring Thayer.

Asper, R. (1984) Epidemiology and socioeconomic aspects of urolithiasis. *Urological Research* 12: 1-5.

Asplin, J., DeGanello, S., Nakagawa, Y.N., *et al.* (1991) Evidence that nephrocalcin and urine inhibit nucleation of calcium oxalate monohydrate crystals. *American Journal of Physiology* 261: F824-F830.

Asplin, J.R., Favus, A.J., Coe, F.L. (2000) Nephrolithiasis. In: *The kidney.* B. M. Brenner (ed). Philadelphia, WB Saunders : pp 1774-1819.

Assimos, D.G., Holmes, R.P. (2000) Role of diet in the therapy of urolithiasis. *Urologic Clinics of North America* 27: 255-268.

Atmani, F., Lacour, B., Strecker, G., *et al.* (1993) Molecular characteristics of uronic-acid-rich protein, a strong inhibitor of calcium oxalate crystallization in vitro. *Biochemical Biophysical Research Communications* 191: 1158-1165.

Auer, B.L., Auer, D., Rodgers, A.L. (1998) The effect of ascorbic acid ingestion on the biochemical and physicochemical risk factors associated with calcium oxalate kidney stone formation. *Clinical Chemistry and Laboratory Medicine* 36: 143-148.

Baggio, B., Plebani, M., Gambaro, G. (1998) Pathogenesis of idiopathic calcium nephrolithiasis: update 1997. *Critical Reviews in Clinical Laboratory Science* 35: 153-187.

Bailey, C.B. (1973) Formation of siliceous urinary calculi in calves given supplements containing large amounts of sodium chloride. *Canadian Journal of Animal Science* 53: 55-60.

Baker, P.W., Rofe, A.M., Bais, R. (1996) Idiopathic calcium oxalate urolithiasis and endogenous oxalate production. *Critical Reviews in Clinical Laboratory Sciences* 33: 39-82.

Balaji, K.C., Menon, M. (1997) Mechanism of stone formation. *Urologic Clinics of North America* 24: 1-11.

Barker, E.S., Singer, R.B., Elkinton, J.R., Clark, J.K. (1957) The renal response in man to acute experimental respiratory alkalosis and acidosis. *Journal of Clinical Investigation* 36: 515-529.

Bartges, J., Osborne, C., Lane, I. (2000) Urolithiasis in cats: Changing composition and changing paradigms. In: *9th International Symposium on Urolithiasis.* A. L. Rodgers, B. E. Hibbert, B. Hess, *et al.* (eds) Cape Town, South Africa, University of Cape Town : pp 812-814.

Bartges, J.W., Osborne, C.A., Felice, L.J., *et al.* (1995a) Diet effect on activity product ratios of uric acid, sodium urate and ammonium urate in urine formed by healthy Beagles. *American Journal of Veterinary Research* 56: 329-333.

Bartges, J.W., Osborne, C.A., Felice, L.J., *et al.* (1995b) Influence of two amounts of dietary casein on uric acid, sodium urate and ammonium urate activity product ratios of healthy Beagles. *American Journal of Veterinary Research* 56: 893-897.

Bartges, J.W., Osborne, C.A., Lulich, J.P., *et al.* (1999) Canine urate urolithiasis. Etiopathogenesis, diagnosis and management. *Veterinary Clinics of North America* 29: 161-191.

Bataille, P., Charransol, G., Gregoire, I., *et al.* (1983) Effect of calcium restriction on renal excretion of oxalate and the probability of stones in the various pathological groups with calcium stones. *Journal of Urology* 130: 218-223.

Bataille, P., Pruna, A., Gregoire, I., *et al.* (1985) Critical role of oxalate restriction in association with calcium restriction to decrease the probability of being a stone former: insufficient effect in idiopathic hypercalciuria. *Nephron* 39: 321-324.

Bateson, E.M. (1973) Renal tract calculi and climate. *Medical Journal of Australia* 2: 111-113.

Batinic, D., Milosevic, D., Blau, N., *et al.* (2000) Value of urinary stone promoters/inhibitors ratios in the estimation of the risk of urolithiasis. *Journal of Chemical Information and Computer Science* 40: 607-610.

Berg, C., Larsson, L., Tiselius, H.G. (1992) The effects of a single evening dose of alkaline citrate on urine composition and calcium stone formation. *Journal of Urology* 148: 979-985.

Berliner, R.W. (1961) Renal mechanisms for potassium excretion. *Harvey Lectures* 55: 141-171.

Bichler, K.H., Kirchner, C., Ideler, V. (1976) Uromucoid excretion of normal individuals and stone-formers. *British Journal of Urology* 47: 733-738.

Bien, E.J., Yu, T.F., Benedict, J.D. (1953) The relation of dietary nitrogen consumption to the rate of uric acid synthesis in normal and gouty men. *Journal of Clinical Investigation* 32: 778-779.

Blacklock, N.J. (1969) The pattern of urolithiasis in the Royal Navy. In: *Proceedings of the Renal Stone Research Symposium.* J. E. A. Wickham (ed). London, Churchill : pp 33-47.

Blount, W.P. (1931) Urinary calculi. *Veterinary Journal* 87: 561-576.

Blumenfeld, J.D., Vaughan, E.D. (1998) Renal physiology. In: *Campbells urology.* P. C. Walsh, A. B. Retik, E. D. Vaughan and A. J. Wein (eds) Missouri, WB Saunders : pp 263-313.

Boistelle, R., Abbona, F., Madsen, H.E.L. (1983) On the transformation of struvite into newberyite in aqueous systems. *Physics and Chemistry of Minerals* 9: 216-222.

Borghi, L., Meschi, T., Amato, F., *et al.* (1993) Hot occupation and nephrolithiasis. *Journal of Urology* 150: 1757-1760.

Borghi, L., Meschi, T., Amato, F., *et al.* (1996) Urinary volume, water and recurrences in idiopathic calcium nephrolithiasis: a 5 year randomized prospective study. *Journal of Urology* 155: 839-843.

Borghi, L., Meschi, T., Schianchi, T., *et al.* (1999) Urine volume: Stone risk factor and preventive measure. *Nephron* 81 (Suppl): 31-37.

Bovee, K.C., McGuire, T. (1984) Qualitative and quantitative analysis of uroliths from dogs: Definitive determination of chemical type. *Journal of the American Veterinary Medical Association* 185: 983-987.

Boyce, W.H. (1968) Organic matrix of human urinary concretions. *American Journal of Medicine* 45: 673-683.

Boyce, W.H., King, J.S., Fieldman, M.L. (1962) Total nondialyzable solids (TNDS) in human urine. Immunological detection of a component peculiar to renal calculous matrix and to urine of calculous patients. *Journal of Clinical Investigation* 41: 1180-1189.

Briggs, D.M., Harley, E.H. (1986) The fate of administered purines in the Dalmatian coach hound. *Journal of Comparative Pathology* 96: 267-278.

Briggs, H. (1994) Anatomy and physiology. In: *Veterinary notes for cat owners.* T. Turner and J. Turner (eds). London, Stanley Paul : pp 129-131.

Brockis, J.G., Levitt, A.J., Cruthers, S.M. (1982) The effects of vegetable and animal protein on calcium, urate and oxalate excretion. *Jounal of Urology* 54: 590-593.

Brodey, R.S. (1955) Canine urolithiasis. A survey and discussion of fifty-two clinical cases. *Journal of the American Veterinary Medical Association* 126: 1-9.

Brown, C.M., Purich, D.L. (1992) Physical-chemical processes in kidney stone formation. In: *Disorders of bone and mineral metabolism.* F. L. Coe and M. J. Favus, (eds). New York, Raven Press : pp 613-624.

Brown, N.O., Parks, J.L., Greene, R.W. (1977) Recurrence of canine urolithiasis. *Journal American Veterinary Medical Association* 1970: 419-422.

Buffington, C.A. (1988) Feline struvite urolithiasis: effect of diet. In: *Proceedings of the 3rd annual symposium of the ESVNU.* Barcelona : pp 73-112.

Buffington, C.A., Chew, D.J. (1999) Calcium oxalate urolithiasis in cats. *Journal of Endourology* 13: 659-663.

Buffington, C.A., Rogers, Q.A., Morris, J.G. (1992) Effect of diet on struvite activity product in feline urine. *American Journal of Veterinary Research* 51: 2025-2030.

Buffington, C.A.T., Chew, D.J., Kendall, M.A., *et al.* (1997) Clinical evaluation of cats with non-obstructive urinary tract disease. *Journal of the American Veterinary Medical Association* 210: 46-50.

Burdette, D.C., Thomas, W.C., Finlayson, B. (1976) Urinary supersaturation with calcium oxalate before and during orthophosphate therapy. *Journal of Urology* 115: 418-422.

Burger, I. (1995a) A basic guide to nutrient requirements. In: *The* WALTHAM™ *book of companion animal nutrition.* I. Burger (ed). Oxford, Pergamon Press : pp 5-24.

Burger, I. (1995b) Updated feeding reccomendations for the canine diet. WALTHAM™ *Focus* 5: 32 (only).

Burger, I., Anderson, R.S., Holme, D.W. (1980) Nutritional factors affecting water balance in the dog and cat. In: *Nutrition of the cat and dog.* Anderson.R.S. (ed) Oxford, Pergamon Press : pp 145-156.

Bush, B.M. (1991) *Interpretation of laboratory results for small animal clinicians.* Oxford, Blackwell.

Bushinsky, D.A., Bashir, M.A., Riordon, D.R., *et al.* (1999) Increased dietary oxalate does not increase urinary calcium oxalate saturation in hypercalciuric rats. *Kidney International* 55: 602-612.

Bushinsky, D.A., Parker, W.R., Asplin, A.R. (2000) Calcium phosphate supersaturation regulates stone formation in genetic hypercalciuric stone-forming rats. *Kidney International* 57: 550-560.

Butz, M. (1986) Rational preventation of calcium urolithiasis. *Urologia Internationalis* 41: 387-392.

Casal, M.L., Giger, U., Bovee, K.C., *et al.* (1995) Inheritance of cystinuria and renal defect in Newfoundlands. *Journal of the American Veterinary Medical Association* 207: 1585-1589.

Case, L.P., Carey, D.P., Hirakawa, D.A. (1995) *Canine and feline nutrition.* St. Louis, Mosby-Year Book Inc.

Caudarella, R., Dormi, A., Rizzoli, E. (1996) Chronic treatment with potassium citrate in recurrent calcium urolithiasis. In: *8th International Symposium on Urolithiasis.* C. Y. C. Pak, M. I. Resnick and G. M. Preminger (eds). Dallas, Millet the Printer : pp 496-497.

Ching, S.V., Fettman, M.J., Hamar, D.W. (1989) The effect of chronic dietary acidification using ammonium chloride on acid-base and mineral metabolism in the adult cat. *Journal of Nutrition* 119: 902-915.

Cizek, L. (1959) Long-term observation on the relationship between food and water ingestion in the dog. *American Journal of Physiology*: 342-346.

Clark, W.T. (1974) Staphylococcal infection of the urinary tract and its relation to urolithiasis in dogs. *The Veterinary Record* 95: 204-206.

Classen, H.G., Schutte, K., Schimatschek, H.F. (1995) Different effects of three high-dose oral calcium salts on acid-base metabolism, plasma electrolytes and urine parameters of rats. *Methods and Findings of Experimental Clinical Pharmacology* 17: 437-442.

Coe, F.I., Parks, J.H., Nakagawa, Y. (1991) Protein inhibitors of crystallization. *Seminars in Nephrology* 11: 98-109.

Coe, F.L., Favus, M.J. (1980) Idiopathic hypercalciuria in calcium nephrolithiasis. *Disease-a-Month* 26: 1-36.

Coe, F.L., Lawton, R.L., Goldstein, R.B., Tembe, V. (1975) Sodium urate accelerates precipitation of calcium oxalate *in vitro*. *Proceedings of the Society of Experimental Biology and Medicine* 149: 926-929.

Coe, F.L., Moran, E., Kavalich, A.G. (1976) The contribution of dietary purine over-consumption to hyperuricosuria in calcium oxalate stone formers. *Journal of Chronic Diseases* 29: 793-800.

Coe, F.L., Raisen, L. (1973) Allopurinol treatment of uric acid disorders in calcium stone-formers. *Lancet* 1: 129-131.

Coe, F.L., Strauss, A.L., Tembe, V., Le Dun, S. (1980) Uric acid saturation in calcium nephrolithiasis. *Kidney International* 17: 662-668.

Cowley, A.W., Skelton, M.M., Merrill, D.C. (1986) Osmoregulation during high salt intake: relative importance of drinking and vasopressin secretion. *American Journal of Physiology* 251: R878-R886.

Cramer, C.F. (1965) Sites of calcium absorption and the calcium concentration of gut contents in the dog. *Canadian Journal of Physiology and Pharmacology* 43: 75-78.

Cummings, N.B. (1977) Overview of bladder stone disease. *Fogarty International Center Proceedings* 37: 1-6.

Cupisti, A., Morelli, E., Lupetti, S., *et al.* (1992) Low urine citrate excretion as main risk factor for recurrent calcium oxalate nephrolithiasis in males. *Nephron* 61: 73-76.

Curhan, G.C. (1997) Dietary calcium, dietary protein and kidney stone formation. *Mineral and Electrolyte Metabolism* 23: 261-264.

Curhan, G.C., Willett, W.C., Rimm, E.B., *et al.* (1993) A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *New England Journal of Medicine* 328: 833-838.

Curhan, G.C., Willett, W.C., Speizer, F.E., *et al.* (1997) Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women. *Annals of Internal Medicine* 126: 497-504.

Dahlberg, P.J., Van Den Berg, C.J., Kurtz, S.B., *et al.* (1977) Clinical features and management of cystinuria. *Mayo Clinic Proceedings* 52: 533-542.

Dawson, K.A., Allison, M.J., Hartman, P.A. (1988) Isolation and some characteristics of anaerobic bacteria from the rumen. *Applied Environmental Microbiology* 40: 833-839.

Dent, C.E., Senior, B. (1955) Studies on the treatment of cystinuria. *British Journal of Urology* 27: 533-542.

Despopoulos, A., Silbernagl, S. (1991) *Colour atlas of physiology.* New York, Thieme Medical Publishers Inc.

Dick, W.H., Lingeman, J.E., Preminger, G.M., *et al.* (1990) Laxative abuse as a cause for ammonium urate renal calculi. *Journal of Urology* 143: 244-247.

Dunn, J.K. (1998) Disorders of water and sodium balance. In: *BSAVA Manual of Small animal endocrinology.* A. G. Torrance and C. P. Mooney (eds). Cheltenham, British Small Animal Veterinary Association : pp 151-161.

Ebisuno, S., Morimoto, S., Yasukawa, S., Ohkawa, T. (1991) Results of long-term rice bran treatment on stone recurrence in hypercalciuric patients. *British Journal of Urology* 67: 237-240.

Edwards, D.G., Kaye, A.E., Druce, E. (1989) Sources and intakes of sodium in the United Kingdom. *European Journal of Clinical Nutrition* 43: 855-861.

Edwards, N.A., Hodgkinson, A. (1965) Metabolic studies in patients with idiopathic hypercalciuria. *Clinical Science* 29: 143-157.

Elion, G.B., Kovensky, A., Hitchings, G.H. (1966) Metabolic studies of allopurinol, an inhibitor of xanthine oxidase. *Biochemical Pharmacology* 15: 863-865.

Ellis, H. (1969) *A history of bladder stone.* Oxford, Blackwell.

Embon, O.M., Rose, G.A., Rosenbaum, T. (1990) Chronic dehydration and stone disease. *British Journal of Urology* 66: 357-362.

Escolar, E. (1990) Structure and composition of canine urinary calculi. *Research in Veterinary Science* 49: 327-333.

Faber, S.R., Feitler, W.W., Bleiler, R.E., *et al.* (1963) The effects of an induced pyridoxine and pantothenic acid deficiency on excretions of oxalic acid and xanthurenic acid in the urine. *American Journal of Clinical Nutrition* 12: 406-411.

Fellstrom, B., Danielson, B.G., Karlstrom, B., *et al.* (1989) Dietary habits in renal stone patients compared with healthy subjects. *Journal of Urology* 63: 575-580.

Finco, D. (1995) Applied physiology of the kidney. In: *Canine and feline nephrology and urology.* C. A. Osborne and D. R. Finco (eds). Philadelphia, Lea & Febiger : pp 29-46.

Finlayson, B. (1977) Where and how does urinary stone disease start? An essay on the expectation of free- and fixed-particle urinary stone disease. In: *Idiopathic urinary bladder stone disease.* R. Van Reen (ed). Washington, DHEW : pp 7-31.

Finlayson, B. (1978) Physicochemical aspects of urolithiasis. *Kidney International* 13: 344-360.

Finlayson, B., Reid, F. (1978) The expectation of free and fixed particles in urinary stone disease. *Investigative Urology* 15: 442-448.

Fischer, C.P., Sonda, L.P. (1979) Cryoprecipitate: Its use and effects in canine coagulum pyelolithotomy. *Investigative Urology* 16: 266-269.

Fleisch, H. (1978) Inhibitors and promotors of stone formation. *Kidney International* 13: 361-371.

Fleisch, H., Bisaz, S., Care, A.D. (1964) Effect of orthophosphate on urinary pyrophosphate excretion and the prevention of urolithiasis. *The Lancet* i: 1065-1067.

Fuselier, H.A., Moore, K., Lindberg, J., *et al.* (1998) Agglomeration inhibition reflected stone-forming activity during long-term potassium citrate theory in calcium stone formers. *Urology* 52: 988-994.

Gaskell, C.J. (1985) Nutrition in diseases of the urinary tract in the dog and cat. *Veterinary Annual* 25: 383-390.

Gershoff, S.N., Faragalla, F.F., Nelson, D.A., Andrus, S.B. (1959) Vitamin B_6 deficiency and oxalate nephrocalcinosis in the cat. *American Journal of Medicine* 27: 72-80.

Gerster, H. (1997) No contribution of ascorbic acid to renal calcium oxalate stones. *Annals of Nutrient Metabolism* 41: 269-282.

Gibbs, D.A., Watts, R.W.E. (1970) The action of pyridoxine in primary hyperoxaluria. *Clinical Science* 38: 277-286.

Giesecke, D., Tiemeyer, W. (1984) Defect of uric acid uptake in Dalmatian dog liver. *Experientia* 40: 1415-1416.

Good, D. (1989) New concepts in renal ammonium excretion. In: *The regulation of acid-base balance.* D. W. Seldin (ed). New York, Raven Press : pp 169-183.

Gould, N., Hallson, P.C., Kasidas, G.P., *et al.* (1995) Rapid computer-assisted infrared analysis of urinary calculi using photoacoustic detection. *Urological Research* 23: 63-69.

Goulden, B.E. (1968) Clinical observations on the role of urinary infection in the aetiology of canine urolithiasis. *The Veterinary Record* 83: 509-514.

Grandage, J. (1975) Some effects of posture on the radiographic appearance of the kidneys of the dog. *Journal of the American Veterinary Medical Association* 166: 165-166.

Graves, F.T. (1982) An experimental study of the anatomy of the renal tubules of the human kidney and its relation to calculus formation. *British Journal of Urology* 54: 569-574.

Grover, P.K., Resnick, M.I. (1995) Evidence for the presence of abnormal proteins in the urine of recurrent stone formers. *Journal of Urology* 153: 1716-1721.

Grover, P.K., Ryall, R.L., V.R., M. (1990) Does Tamm-Horsfall mucoprotein inhibit or promote calcium oxalate crystallization in human urine. *Clinica Chimica Acta* 190: 223-228.

Guyton, A.C., Hall, J.E. (1996) *Textbook of Medical Physiology.* Philadelphia, WB Saunders.

Halabe, A., Sutton, R.A.L. (1987) Primary hyperparathyroidism and idiopathic hypercalciuria. *Mineral and Electrolyte Metabolism* 13: 235-241.

Hallson, P.C. (1988) Oxalate crystalluria. In: *Oxalate metabolism in relation to urinary stone.* G. A. Rose (ed). London, Springer-Verlag : pp 131-166.

Hallson, P.C., Rose, G.A., Sulaiman, S. (1982) Magnesium reduces calcium oxalate formation in human whole urine. *Clinical Science* 62: 17-19.

Hamar, D., Chow, F.C.H., Dysart, M.I., *et al.* (1976) Effect of sodium chloride in prevention of experimentally produced uroliths in male cats. *Journal of the American Animal Hospital Association* 12: 514-517.

Hardy, R.M., Klausner, J.S. (1983) Urate calculi associated with portal vascular anomalies. In: *Current veterinary therapy VII.* R. W. Kirk (ed). Philadelphia, WB Saunders : pp 1073-1076.

Harrison, A.R., Kasidas, G.P., Rose, G.A. (1981) Hyperoxaluria and recurrent stone formation apparently cured by short courses of pyridoxine. *British Medical Journal* 282: 2097-2098.

Harvey, J.A., Zobitz, M.M., Pak, C.Y.C. (1988) Dose dependency of calcium absorption: A comparison of calcium carbonate and calcium citrate. *Journal of Bone Mineral Research* 3: 253-258.

Hatch, M., Mulgrew, S., Bourke, E., *et al.* (1980) Effect of megadoses of ascorbic acid on serum and urinary oxalate. *European Urology* 6: 166-169.

Hauschildt, S., Rudolph, R., Feldheim, W. (1972) Oxalate metabolism and thiamin-pyridoxine status in the rat. *International Journal for Vitamin and Nutrition Research* 42: 457-467.

Hellman, L., Burns, J.J. (1958) Metabolism of L-ascorbic acid 1-C14. *Journal of Biological Chemistry* 233: 923-924.

Her Majesty's Stationery Office. (1991) *Feeding stuffs sampling and analysis regulations.* London, The HMSO Publications Centre.

Hess, B. (1994) Tamm-Horsfall glycoprotein and calcium nephrolithiasis. *Mineral and Electrolyte Metabolism* 20: 393-398.

Hess, B., Jost, C., Zipperle, L., *et al.* (1998) High-calcium intake abolishes hyperoxaluria and reduces urinary crystallization during a 20-fold normal oxalate load in humans. *Nephrology Dialysis Transplantation* 13: 2241-2247.

Hesse, A. (1990) Canine urolithiasis: epidemiology and analysis of urinary calculi. *Small Animal Practice* 31: 599-604.

Hesse, A., Frenk, M., Wolters, M., Laube, N. (2000) The changing incidence of calcium oxalate stones in cats. In: *9th International Symposium on Urolithiasis.* A. L. Rodgers, B. E. Hibbert, B. Hess, *et al.* (eds). Cape Town, South Africa, University of Cape Town : pp 815-817.

Hesse, A., Heimbach, D. (1999) Causes of phosphate stone formation and the importance of metaphylaxis by urinary acidification: a review. *World Journal of Urology* 17: 308-315.

Hesse, A., Sanders, G. (1985) A survey of urolithiasis in cats. *Journal of Small Animal Practice* 26: 465-476.

Hesse, A., Siener, R., Heynck, H., Jahnen, A. (1993) The influence of dietary factors on the risk of urinary stone formation. *Scanning Microscopy* 7: 1119-1128.

Hesse, A., Steffes, H., Graf, C., *et al.* (1997) Current composition and breed distribution of canine urinary stones. *Experimentelle Urologie* 110: 436-439.

Hesse, A., Steffes, H.J., Graf, C. (1998) Pathogenic factors of urinary stone formation in animals. *Animal Physiology and Animal Nutrition* 80: 108-119.

Hesse, A., Wuzel, H., Vahlensieck, W. (1986) The excretion of glycosaminoglycans in the urine of calcium-oxalate-stone patient and healthy person. *Urologia Internationalis* 41: 81-87.

Hesse, A., Wuzel, H., Vahlensieck, W. (1991) Significance of glycosaminoglycans for the formation of calcium oxalate stones. *American Journal of Kidney Diseases* 17: 414-419.

Hodgkinson, A. (1978) Evidence of increased oxalate absorption in patients with calcium-containing renal stones. *Clinical Science and Molecular Medicine* 54: 291.

Hojaard, I., Fornander, A.M., Nilsson, M.A., *et al.* (1999) Crystallization during volume reduction of solutions with a composition corresponding to that in the collecting duct: the influence of hydroxyapatite seed crystals and urinary macromolecules. *Urological Research* 27: 417-425.

Holmes, R.P., Goodman, H.O., Assimos, D.G. (1995) Dietary oxalate and its intestinal absorption. *Scanning Microscopy* 9: 1109-1120.

Holmes, R.P., Goodman, H.O., Assimos, D.G. (2001) Contribution of dietary oxalate to urinary oxalate excretion. *Kidney International* 59: 270-276.

Holmes, R.P., Kennedy, M. (2000) Estimation of the oxalate content of foods and daily oxalate intake. *Kidney International* 57: 1662-1667.

Hoppe, A. (1994) Canine lower urinary tract diseases. In: *The* WALTHAM™ *book of clinical nutrition of the cat and dog.* J. M. Wills and J. W. Simpson (eds). Oxford, Pergamon : pp 335-352.

Horner, H.T., Wagner, B.L. (1995) Calcium oxalate formation in higher plants. In: *Calcium oxalate in biological systems.* S. R. Khan and B. Raton (eds). Boca Raton, CRC Press : pp 53-72.

Houston, D., Patterson, J., Moore, A., *et al.* (2000) Preliminary results from the Canadian veterinary urolith centre. *Canadian Veterinary Journal* 41: 318-319.

Hoyer, J.R. (1994) Uropontin in urinary calcium stone formation. *Mineral Electrolyte Metabolism* 20: 385-392.

Hughes, C., Dutton, S., Stewart - Truswell, A. (1981) High intakes of ascorbic acid and urinary oxalate. *Human Nutrition* 35: 274-280.

Jackson, O.F. (1977) Water balance studies in domestic cats. *Feline Practice* 7: 30-33.

Jaeger, P., Portmann, L., Jacquet, A.F., *et al.* (1985) Influence of the calcium content of the diet on the incidence of mild hyperoxaluria in idiopathic renal stone formers. *American Journal of Nephrology* 5: 40-44.

Jahnen, L.F., Heynck, H., Gertz, A., *et al.* (1992) Dietary fibre: the effectiveness of a high bran diet in reducing calcium excretion. *Urological Research* 20: 3-6.

James, L.F., Butcher, J.E. (1972) Halogeton poisoning of sheep; effect of high oxalate intake. *Journal of Animal Science* 35: 1233-1238.

Johansson, G., Backman, U., Danielson, B.G., *et al.* (1980) Magnesium metabolism in renal stone formers. Effects of therapy with magnesium hydroxide. *Scandinavian Journal of Urology and Nephrology* 53: 125-130.

Joly, J.S. (1929) *Stone and calculous disease of the urinary organs.* St.Louis, C.V.Mosby.

Jones, B.R., Sanson, R.L., Morris, R.S. (1997) Elucidating the risk factors of feline lower urinary tract disease. *New Zealand Veterinary Journal* 45: 100-108.

Keene, B.W. (1992) L-Carnitine deficiency in canine dilated cardiomyopathy. In: *Current veterinary therapy X1: Small animal practice.* R. W. Kirk (ed). Philadelphia, WB Saunders. : pp 780-783.

Khan, S.R., Glenton, P.A. (1995) Deposition of calcium phosphate and calcium oxalate crystals in the kidneys. *Journal of Urology* 153: 811-817.

Khan, S.R., Shevock, P.N., Hackett, R.L. (1993) Magnesium oxide administration and prevention of calcium oxalate nephrolithiasis. *Journal of Urology* 149: 412-416.

King, J.S., Boyce, W.H. (1957) Amino acid and carbohydrate composition of the mucoprotein matrix in various calculi. *Proceedings of the Society of Experimental Biology and Medicine* 95: 183-187.

Kirk, C.A., Ling, G.V., Franti, C.E., *et al.* (1995) Evaluation of the factors associated with development of calcium oxalate in cats. *Journal of the American Veterinary Medical Association* 207: 1429-1434.

Kirk, H. (1925) *The diseases of the cat.* London, Balliere, Tindall and Cox.

Klausner, J.S., Osborne, C.A., O'Leary, T.P., *et al.* (1980) Experimental induction of struvite uroliths in miniature schnauzer and beagle dogs. *Investigative Urology* 18: 127-132.

Kleeman, C.R., Bohannan, J., Bernstein, D., *et al.* (1964) Effects of variations in sodium intake on calcium excretion in normal humans. *Proceedings of the Society of Experimental Biology and Medicine* 115: 29-32.

Klohn, M., Bolle, J.F., Reverdin N.P, *et al.* (1986) Ammonium urate urinary stones. *Urological Research* 14: 15-318.

Knickelbein, R.G., Aronson, P.S., Dobbins, J.W., *et al.* (1968) Oxalate transport by anion exchange across rabbit ileal brush border. *Clinical Investigation* 77: 170-175.

Knorle, R., Schnierle, P., Koch, A., *et al.* (1994) Tamm-Horsfall glycoprotein: Role in inhibition and promotion of renal calcium oxalate stone formation studied with Fourier-transform infrared spectroscopy. *Clinical Chemistry* 40: 1739-1743.

Kohri, K., Garside, J., Blacklock, N.J. (1988) The role of magnesium in calcium oxalate urolithiasis. *British Journal of Urology* 61: 107-112.

Kok, D.J., Irestra, J.A., Doorenbos, C.J., *et al.* (1990a) The effect of dietary excesses in animal protein and in sodium on the composition and the crystallisation

kinetics of calcium oxalate monohydrate in urines of healthy men. *Journal of Clinical Endocrinology and Metabolism.* 71: 861-866.

Kok, D.J., Khan, S.R. (1994) Calcium oxalate nephrolithiasis, a free or fixed particle disease. *Kidney International* 46: 847-854.

Kok, D.J., Papapoulos, S.E., Bijvoet, O.L.M. (1990b) Crystal agglomeration is a major element in calcium oxalate urinary stone formation. *Kidney International* 37: 51-56.

Larsson, L., Tiselius, H.G. (1987) Hyperoxaluria. *Mineral and Electrolyte Metabolism* 13: 242-250.

Lawler, D.F., Sjolind, W., Collins, J.E. (1985) Incidence rates of feline lower urinary tract disease in the United States. *Feline Practice* 15: 13-16.

Lee, P.M. (1997) *Bayesian statistics.* London, Arnold.

Lee, R., Leowijuk, C. (1982) Normal parameters in abdominal radiology of the dog and cat. *Journal of Small Animal Practice* 23: 251-269.

Lees, G.E., Osbourne, C.A., Stevens, J.B. (1979) Antibacterial properties of urine; studies of feline urine specific gravity, osmolality, and urine pH. *American Animal Hospital Association* 15: 135-141.

Lekcharoensuk, C., Lulich, J., Osborne, C., *et al.* (2000a) Association between patient-related factors and risk of calcium oxalate and magnesium ammonium phosphate urolithiasis in cats. *Journal of the American Veterinary Medical Assocation* 217: 520-525.

Lekcharoensuk, C., Lulich, J.P., Osborne, C.A., *et al.* (2000b) Patient and environmental factors associated with calcium oxalate urolithiasis in dogs. *Journal of the American Veterinary Medical Association* 217: 515-519.

Lekcharoensuk, C., Osborne, C., Lulich, J. (2001) Epidemiologic study of the risk factors for lower urinary tract diseases in cats. *Journal of the American Veterinary Medical Association* 218: 1429-1435.

Lemann, J., Adams, N.D., Gray, R.W. (1979) Urinary calcium excretion in human beings. *The New England Journal of Medicine* 301: 535-541.

Lewis, L.D., Chow, F.H.C., Taton, L.F., *et al.* (1978) Effect of various dietary mineral concentrations on the occurrence of feline urolithiasis. *Journal of the American Veterinary Medical Association* 172: 559-563.

Li, M.K., Blacklock, N.J., Garside, J. (1985) Effect of magnesium on calcium oxalate crystallisation. *Urological Research* 133: 123-128.

Liebman, M., Chai, W. (1997) Effect of dietary calcium on urinary oxalate excretion after oxalate loads. *American Journal of Clinical Nutrition* 65: 1453-1459.

Liebman, M., Costa, G. (2000) Effects of calcium and magnesium on urinary oxalate excretion after oxalate loads. *Journal of Urology* 163: 1565-1569.

Lindberg, J., Cole, F.E., Neusetzer, S. (1996) Treatment of calcium oxalate stone formers with potassium citrate. In: *8th International Symposium on Urolithiasis.* C. Y. C. Pak, M. I. Resnick and G. M. Preminger (eds). Dallas, Millet the Printer : pp 490-491.

Ling, G.V., Franti, C.E., A.L., R., *et al.* (1998a) Urolithiasis in dogs II: Breed prevalence and interrelationships of breed, sex, age and mineral composition. *American Journal of Veterinary Research* 59: 624-629.

Ling, G.V., Franti, C.E., Johnson, D.L., Ruby, A.L. (1998b) Urolithiasis in dogs III: Prevalence of urinary tract infection and interrelations of infection, age, sex and mineral composition. *American Journal of Veterinary Research* 5: 643-649.

Ling, G.V., Franti, C.E., Ruby, A.L., *et al.* (1998c) Urolithiasis in dogs I: Mineral prevalence and interrelationships of mineral composition, age and sex. *American Journal of Veterinary Research* 59: 624-629.

Ling, G.V., Franti, C.E., Ruby, A.L., Johnson, D.L. (1990) Epizootiologic evaluation and quantitative analysis of urinary calculi from 150 cats. *Journal of the American Veterinary Medical Association* 196: 1459-1462.

Ling, G.V., Ruby, A.L. (1986) Canine uroliths: Analysis of data derived from 813 specimens. *Veterinary Clinics of North America* 16: 303-316.

Lloyd, E., Sullivan, D.J. (1984) Effects of orally administered ammonium chloride and methionine on feline urinary acidity. *Veterinary Medicine* 79: 773-778.

Loveridge, G. (1994) Provision of environmentally enriched housing for cats. *Animal Technology* 45: 69-87.

Low, R.K., Stoller, M.L. (1997) Endoscopic mapping of renal papillae for Randalls plaques in patients with urinary stone disease. *Journal of Urology* 158: 2062-2064.

Lulich, J.P., Osborne, C.A. (1995) Canine calcium oxalate uroliths. In: *Kirks Current veterinary therapy XII: Small animal practice.* J. D. Bonagura (ed). London, WB Saunders : pp 992-996.

Lulich, J.P., Osborne, C.A. (1996) Overview of diagnosis of feline lower urinary tract disorders. *Veterinary Clinics of North America* 26: 339-352.

Lulich, J.P., Osborne, C.A., Bartges, J.W., *et al.* (2000) Canine lower urinary tract disorders. In: *Textbook of veterinary internal medicine.* S. Ettinger and M. Feldman (eds). Philadelphia, WB Saunders : pp 1747-1783.

Lulich, J.P., Osborne, C.A., Lekcharoensuk, C., *et al.* (1999a) Canine calcium oxalate urolithiasis. *Veterinary Clinics of North America* 29: 123-139.

Lulich, J.P., Osborne, C.A., Nagode, L.A., et. al. (1991a) Evaluation of urine and serum metabolites in Miniature Schnauzers with calcium oxalate urolithiasis. *American Journal of Veterinary Research* 52: 1583-1589.

Lulich, J.P., Osborne, C.A., Polzin, D.J., *et al* (1992a) Calcium oxalate urolithiasis. In: *16th annual* WALTHAM™*/OSU Symposium for treatment of Small Animal Diseases.* C. A. T. Buffington, S. P. DiBartola, M. P. Wyman, *et al.* (eds) Ohio State University College of Veterinary Medicine, Kal Kan Foods Inc.: pp 69-74.

Lulich, J.P., Osborne, C.A., Polzin, D.J., *et al* (1992b) Effects of oral NaCl administration on urinary oxalate excretion (Abstract). In: *10th. ACVIM Forum.* W. B. Morrison (ed) Wisconsin, Omnipress : pp 119 (only).

Lulich, J.P., Osborne, C.A., Thumchai, R. (1999b) Epidemiology of canine calcium oxalate uroliths - identifying risk factors. *Veterinary Clinics of North America* 29: 113-122.

Lulich, J.P., Osborne, C.A., Unger, L.K., *et al.* (1991b) Prevalence of calcium oxalate uroliths in Miniature Schnauzers. *American Journal of Veterinary Research* 52: 1579-1582.

Malnic, G., De Mello, A., Giebisch, G. (1971) Potassium transport across renal distal tubules during acid-base disturbances. *American Journal of Physiology* 221: 1192-1208.

Manning, R.A., Blaney, B.J. (1986) Identification of uroliths by infrared spectroscopy. *Australian Veterinary Journal* 63: 393-396.

Markwell, P.J., Buffington, C.T. (1994) Feline lower urinary tract disease. In: *The* WALTHAM™ *book of clinical nutrition of the cat and dog.* J. M. Wills and K. W. Simpson (eds). Oxford, Pergamon : pp 293-311.

Markwell, P.J., Robertson, W.G., Stevenson, A.E. (2000) Urolithiasis: a comparison of humans, cats and dogs. In: *9th International Symposium on Urolithiasis.* A. L. Rodgers, B. E. Hibbert, B. Hess, *et al.* (eds) Cape Town, South Africa, University of Cape Town : pp 785-788.

Markwell, P.J., Smith, B.H.E., McCarthy, K.P. (1999) A non-invasive method for assessing the effect of diet on urinary calcium oxalate and struvite relative supersaturation in the cat. *Animal Technology* 50: 61-67.

Marshall, D.H., Nordin, B.E.C., Speed, R. (1976) Calcium, phosphorus and magnesium requirements. *Proceedings of the Nutrition Society* 35: 163-173.

Marshall, R.W., Cochran, M., Hodgkinson, A. (1972) Relationships between calcium and oxalic acid intake in the diet and their excretion in the urine of normal and renal-stone-forming subjects. *Clinical Science* 43: 91-99.

Marshall, W., Robertson, W.G. (1976) Nomograms for the estimation of the saturation of urine with calcium oxalate, calcium phosphate, magnesium ammonium phosphate, uric acid, sodium acid urate, ammonium acid urate and cystine. *Clinica Chimica Acta* 72: 253-260.

Martini, L.A., Cuppari, L., Colugnati, F.A., *et al.* (2000) High sodium chloride intake is associated with low bone density in calcium stone-forming patients. *Clinical Nephrology* 54: 85-93.

Masai, M., Ito, H., Kotake, T. (1995) Effect of dietary intake on urinary oxalate excretion in calcium renal stone formers. *British Journal of Urology* 76: 692-696.

McKerrell, R.E., Blakemore, W.F., Heath, M.F., *et al.* (1989) Primary oxaluria (L-glyceric aciduria) in the cat: A newly recognised inherited disease. *The Veterinary Record* 125: 31-34.

McKinley, M.J., Denton, D.A., Weisinger, R.S. (1978) Sensors for antidiuresis and thirst osmoreceptors or CSF sodium detectors. *Brain Research* 141: 89-103.

McKinley, M.J., Oldfield, B.J., Vivas, L. (1992) Osmotic and hormonal regulation of thirst in domestic animals. *Domestic Animal Endocrinology* 9: 1-11.

Menon, M., Mahle, C.J. (1982) Oxalate metabolism and renal calculi. *Journal of Urology* 127: 148-151.

Menon, M., Mahle, C.J. (1983) Oxalate transport by intestinal brush border membrane vesicles. *Western Journal of Urology* 1: 163-169.

Menon, M., Parular, B.G., Drach, G.W. (1998) Urinary lithiasis: etiology, diagnosis, and medical management. In: *Campbells urology.* P. C. Walsh, A. B. Retik, E. D. Vaughan and *et al.* (ed). Philadelphia, WB Saunders : pp 2661-2733.

Messa, P., Marangella, M., Paganin, L., *et al.* (1997) Different dietary calcium intake and relative supersaturation of calcium oxalate in the urine of patients forming renal stones. *Clinical Science* 93: 257-263.

Messa, P., Miono, G., Franzon, R., *et al.* (1992) Factors affecting fasting urinary calcium excretion in stone former patients on different dietary calcium intakes. *Scanning Microscopy* 6: 239-246.

Metcalfe-Gibson, A., MacCallum, F.M., Morrison, R.B.I., et. al. (1965) Urinary excretion of hydrogen ions in patients with uric acid calculi. *Clinical Science* 28: 325-345.

Meyer, J.L. (1981) Nucleation kinetics in the calcium oxalate-sodium urate monohydrate system. *Investigative Urology* 19: 197-201.

Meyer, J.L., Smith, L.H. (1975) Growth of calcium oxalate crystals. II. Inhibition by natural urinary crystal growth inhibitors. *Investigative Urology* 13: 36-39.

Meyer, W.J., Transbol, I., Bartter, F.C., Delea, C. (1976) Control of calcium absorption: Effect of sodium chloride loading and depletion. *Metabolism* 25: 989-993.

Michell, A.R. (1989) Urolithiasis - historical, comparative and pathophysiological aspects: a review. *Journal of the Royal Society of Medicine* 82: 669-672.

Miller, M.E. (1962) *Guide to the dissection of the dog.* New York, Ithaca.

Milosevic, D., Batinic, D., Blau, N., *et al.* (1998) Determination of urine saturation with computer program Equil 2 as a method for estimation of the risk of urolithiasis. *Journal of Chemistry, Information and Computing Science* 38: 646-650.

Mitwalli, A. (1989) Control of hyperoxaluria with large doses of pyridoxine in patients with kidney stones. *Annals of Saudi Medicine* 9: 541-546.

Moore, E., Coe, F., McMann, B., *et al.* (1978) Idiopathic hypercalciuria in children: prevalance, and metabolic characteristics. *Journal of Pediatricians* 92: 906-910.

Moreau, P.M. (1982) Neurogenic disorders of micturition in the dog and cat. *Compendium on Continuing Education for the Practicing Veterinarian* 4: 2-21.

Morozumi, M., Ogawa, Y. (2000) Impact of dietary calcium and oxalate ratio on urinary stone formation in rats. *Molecular Urology* 4: 313-320.

Moxham, G. (2001) The WALTHAM™ feces scoring system - a tool for veterinarians and pet owners: How does your pet rate? *WALTHAM™ Focus* 11: 24-25.

Murayama, T., Taguchi, T. (1993) The role of the diurnal variation of urinary pH in determining stone compositions. *Journal of Urology* 150: 1437-1439.

Nakada, T., Sasagawa, I., Furuta, H., *et al.* (1988) Effect of high-calcium diet on urinary oxalate excretion in urinary stone formers. *European Urology* 15: 264-270.

Nakagawa, Y., Abram, V., Parks, J.H., *et al.* (1985) Urine glycoprotein crystal growth inhibitors: evidence for a molecular abnormality in calcium oxalate nephrolithiasis. *Journal of Clinical Investigation* 76: 1455-1462.

Nakagawa, Y., Margolis, H.C., Yokoyama, S., *et al.* (1981) Purification and characterisation of a calcium oxalate monohydrate crystal growth inhibitor from human kidney tissue culture medium. *Journal of Biological Chemistry* 256: 3936-3944.

Nakagawa, Y., Renz, C.L., Ahmed, M., *et al.* (1991) Isolation of nephrocalcin from the kidney tissue of nine vertebrate species. *American Journal of Physiology* 260: F243-F248.

Nash, A. (1990) The urinary system. In: *Veterinary Notes for Dog Owners.* T. Turner (ed). London, Popular dogs : pp 190-193.

Nash, A. (1994) The urinary system. In: *Veterinary Notes for Cat Owners.* T. Turner and J. Turner (eds). London, Stanley Paul : pp 258-259.

Nicar, M.J., Skurla, C., Sakhaee, K., *et al.* (1983) Low urinary citrate excretion in nephrolithiasis. *Urology* 21: 8-14.

Niki, E. (1990) Vitamin C as an antioxidant. *World Reviews of Nutrition and Diet* 64: 1-30.

Norris, C.R., Williams, B.J., Ling, G.V., *et al.* (2000) Recurrent and persistent urinary tract infections in dogs: 383 cases (1969-1995). *Journal of the American Animal Hospital Association* 36: 484-492.

Ogawa, Y. (1994) Impact of sodium-potassium citrate on the diurnal variations in urinary calcium oxalate and calcium phosphate saturation concentrations in normal individuals. *Journal of Urology* 76: 136-141.

Ogawa, Y., Takahashi, S., Kitagawa, R., *et al.* (1983) Diurnal variation in calcium-oxalate supersaturation concentration in normal and stone-forming urine. *Japanese Journal of Nephrology* 25: 1127-1130.

Ohkawa, T., Ebisuno, S., Kitagawa, M., et. al. (1984) Rice bran treatment for patients with hypercalciuric stones: experimental and clinical studies. *Urology* 132: 1140-1145.

Osborne, C., Bartges, J., Lulich, J., *et al* (2000a) Urolithiasis in dogs. In: *9th International Symposium on Urolithiasis.* A. L. Rodgers, B. E. Hibbert, B. Hess, *et al.* (eds). Cape Town, South Africa, University of Cape Town : pp 789-791.

Osborne, C.A., Bartges, J.W., Luich, J.P., *et al.* (2000b) Canine urolithiasis. In: *Small animal clinical nutrition.* M. S. Hand, C. D. Thatcher, R. L. Remillard *and et al.* (eds). Missouri, Walsworth Publishing : pp 605-688.

Osborne, C.A., Clinton, C.W. (1986) Urolithiasis - terms and concepts. *Veterinary Clinics of North America* 16: 3-18.

Osborne, C.A., Clinton, C.W., Moran, H.C., Bailie, N.C. (1986a) Comparison of qualitative and quantitative analyses of canine uroliths. *Veterinary Clinics of North America* 16: 317-323.

Osborne, C.A., Fletcher, T.F. (1995) Applied anatomy of the urinary system with clinicopathologic correlation. In: *Canine and feline nephrology and urology.* C. A. Osborne and D. R. Finco (eds). Philadelphia, Lea & Febiger : pp 3-28.

Osborne, C.A., Kruger, J.M., Lulich, J.P. (1996a) Feline lower urinary tract disorders - definition of terms and concepts. *Veterinary Clinics of North America* 26: 169-179.

Osborne, C.A., Kruger, J.M., Lulich, J.P., et. al. (2000c) Feline lower urinary tract diseases. In: *Textbook of veterinary internal medicine.* S. Ettinger and M. Feldman (eds). Philadelphia, WB Saunders : pp 1710-1714.

Osborne, C.A., Kruger, J.M., Lulich, J.P., *et al.* (1995a) Disorders of the feline lower urinary tract. In: *Canine and feline nephrology and urology.* C. A. Osborne and D. R. Finco (eds). Philadelphia, Lea & Febiger. : pp 625-629.

Osborne, C.A., Lees, G.E. (1995) Bacterial infections of the canine and feline urinary tract. In: *Canine and feline nephrology and urology.* C. A. Osborne and D. R. Finco (eds). Philidelphia, Lea & Febiger : pp 759-797.

Osborne, C.A., Lulich, J.P., Bartges, J.W., *et al.* (1995b) Canine and feline urolithiasis: relationship of etiopathogenesis to treatment and prevention. In: *Canine and feline nephrology and urology.* C. A. Osborne and D. R. Finco (eds). Philadelphia, Lea & Febiger : pp 798-888.

Osborne, C.A., Lulich, J.P., Polzin, D.J., *et al.* (1999a) Analysis of 77,000 canine uroliths. *Veterinary Clinics of North America* 29: 17-38.

Osborne, C.A., Lulich, J.P., Polzin, D.J., *et al.* (1999b) Medical dissolution and prevention of canine struvite urolithiasis. *Veterinary Clinics of North America* 29: 17-38.

Osborne, C.A., Lulich, J.P., Thumachai, R. (1995c) Etiopathogenesis and therapy of feline calcium oxalate uroliths. In: *13th ACVIM Forum.* R. DeNovo (ed). Wisconsin, Omnipress : pp 487-489.

Osborne, C.A., Lulich, J.P., Thumchai, R., *et al.* (1996b) Feline urolithiasis: etiology and pathophysiology. *Veterinary Clinics of North America* 26: 217-232.

Osborne, C.A., Lulich, J.P., Thumchai, R., *et al.* (1997) Changing demographics of feline urolithiasis: perspectives from the Minnesota Urolith Center. In: *Consultations in feline internal medicine.* J. R. August (ed). Philadelphia, WB Saunders : pp 349-360.

Osborne, C.A., Poffenbarger, E.M., Klausner, J.S., *et al.* (1986b) Etiopathogenesis, clinical manifestations and management of canine calcium oxalate uroliths. *Veterinary Clinics of North America* 16: 133-170.

Osborne, C.A., Polzin, D.J., Kruger, J.M., *et al.* (1989a) Relationship of nutritional factors to the cause, dissolution and prevention of feline uroliths and urethral plugs. *Veterinary Clinics of North America* 19: 561-581.

Osborne, C.A., Sanna, J.J., Unger, L.K., *et al.* (1989b) Analysing the mineral composition of uroliths from dogs, cats, horses, cattle, sheep, goats and pigs. *Veterinary Medicine*: 750-764.

Othnes, B. (1983 [supplement]) Urinary stone analysis: methods, materials and value. *Scandanavian Journal of Urology and Nephrology* 71: 7-109.

Pak, C.Y.C. (1987) Citrate and renal calculi. *Mineral and Electrolyte Metabolism* 13: 257-266.

Pak, C.Y.C. (1991) Etiology and treatment of urolithiasis. *American Journal of Kidney Diseases* 18: 624-637.

Pak, C.Y.C., Cox, J.W., Powell, E., Bartter, F.C. (1978a) Effect of the oral administration of ammonium chloride, sodium phosphate, cellulose phosphate and

parathyroid extract on the activity product of brushite in urine. *American Journal of Medicine* 50: 147-154.

Pak, C.Y.C., Fuller, C., Sakhee, K., *et al.* (1985) Long-term treatment of calcium nephrolithiasis with potassium citrate. *Journal of Urology* 134: 11-19.

Pak, C.Y.C., Galosy, R.A. (1980) Propensity for spontaneous nucleation of calcium oxalate. *American Journal of Medicine* 69: 681-689.

Pak, C.Y.C., Hayashi, Y., Finlayson, B., *et al.* (1977a) Estimation of the state of saturation of brushite and calcium oxalate in urine: a comparison of three methods. *Journal of Laboratory and Clinical Medicine* 89: 891-901.

Pak, C.Y.C., Holt, K., Zerwekh, J., Barilla, D.E. (1978b) Effects of orthophosphate therapy on the crystallization of calcium salts. *Mineral and Electrolyte Metabolism* 1: 147-154.

Pak, C.Y.C., Ohata, M., Lawrence, E.C., *et al.* (1974) The hypercalciurias: courses, parathyroid functions and idiopathic criteria. *Journal of Clinical Investigation* 54: 387-400.

Pak, C.Y.C., Sakhaee, K., Crowther, C., Brinkley, R.D. (1980) Evidence justifying a high fluid intake in treatment of nephrolithiasis. *Annals of Internal Medicine* 93: 36-39.

Pak, C.Y.C., Waters, O., Arnold, L., *et al.* (1977b) Mechanisms for calcium urolithiasis among patients with hyperuricosuria: supersaturation of urine with respect to monosodium urate. *Journal of Clinical Investigation* 59: 426-431.

Parivar, F., Low, R.K., Stoller, M.L. (1996) The influence of diet on urinary stone disease. *Journal of Urology* 155: 432-440.

Parks, J.H., Coward, M., Coe, F.L. (1997) Correspondence between stone composition and urine saturation in nephrolithiasis. *Kidney International* 51: 894-900.

Pastoor, F.J.H., Opitz, R., Van't Klooster, A.T., Beynen, A.C. (1994a) Substitution of dietary calcium chloride for calcium carbonate reduces urinary pH and urinary phosphorus excretion in adult cats. *The Veterinary Quarterly* 16: 157-160.

Pastoor, F.J.H., Van t'Klooster, A.T., Mathot, J.N.J.J., Beynen, A.C. (1994b) Increasing dietary calcium intake lower urinary concentrations of phosphorus and magnesium in adult ovariectomized cats. *Journal of Nutrition* 124: 299-304.

Peacock, M., Robertson, W.G. (1979) The biochemical aetiology of renal lithiasis. In: *Urinary calculous disease.* J. E. A. Wickham (ed). Edinburgh, Churchill Livingstone : pp 69-95.

Petfood Manufacturers Association. (1995) *Summary of information to support the inclusion of the following additives as "urine pH modifying substances" in relation to the list of nutritional purposes being drawn up under directive 93/74: ammonium chloride, DL-methionine, calcium sulphate, potassium citrate, calcium carbonate. Resume dossier.* London, Pet Food Manufacturers Association.

Pierce, L.W., Bloom, B. (1945) Observation on urolithiasis among American troops in a desert area. *Journal of Urology* 54: 466-470.

Pitts, R.F. (1948) Renal excretion of acid. *Federation Proceedings* 7: 418-426.

Porter, P. (1963) Urinary calculi in the dog. II. Urate stones and purine metabolism. *Journal of Comparative Pathology.* 73: 119-125.

Prince, C.L., Scardino, P.L., Wolan, C.T. (1956) The effect of temperature, humidity, and dehydration on the formation of renal calculi. *Journal of Urology* 75: 209-215.

Rabin, S., Stillian, J., Barreto, V. (1993) New membrane-based elecrolyte suppressor device for suppressed conductivity detection in ion chromatography. *Journal of Chromaotgraphy* 640: 97.

Randall, A. (1940) Papillary pathology as a precursor of primary renal calculus. *Journal of Urology* 44: 580-589.

Randall, D., Burggren, W., French, K. (1997) *Eckert animal physiology, mechanisms and adaptations.* New York, WH Freeman.

Rao, P.N., Prendiville, A., Buxton, D.G., *et al.* (1982) Dietary management of urinary risk factors in renal stone formers. *British Journal of Urology* 54.

Reed. C.F. (2001) Urinary inhibitors of calcium oxalate formation in cats. PhD Thesis, University of Nottingham.

Resnick, M., Pridgen, D.B., Goodman, H.O. (1968) Genetic predisposition to formation of calcium oxalate renal calculi. *New England Journal of Medicine* 278: 1313-1318.

Resnick, M.I., Sorrell, M.E., Bailey, J.A., Boyce, W.H. (1982) Inhibitory effects of urinary calcium-binding substances on calcium oxalate crystallisation. *Journal of Urology* 127: 568-571.

Revusova, V., Gratzlova, J., Zvara, V. (1977) The evaluation of some biochemical parameters in pyridoxine-treated calcium oxalate renal stone-formers. *Urologia Internationalis* 32: 348-352.

Rhodes, D.C.J., Hinsman, E.J., Rhodes, J.A. (1993) Cation-induced aggregation of cat Tamm-Horsfall glycoprotein and its possible role in feline urolithiasis. *Journal of the Veterinary Medical Association* 40: 283-291.

Rhodes, D.C.J., Hinsman, E.J., Rhodes, J.A., Hawkins, E.C. (1992) Urinary Tamm-Horsfall glycoprotein concentrations in normal and urolithiasis-affected male cats determined by ELISA. *Journal of the Veterinary Medical Association* 39: 621-634.

Rich, L.J., Dysart, I., Chow, F.C. (1974) Urethral obstruction in male cats: experimental production by additon of magnesium and phosphorus to diet. *Feline Practice* 4: 44-47.

Roberts, S.H., Resnick, M.I. (1986) Glycosaminoglycan content of stone matrix. *Journal of Urology* 135: 1078-1083.

Robertson, W.G. (1969) Measurement of ionized calcium in biological fluids. *Clinica Chimica Acta* 24: 149-157.

Robertson, W.G. (1977) Saturation-inhibition index as a measure of the risk of calcium oxalate stone formation. In: *Idiopathic urinary bladder stone disease.* R. Van Reen (ed). Washington, DHEW : pp 55-71.

Robertson, W.G. (1993) Urinary tract calculi. In: *Metabolic bone and stone disease.* B. E. C. Nordin, A. G. Need and H. A. Morris (ed). New York, Churchill Livingstone : pp 249-311.

Robertson, W.G., Jones, J.S., Heaton, M.A., *et al.* (in press) Predicting the crystallisation potential of urine from cats and dogs with respect to calcium oxalate and magnesium ammonium phosphate (struvite). *Journal of Nutrition.*

Robertson, W.G., Markwell, P.J. (1999) Predicting the calcium oxalate crystallization potential of cat urine. *WALTHAM™ Focus* 9: 32-33.

Robertson, W.G., Nisa, M., Hussain, I., *et al.* (1989) The importance of diet in the aetiology of primary calcium and uric acid stone-formation: the Arabian experience. In: *Urolithiasis.* V. R. Walker, R. A. L. Sutton, E. C. B. Cameron, *et al.* (eds). New York, Plenum Press : pp 735-739.

Robertson, W.G., Nordin, B.E.C. (1976) Physico-chemical factors governing stone formation. In: *Scientific foundations of urology.* D. I. Williams and G. D. Chisolm, (eds). London, Heinemann. 1 : pp 254-267.

Robertson, W.G., Peacock, M. (1980) The cause of idiopathic calcium stone disease: hypercalciuria or hyperoxaluria? *Nephron* 26: 105-110.

Robertson, W.G., Peacock, M. (1985) Pathogenesis of urolithiasis. In: *Handbook of urology.* H. J. Schneider (ed). Berlin, Springer-Verlag : pp 185-334.

Robertson, W.G., Peacock, M., Heyburn, M., *et al.* (1981a) Risk factors in calcium stone disease. In: *Urinary calculus.* J. G. Brokis and B. Finlayson (eds). Littleton, PSG Publishing : pp 265-273.

Robertson, W.G., Peacock, M., Heyburn, P.J., al., e. (1981b) The risk of calcium stone formation in relation to affluence and dietary animal protein. In: *Urinary calculus.* J. G. Brockis and B. Finlayson (eds). Littleton, PSG Publishing : pp 3-12.

Robertson, W.G., Peacock, M., Heyburn, P.J., *et al.* (1978) Risk factors in calcium stone disease of the urinary tract. *British Journal of Urology* 50: 449-454.

Robertson, W.G., Peacock, M., Heyburn, P.J., *et al.* (1979a) Should recurrent calcium oxalate stone formers become vegetarians? *British Journal of Urology* 51: 427-431.

Robertson, W.G., Peacock, M., Hodgkinson, A. (1979b) Dietary changes and the incidence of urinary calculi in the UK between 1958 and 1976. *Journal of Chronic Diseases* 32: 469-476.

Robertson, W.G., Peacock, M., Marshall, R.W., *et al.* (1976) Saturation-inhibitor index as a measure of the risk of calcium oxalate stone-formation in the urinary tract. *New England Journal of Medicine* 294: 249-252.

Robertson, W.G., Peacock, M., Nordin, B.E.C. (1968) Activity products in stone-forming and non-stone-forming urine. *Clinical Science* 34: 579-594.

Robertson, W.G., Peacock, M., Nordin, B.E.C. (1969) Calcium crystalluria in recurrent renal stone-formers. *The Lancet* 2: 21-24.

Robertson, W.G., Peacock, M., Nordin, B.E.C. (1971) Calcium oxalate crystalluria and urine saturation in recurrent renal stone-formers. *Clinical Science* 40: 365-374.

Robertson, W.G., Scurr, D.S. (1986) Modifiers of calcium oxalate crystallization found in urine. 1. Studies with a continuous crystallizer using an artificial urine. *Journal of Urology* 135: 1322-1326.

Roch-Ramel, F., Wong, N.L.M., Dirks, J.H. (1976) Renal excretion of urate in mongrel and Dalmatian dogs: A micropuncture study. *American Journal of Physiology* 231: 326-331.

Rothuizen, J., Van Den Ingh, T.S.G.A.M., Voorhout, G., *et al.* (1982) Congenital portosystemic shunts in sixteen dogs and three cats. *Journal of Small Animal Practice* 23: 67-81.

Ruby, A.L., Ling, G.V. (1986) Methods of analysis of canine uroliths. *Veterinary Clinics of North America* 16: 293-302.

Russell, R.G.G., Edwards, N.A., Hodgkinson, A. (1964) Urinary pyrophosphate and urolithiasis. *The Lancet* 1: 1446.

Ryall, R.L. (1993) The scientific basis of calcium oxalate urolithiasis. *World Journal of Urology* 11: 59-65.

Ryall, R.L. (1997) Urinary inhibitors of calcium oxalate crystallisation and their potential role in stone formation. *World Journal of Urology* 15: 155-164.

Ryall, R.L., Grover, P.K., Stapleton, A.M.F., *et al.* (1995) The urinary F1 activation peptide of human prothrombin is a potent inhibitor of calcium oxalate crystallization in undiluted human urine in vitro. *Clinical Science* 89: 181-188.

Ryall, R.L., Harnett, R., M. , Hibberd, C.M., *et al.* (1991) Effects of chondroitin sulphate, serum albumin and Tamm-Horsfall mucoprotein on calcium oxalate crystallization in undiluted human urine. *Urological Research* 19: 181-188.

Sakhaee, K., Harvery, J.A., Padalino, P.K. (1993) The potential role of salt abuse on the risk of kidney stone formation. *Journal of Urology* 150: 310-312.

Sakhaee, K., Nicar, M., Hill, K., *et al.* (1983) Contrasting effects of potassium citrate and sodium citrate therapies on urinary chemistries and crystallization of stone-forming salts. *Kidney International*: 348-352.

Samuell, C.T. (1979) Uromucoid excretion in normal subjects, calcium stone-formers and patients with chronic renal failure. *Urological Research* 7: 5-12.

Schaible, R.H. (1986) Genetic predisposition to purine uroliths in Dalmatian dogs. *Veterinary Clinics of North America* 16: 127-131.

Schneider, H.J. (1985) Morphology of urinary tract concretions. In: *Handbook of urology.* H.J. Schneider (ed). Berlin, Springer-Verlag : pp1-136.

Schnierle, P., Sialm, F., Seiler, H., *et al.* (1992) Investigations on macromolecular precipitation inhibitors of calcium oxalate. *Urological Research* 20: 7-11.

Schrier, E.E., Lee, K.E., J.L., R., *et al.* (1979) Macromolecular inhibitors of calcium oxalate crystal growth and aggregation in urine. In: *Oxalate in human biochemistry.* G. A. Rose, W. G. Robertson and R. W. E. Watts (eds). London, Wellcome Foundation : pp 22-27.

Schulz, E., Hengst, E., Brundig, P., *et al.* (1987) Disturbed urinary transport in the pelvicalyceal system in calcium oxalate stone patients. *Urological Research* 15: 109-113.

Schwille, P.O., Rumenapf, G., Wolfel, G., *et al.* (1988) Urinary pyrophosphate in patients with recurrent calcium urolithiasis and in healthy controls - a reevaluation. *Journal of Urology* 140: 239-245.

Senior, D.F. (1992) Urate urolithiasis. In:*16th Annual WALTHAM™/OSU Symposium for Treatment of Small Animal Diseases.* C. A. T. Buffington, S. P. DiBartola, M. P. Wyman, *et al.* (eds). Ohio State University College of Veterinary Medicine, Kal Kan Foods Inc : pp 59-67.

Sharma, V., Murthy, M.S.R., Thind, S.K., *et al.* (1984) Regulation of oxalate synthesising enzymes by sex hormones in weanling rats (Abstract). *Urological Research* 12: 75.

Shortt, C., Flynn, A. (1990) Sodium-calcium inter-relationships with specific reference to osteoporosis. *Nutrition Research Reviews* 3: 101-115.

Siegel, S. (1956) *Nonparametric statistics for the behavioural sciences.* Tokyo, McGraw-Hill Inc.

Siener, R., Hesse, A., Anke, M., *et al.* (1991) Influence of an oral sodium chloride load on calcium excretion in urine. *Experimentelle Urologie* 11: 33-40.

Simpson, D.P. (1983) Citrate excretion; a window on renal metabolism. *American Journal of Physiology.* 244: F223-F234.

Singh, D.P., Teotia, M., Teotia, S.P.S., *et al.* (1985) Urinary pyrophosphate excretion from stone formers. *Indian Journal of Medical Research* 82: 341-345.

Singh, P.P., Pendse, A.K., Hada, P., *et al.* (1987) Sodium is neither a risk or protective factor in urolithiasis? *Urological Research* 15: 105-108.

Slatopolsky, E., Weerts, C., Norwood, K., *et al.* (1989) Long-term effects of calcium carbonate and 2.5mEq/liter calcium dialysate on mineral metabolism. *Kidney International* 36: 897-903.

Smith, L.H. (1991) Diet and hyperoxaluria in the syndrome of idiopathic calcium oxalate urolithiasis. *American Journal of Kidney Diseases* 17: 370-375.

Smith, L.H., Van Den Berg, C.J., Wilson, D.M. (1978) Current concepts in nutrition: nutrition and urolithiasis. *New England Journal of Medicine* 298: 87-89.

Smith, L.H., Werness, P.G., Lee, K.E., *et al.* (1979) Inhibitors of crystal growth and aggregation in calcium urolithiasis. *Clinical Research* 26: 727a.

Smith, L.H., Werness, P.G., Wilson, D.M. (1980) Metabolic and clinical disturbances in patients with calcium urolithiasis. *Scandinavian Journal of Urology and Nephrology 53 (Supplement)*: 213-223.

Sojka, N.J. (1980) Feline serum collection, evaluation, and artificial insemination. In: *Current therapy in theriogenology.* D. A. Morrow (eds). Philadelphia, WB Saunders : pp 848-850.

Stevenson, A.E., Blackburn, J.M., Markwell, P.J. (2000a) Dietary management of calcium oxalate urolithiasis in dogs (Abstract). *Journal of Veterinary Internal Medicine* 14: 383 (only).

Stevenson, A.E., Markwell, P. (2001) A comparison of urine parameters produced by healthy Labrador Retrievers and Miniature Schnauzers. *American Journal of Veterinary Research* 62: 1782-1786.

Stevenson, A.E., Markwell, P.J., Kasidas, G.P. (2000b) Preliminary data from quantitative analysis of canine urolithiasis in Great Britain. In: *9th International Symposium on Urolithiasis.* A. L. Rodgers, B. E. Hibbert, B. Hess, *et al.* (eds). Cape Town, South Africa, University of Cape Town : pp 792-793.

Stevenson, A.E., Smith, B.H.E., Markwell, P.J. (1998a) The effect of potassium citrate supplementation on urine pH and relative supersaturations of the dog

(Abstract). *Proceedings of 41st British Small Animal Veterinary Association Conference* : 305 (only).

Stevenson, A.E., Smith, B.H.E., Markwell, P.J. (1998b) A system to monitor urinary tract health in the dog. *Journal of Nutrition* 28: 2761s-2762s.

Stevenson, A.E., Smith, P.J., Markwell, P.J. (1996) Use of computer systems to monitor urinary tract health in cats. In: *Proceedings of 6th FELSA Symposium.* P. N. O'Donoghue (ed). London, The Royal Society of Medicine Press : pp 95-96.

Stevenson, A.E., Wrigglesworth, D.J., Markwell, P.J. (2000c) Dietary sodium chloride, urinary calcium and urinary oxalate in healthy dogs. In: *9th International Symposium on Urolithiasis.* A. L. Rodgers, B. E. Hibbert, B. Hess, *et al.* (eds) Cape Town, South Africa, University of Cape Town : pp 794-796.

Stevenson, A.E., Wrigglesworth, D.J., Markwell, P.J. (2000d) Urine pH and urinary relative supersaturation in healthy adult cats. In: *9th International Symposium on Urolithiasis.* A. L. Rodgers, B. E. Hibbert, B. Hess, *et al.* (eds). Cape Town, South Africa, University of Cape Town : pp 818-820.

Stevenson, A.E., Wrigglesworth, D.J., Smith, B.H.E., Markwell, P.J. (2000e) Effects of dietary potassium citrate supplementation on urine pH and urinary relative supersaturation of calcium oxalate and struvite in healthy dogs. *American Journal of Veterinary Research* 61: 430-435.

Sweeny, R.A. (1989) Generic combustion method for the determination of crude protein in feeds: collaborative study. *Journal of Applied Chemistry* 72: 770-774.

Taton, G.F., Hamae, D.W., Lewis, L.D. (1984) Urinary acidification in the prevention and treatment of feline struvite urolithiasis. *Journal of the American Veterinary Medical Association* 184: 437-443.

Teotia, M., Sutor, D.J. (1971) Crystallization of ammonium acid urate and other uric acid derivatives from urine. *British Journal of Urology* 43: 3381-386.

Thalut, K., Rizal, A., Brockis, J.G., *et al.* (1976) The endemic bladder stone of Indonesia - epidemiology and clinical features. *British Journal of Urology* 48: 617-621.

Thomas, N.E., Moorthy, H.K., Valthsala, Y.M.F., *et al.* (1994) Urinary citrate in relation to dietary citrate (Abstract). In: *Urolithiasis.* New York., Plenum Press : pp 2 (only).

Thumchai, R., Lulich, J., Osborne, C., *et al.* (1996) Epizootiologic evaluation of urolithiasis in cats: 3498 cases (1982-1992). *Journal of the American Veterinary Medical Association* 208: 547-551.

Tiselius, H.G. (1985) Measurement of the risk of calcium oxalate crystallisation in urine. *Urological Research* 13: 297-300.

Tiselius, H.G. (1987) Measurement of the risk of calcium phosphate crystallisation in urine. *Urological Research* 15: 79-81.

Tiselius, H.G. (1997) Risk formulas in calcium oxalate urolithiasis. *World Journal of Urology* 15: 176-185.

Tiselius, H.G., Almgard, L.E. (1977) The diurnal urinary excretion of oxalate and the effect of pyridoxine and ascorbate on oxalate excretion. *European Urology* 3: 41-46.

Tisher, C.C., Madsen, K.M. (2000) Anatomy of the kidney. In: *The kidney.* B. M. Brenner (ed). Philadelphia, WB Saunders. 1 : pp 3-67.

Toussaint, C., Vereerstraeten, P. (1962) Effects of blood pH on potassium excretion in the dog. *American Journal of Physiology* 202: 768-772.

Treacher, R.J. (1962) Amino-acid excretion in canine cystine-stone disease. *Veterinary Record* 74: 503-504.

Trinchieri, A., Mandressi, A., Luongo, P., *et al.* (1991) The influence of diet on urinary risk factors for stones in healthy subjects and idiopathic renal calcium stone formers. *British Journal of Urology* 67: 230-236.

Tsujihata, M., Miyake, O., Yoshimura, K., *et al.* (2000) Fibronectin as a potent inhibitor of calcium oxalate urolithiasis. *Journal of Urology* 164: 1718 - 1723.

Udall, R.H., Chow, F.C.H. (1962) The etiology and control of urolithiasis. *Advances in Veterinary Science* 13: 29-57.

Umekawa, T., Kurita, T. (1994) Calprotectin-like protein is related to soluble organic matrix in calcium oxalate urinary stone. *Biochemical and Molecular Biology International* 34: 309-313.

Vermeulen, C.W., Ellis, J.E., Hsu, T.C. (1966) Experimental observations on the pathogenesis of urinary calculi. *Journal of Urology* 95: 681-690.

Vermeulen, C.W., Lyon, E.S. (1968) Mechanism of genesis and growth of calculi. *American Journal of Medicine* 45: 684-692.

Walker, A.D., Weaver, A.D., Anderson, R.S., *et al.* (1977) An epidemiological survey of the feline urological syndrome. *Journal of Small Animal Practice* 18: 283-301.

Wallerstrom, B.I., Wagberg, T.I. (1992) Canine urolithiasis in Sweden and Norway: retrospective survey of prevalence and epidemiology. *Journal of Small Animal Practice* 33: 534-539.

Walser, M. (1961) Calcium clearance as a function of sodium clearance in the dog. *American Journal of Physiology* 200: 1099-1104.

Wanzilack, T., D'Andre, S., Davis, P., *et al.* (1994) Effect of high dose vitamin C on urinary oxalate concentrations. *Journal of Urology* 151: 834-837.

Weaver, A.D., Pillinger, R. (1975) Relationship of bacterial infection in urine and calculi to canine urolithiasis. *The Veterinary Record* 97: 48-50.

Weber, H., Steffes, H., Steinlechner, S., Hesse, A. (2000) Ammonium urate lithiasis, specific occurence in Eurasian otters, *Lutra lutra. 9th International Symposium on Urolithiasis.* A. L. Rodgers, B. E. Hibbert, B. Hess, *et al.* (eds). Cape Town, South Africa, University of Cape Town : pp 836-838.

Werness, P.G., Bergert, J.H., Smith, L.H. (1981) Crystalluria. *Journal of Crystal Growth* 53: 166-181.

Werness, P.G., Brown, C.M., Smith, L.H. (1985) Equil 2: A basic computer program for the calculation of urinary saturation. *Journal of Urology* 134: 1242-1244.

Whalley, N.A., Mayers, A.M., Martins, M., *et al.* (1996) Long-term effects of potassium citrate therapy on the formation of new stones in groups of recurrent stone formers with hypocitraturia. *British Journal of Urology* 78: 10-14.

White, E.G. (1996) Symposium on urolithiasis in the dog - introduction and incidence. *Journal of Small Animal Practice* 7: 529-535.

Wikstrom, B., Wieslander, J. (1981) Excretion of Tamm-Horsfall urinary glycoprotein (uromucoid) in renal calcium stone-formers. In: *Urolithiasis - basic and clinical research.* L. H. Smith, W. G. Robertson and B. Finlayson (eds). New York, Plenum Press : pp 685-689.

Wilkinson, R. (1976) Absorption of calcium, phosphorus and magnesium. In: *Calcium, phosphate and magnesium metabolism.* B. E. C. Nordin (ed). London, Churchill Livingstone : pp 36-112.

Willeberg, P. (1975) Outdoor activity concentration as a factor in the feline urological syndrome. *Nordisk Veterinaermedicin* 27: 523-524.

Willeberg, P. (1976) Interaction effects of epidemiologic factors in the feline urological syndrome. *Nordisk Veterinaermedicin* 28: 193-200.

Willeberg, P., Priester, W. (1976) Feline urological syndrome: Associations with some time, space and individual patient factors. *American Journal of Veterinary Research* 37: 975-978.

Williams, A.W., Wilson, D.M. (1990) Dietary intake, absorption, metabolism and excretion of oxalate. *Seminars in Nephrology* 10: 2-8.

Wills, J. (1996) Feeding healthy animals - dog and cat: adult maintenance. In: *BSAVA manual of companion animal nutrition and feeding.* N. C. Kelly and J. M. Wills (eds). Cheltenham, British Small Animal Veterinary Association : pp 44-47.

Wilson, C.W.M., Loh, H.S., Foster, F.G. (1973) The beneficial effects of Vitamin C on the common cold. *European Journal of Clinical Pharmacology* 6: 26-32.

Wolf, A.V. (1986) Osmometric analysis of thirst in man and dog. *American Journal of Physiology* 161: 75-86.

Worcester, E.M. (1996) Inhibitors of stone formation. *Seminars in Nephrology* 16: 474-486.

Wrigglesworth, D.J., Stevenson, A.E., Smith, B.H.E., Markwell, P.M. (1999) Effect of pyridoxine hydrochloride on feline urine pH and urinary relative supersaturation of calcium oxalate (Abstract). *Proceedings of 42nd British Small Animal Veterinary Association Conference*: 324 (only).

Wyngaarden, J.B., Kelly, W.N. (1999) *Gout and hyperuricosuria.* New York, Grune and Stratton.

Young, C.M., Hagan, G.C., Tucker, R.E., Foster, W.D. (1952) A comparison of dietary study methods. *Journal of the American Dietetic Association* 28: 218-221.

Zahrani, H., Norman, R.W., Thompson, C., Weerasinghe, S. (2000) The dietary habits of idiopathic stone-formers and normal control subjects. *British Journal of Urology* 85: 616-620.

Zar, J.H. (1999) *Biostatistical analysis.* New Jersey, Prentice-Hall.

Zarembski, P.M., Hodgkinson, A. (1969) Some factors influencing the urinary excretion of oxalic acid in man. *Clinica Chimica Acta* 25: 1-10.

Zarkadas, M., Gougeon-Reyburn, R., Marliss, E.B., *et al.* (1989) Sodium chloride supplementation and urinary calcium excretion in post-menopausal women. *American Journal of Clinical Nutrition* 50: 1088-1094.