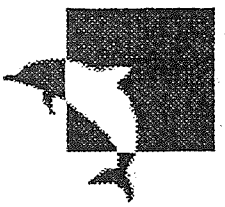

University
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ALTERED THYROID FUNCTION AND BEHAVIOURAL CHANGE IN THE DOMESTIC DOG
Canis familiaris

by

Theresa Anne Barlow

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ABSTRACT

FACULTY OF SCIENCE

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ALTERED THYROID FUNCTION AND BEHAVIOURAL CHANGE IN THE DOMESTIC DOG *Canis familiaris*

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The main goal of this thesis was to examine the relationship between thyroid hormone and problematic behaviour of the domestic dog *Canis familiaris*. It focuses specifically on the relationship between thyroid function, stress and the incidence of dog behaviour disorders, as anecdotal evidence put forward by veterinary clinicians has suggested that altered thyroid state is linked to canine aggression.

The behaviour patterns of a clinical population of 82 dogs diagnosed with a behaviour disorder were measured in both familiar and unfamiliar environments in order to establish a representative record of their behavioural repertoire. The observed behaviour patterns and clinical diagnoses were then compared to the dogs' thyroid status. In a separate study, the behaviour, thyroid hormones and plasma cortisol titres of 11 dogs with problematic behaviour was monitored for a twelve-week period during the implementation of behaviour modification programmes. Lastly, the incidence of behaviour disorders in a population of 218 dogs with different profiles of thyroid function was also examined.

A relationship was found between thyroxine and the incidence of aggressive behaviour in dogs; however this relationship indicated that a low level of thyroxine was associated with low rather than high levels of aggression. Reduced levels of thyroid hormone were generally associated with reduced behavioural activity, both directly observed and as reported by owners. Reporting of separation related disorders was reduced in the antibody positive forms of hypothyroidism, probably due to a reduction in overall activity, whilst training disorders and coprophagia were associated with the sub clinical form of hypothyroidism, possibly mediated through stress hormones.

Reduced thyroid function appears to be associated with inactive behaviour patterns, which is consistent with the observation that the principal symptom of hypothyroidism is lethargy. The findings of this thesis do not support proposal that lowered thyroid function is related to aggressive behaviour in the dog. The link between behaviour, thyroid hormone titre and cortisol was explored, but insufficient physiological data was available and this connection warrants further investigation.

Comparisons of diagnoses by three clinicians of 15 cases from the clinical population indicated only 60% agreement, pointing to a need for a more transparent and consistent system for the classification of behavioural disorders in dogs.

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This thesis is dedicated to Bruiser (1986 - 2000), as he was a truly exceptional canine companion who inspired me to question: "Why do dogs do the things they do?"

Bruiser, my love and gratitude always.

"It is a good morning exercise for a research scientist to discard a pet hypothesis every day before breakfast. It keeps him young." Lorenz (1966 p.8)

Chapter 1

Introduction

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1.1 Rationale

Each year, thousands of dogs in the UK are euthanised because they exhibit behaviour patterns that are considered unacceptable by their owners. In a 12-month study, Stead (1982) found that 39% of dogs' euthanised at veterinary clinics in Scotland were physically healthy, and Podberscek (1997) states that the most common reason for the abandonment or euthanasia of pets is due to the exhibition of inappropriate or unacceptable behaviour. Behaviour disorders have a range of causes, but are frequently motivated by fear, anxiety or stress, for which treatment can be complex (Overall 1997).

The veterinary literature suggests that hypothyroidism (insufficient production of active thyroid hormone) is the most common endocrine disease in dogs (Swenson and Reece 1993; Turner 1994; Behrend *et al.* 1998). Over the past 20 years, both veterinarians and behaviourists have suggested that hypothyroidism could be a cause of canine aggression. The late Tobjorn Owren, veterinarian and behaviourist, was the first to describe his observations of the association between aggressive behaviour patterns and hypothyroidism in the dog (Scott 1999). However, none of his findings were published and the relationship between hypothyroidism and clinical aggression has been relatively unexplored.

Behaviour has long been used as an indicator of canine physical health (Gerzovich 1995) but there is often insufficient integration of veterinary and behavioural approaches when investigating the cause of behaviour disorders. In order to deduce the relationship between hypothyroidism and dog behaviour, interdisciplinary communication among veterinarians, behavioural practitioners and research scientists is required.

This thesis takes a multidisciplinary approach and describes an applied research project which aims to deduce associations between dog behaviour and the development of hypothyroidism, with the ultimate aim of improving the welfare of dogs. Each chapter aims to answer one question pertaining to the association between hypothyroidism and behavioural change. To introduce the relevance of each research question I will initially review the literature on canine behaviour disorders (Section 1.2), how behaviour and hormone levels are related (Section 1.3) and the relevance of stress physiology (Section 1.4). As this research is based in the disciplines of both ethology and endocrinology, the rudiments of behavioural endocrinology (Section 1.3) are also considered, and in particular that of the thyroid gland (Section 1.3.2); *i.e.*, how thyroid malfunction can be related to behaviour disorders and the welfare implications associated with such conditions. Due to the limited research on the relationship between hypothyroidism and behaviour in non-human mammals, a comparative approach, where taken, will draw reference to research in human rather than veterinary medicine.

Thus, the biological pathways linked with hypothyroidism (Section 1.6), and behavioural changes associated with altered thyroid function (Section 1.7) as well as the welfare implications of hypothyroidism and behavioural change (Section 1.8) will also be reviewed. An overview and a research outline end the introduction (Section 1.9).

1.2 Canine behaviour and behaviour disorders

1.2.1 The value of canine ethological research

The association between man and dog has been long and varied; dogs have, and still do, fulfil numerous roles in human society (Clutton-Brock 1995). Given this long-term close association between dogs and humans, interspecific communication is well developed (O'Farrell 1996); however, frequent misunderstandings do still occur (Rooney *et al.* 2001). Research into canine behaviour is essential for the effective comprehension and therefore improvement of interspecific communication and the preservation of amicable social bonds between humans and dogs. Thus, the domestication of the dog, the dog owner relationship, and what happens when this relationship goes wrong will all be considered further.

1.2.2 The domestication and evolution of the dog

Archaeological evidence indicates that the dog was domesticated 12,000 years ago (Clutton-Brock 1988); however, recent mitochondrial DNA evidence suggests that dogs may have diverged from the wolf (*Canis lupus*) more than 100,000 years ago (Vila *et al.* 1997). The dog's closest relatives are considered to be the wolf, golden jackal (*C. aureus*) and the coyote (*C. latrans*). Although the dog can mate with all of these canids and produce fertile offspring, mitochondrial DNA implicates the wolf as the sole ancestor of the dog (Vila *et al.* 1997). In addition to genetic evidence, both the wolf and the dog have very similar laryngeal anatomy and vocal capabilities. The varieties of dog breeds that exist today do not originate from the same sub-species of wolf (Clutton-Brock 1988). Several distinct types of dog have been identified from fossils, dating back to the Bronze Age c.4500BC; the Indian wolf (*C. lupus pallipes*), the Chinese wolf (*C. lupus chanco*) the European wolf (*C. lupus*) and the North American wolf (*C. lupus*) are all considered to be ancestors of dogs (Clutton Brock 1995).

There is no absolute consensus as to how dogs were domesticated, but three main ideas exist. The first suggests that the puppies of wild canids were stolen and bonded to tribeswomen as they suckled at the breasts of lactating mothers (Thurston 1996). The second theory suggests that dogs were domesticated by scavenging near villages (Zeuner 1963). The dogs could have been encouraged, as their presence would have had several advantages for the humans. For example, dogs would have kept the settlement clean in their consumption of human faeces, would have vocalised at the approach of strangers and these dogs could also have been a valuable source of meat and fur in times of need

(Serpell 1995). An association with humans has played a vital role in canine evolution (Feddersen 1991) as the third theory of domestication refers to a time when people adapted to a more settled existence, with the planting of crops and storage of grain and the development of tools into catapulting and bow and arrows types, leading to the commencement of hunting. Dogs then became a very valuable commodity as they could be trained and used for tasks, such as the retrieval of prey. However, it is possible that all three methods of domestication may have occurred at different times (Serpell 1995).

Domestication has resulted in alterations to canine morphology, physiology and behaviour (Hafez 1969). This is due to natural genetic mutation and selective breeding by humans, which have produced the breeds that exist today (Clutton-Brock 1988). Domestication has led to both intensification and inhibition of particular behaviour patterns through such selective breeding (Fox 1976) and in some instances humans have taken the selection of dog breeds to extreme, for aesthetic and/or behavioural purposes. Selective breeding has resulted in health problems for specific breeds, for example, nasal congestion in the Bulldog due to the shortened snout, and the inability to thermoregulate efficiently in the Old English Sheepdog due to its very dense coat (Sylvester 1982). In some countries where the number of individuals of certain breeds is low, it has been difficult for breeders to avoid mating close relatives and carriers of deleterious genes (McGreevy and Nicholas 1999). Behaviourally, too, there are obstacles, as selective breeding for purely aesthetic purposes has resulted in changes in morphology which can impede effective interspecific and intraspecific communication systems, and this can be a cause of inappropriate behaviour. For example, the 'perfect double curl' in the tail of a Pug makes canine communication difficult.

Of the dogs euthanised due to behaviour disorders, the majority were as a result of inappropriate aggression. In cases where dogs have been bred for heightened aggressive tendencies (e.g. the Pit Bull Terrier), this also has consequences for the safety of humans. An inappropriately aggressive dog is a problem because of the damage that it can cause to human beings, other animals (Podberscek 1991), property and to itself. McGreevy and Nicholas (1999) theorise that selecting for the absence of specific behaviour disorders such as separation anxiety, one may inadvertently select for another behaviour disorder such as dominance aggression because behaviour patterns are the consequence of underlying traits triggered by stimuli from their external environment.

Today the dog continues to be a valuable resource throughout the world as it retains its multiplicity of uses, for example in hunting, shepherding, guarding, tracking, religious worship (Thurston 1996), witchcraft, scientific research and also assistance roles that include forewarning of seizure onset in people with epilepsy. However, the dog's primary role today is as our companion and pet.

1.2.3 The human - canine bond

Historically dogs may have been chosen as pets because they were able to transfer their normal social attachments to man and behave towards him in a manner that he interpreted to be friendly, affectionate and companionable (Messent and Serpell 1981). Dogs also remain in the vicinity of humans without the need for tethering or other restrictions, and display signals indicative of motivational state (Halliday and Slater 1983) typically interpreted correctly by humans (Figure 1.1).

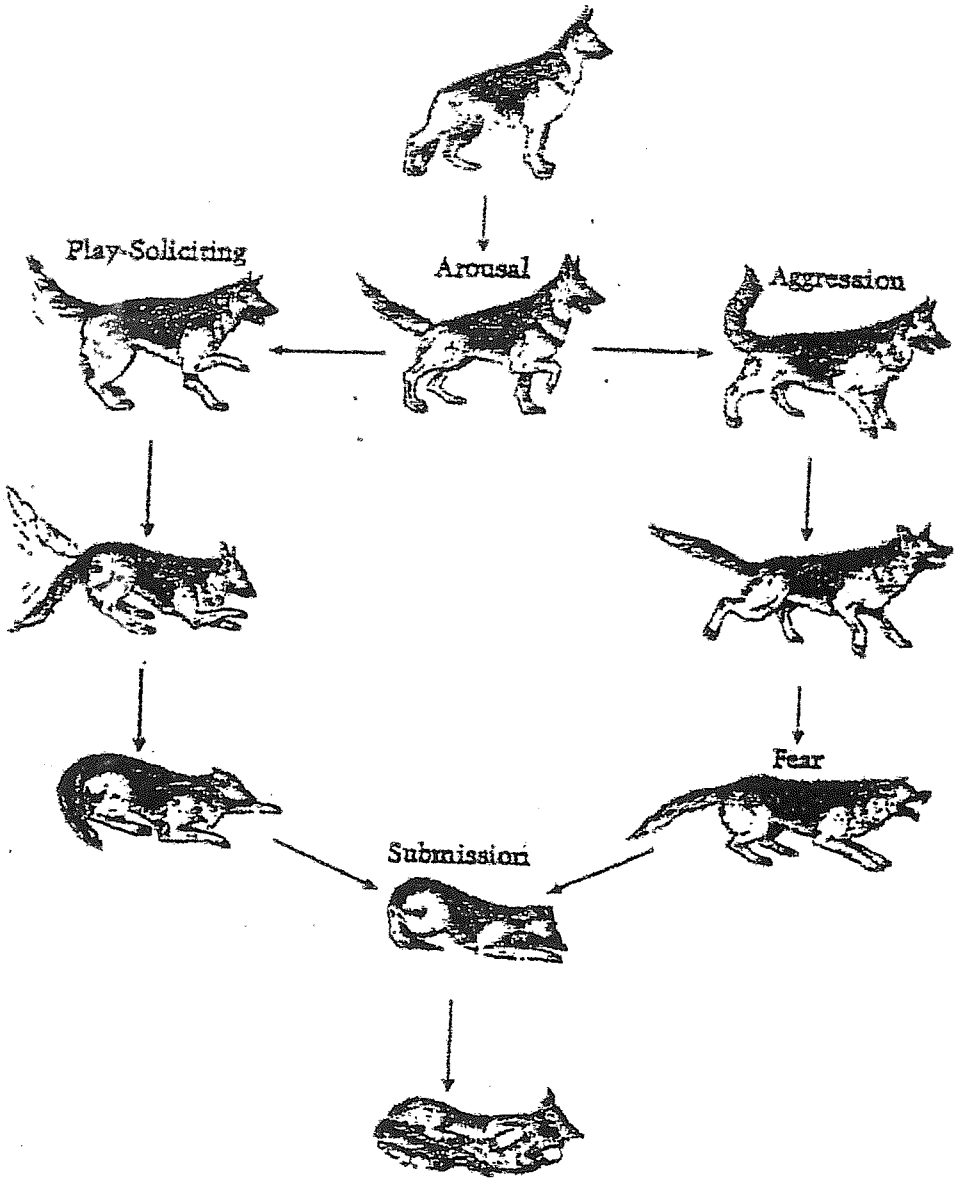

Figure 1.1 Behaviour patterns of the dog (Overall 1997)

Today just under 50% of UK households own a pet, the second most numerous is the dog (Anon. 2002). It has been suggested that owning a dog can have advantageous effects on the health of owners. For example, pets can assist in child development, helping children to build confidence and aiding cognitive development (Poresky and Hendrix 1988). Pet ownership can also have beneficial effects on elderly people living alone (Robinson 1995). Pet-owning elderly people have been shown to experience less depression following the death of a spouse (Garrity *et al.* 1989), exhibit less stress (Allen *et al.* 1991; Wilson 1991) and take less medication than non-pet owners (Robinson 1995). Other physiological and psychological benefits have also been suggested to be associated with dog ownership. Dog owners are said to experience less anxiety, which helps to maintain an effective immune system, and they may therefore have a decreased risk of contracting diseases such as eczema, colds, coronary heart disease, headaches, hypertension, impotence, stomach ulcers and cancer (Fraser 1991; Biswas and Ahmedzai 1992; Robinson 1995), all of which are associated with a stressful lifestyle.

Due to the close association between owners and pets, interspecific and intraspecific misunderstandings occur which may threaten the agreeable dog-owner relationship. Dogs require the cohesion of a predictable social group and when owners cease to provide such an environment, behaviour disorders can occur. At its most serious, abnormal unacceptable behaviour can be life threatening for the dog in the sense that if it cannot be tolerated, euthanasia is the owner's only option (O'Farrell 1996).

1.2.4 Dog behavioural development

Behaviour can be affected by a number of variables including the environment, physiology, experience, learning and genetic predisposition of the dog (Borchelt and Voith 1986). A very significant period of learning occurs between the fourth and the fourteenth week of life and is termed the Socialisation Period (Fox and Stelzner 1966). Behaviour disorders including a heightened fear response due to an aversive experience, or anxiety due to the anticipation of an aversive stimulus are the most common sort seen by behaviour counsellors. These are largely influenced by previous learning experiences and particularly by ineffective socialisation during a puppy's socialisation period (Appleby *et al.* 2002). The peak of the socialisation period is generally accepted as between six and eight weeks of age, and during this period the puppy learns to accept the stimuli in its environment as normal. This is therefore considered as the best time to remove a puppy from its litter (Scott and Fuller 1965), enabling the puppy to become familiar with the environment and people that it will be with as an adult. The socialisation period is important from a clinical standpoint in that whatever stimuli the puppy is not exposed to during this period is more likely to trigger a fear response in adulthood (Houpt 1998).

1.2.5 Dog behaviour disorders

Today, many people keep dogs strictly as companion animals rather than for protection or other functional purposes; as a result there is increased necessity for the behaviour of the dog to be socially acceptable. Misunderstandings occur because owners often regard their dog as an honorary human member of the family and expect it to assume social responsibilities to match that position (Wilbur 1976).

Mugford (1981) describes a behaviour disorder as consisting of behaviours that do not fulfil "*reasonable*" human expectations. Owren and Matre (1994 p.50S) define behaviour problems as types of behaviour that are, "*a source of strain and conflict for the owner, the dog or others in the establishment*". However, as the terminology *"reasonable"* and *"source of strain"* mentioned in these definitions respectively can be considered vague and ambiguous, I would suggest that any behaviour pattern exhibited by a dog, and considered as a problem by its owner, is a behaviour disorder. This definition fits the selection criteria I have used in recruiting a sample of dogs with behaviour disorders. However, it should be noted that within a population of dogs with behaviour disorders there are subpopulations of dogs that are showing normal behaviour patterns which are inappropriately directed and considered unacceptable by their owners, e.g., mounting people and digging on the lawn, and those behaviour disorders that are often motivated by fear and anxiety, e.g., aggression and separation related disorders.

Behaviour disorders include separation related behaviour, training problems, phobias, stereotypies and play related disorders all of which shall be defined. Additionally, the most common behaviour disorder is aggression which shall be considered further due to its multifactorial causes and because aggressive behaviour has been linked to abnormal thyroid function and is therefore of particular relevance. Although the behaviour disorders are considered individually it should be noted that this list is neither mutually exclusive nor exhaustive.

Separation related behaviour refers to a particular set of behaviour patterns that occur exclusively when a dog is left alone. These include destructive chewing, excessive vocalising, digging, consumption of non-foodstuffs, urination and/or defecation, panting or drooling, and may also include aggressive behaviour patterns directed towards the owner on leaving their dog. These behaviours can have varied motivations, such as: over attachment to owners; lack of habituation to separation from members of their social group; fear of specific stimuli (such as noises) that generalise to a particular context; and operant learning, where the dog's behaviour is successful in controlling their owner's behaviour.

Training problems are defined as any normal dog behaviour patterns that occur inappropriately, or any desired behaviour not readily carried out by the dog on command. Training problems can include

loss of toilet training, chewing, pulling on the lead and not returning to the owner when called. Phobias can be defined as excessive or extreme fear responses to stimuli and phobic responses often involve whining, panting, shaking, running or hiding in response to particular stimuli. Phobias are often elicited by loud noises but can also result from stimuli such as unfamiliar people, insects, hot air balloons or other specific contexts. Stereotypies are defined as repetitive behaviour patterns with no obvious function (Beaver 1994), e.g., flank sucking, tail chasing and fly snapping. Beaver (1994) suggests that stereotypies develop as a coping strategy during chronically stressful situations. Play related disorders are poorly signalled inappropriate behaviour patterns that occur during bouts of play e.g., aggressive behaviour during periods of play.

The definition of aggression varies with authors (Berkowitz 1962; Kaufmann 1965; Montagu 1966; Welch 1969) probably because its motivation is multifactorial (Washburn and Hamburg 1968). Argyle (1975 p.220-221) maintains, "*aggression is the innate response to attack, frustration and competition for resources*" and Frederiksen and Gerald (1977) discuss various methods of defining aggression. However, Moyer (1976) refers to aggression as overt behaviour involving intent to inflict noxious stimulation or to behave destructively toward another organism; this can be direct or indirect towards inanimate objects, and can only be described as aggression if frustration or aversive stimulation is involved. Several overlapping types of aggressive behaviour disorders are recognised: these include confident, predatory, territorial, possessive/protective, fear-induced, play related, interspecific/intraspecific and learned aggression. In a clinical setting however, aggression most often occurs as a consequence of fearfulness.

Confident aggression (Figure 1.1) has often been explained using theories of dominance relationships, but this may not be a sound approach since the function of dominance relationships in social groups is to assure social stability and prevent fighting (Lorenz 1966; Candland *et al.* 1970; Johnson 1972; Bradshaw 1995; Drews 1993). Line and Voith (1986) suggest that dominant dogs are predominantly male purebreds, although a subject that is dominant in every situation is exceptional (Fonberg 1988). Non-confrontational behaviour modification programmes are effective in reducing owner directed confidence aggression (Dodman *et al.* 1996; Cameron 1997; Uchida *et al.* 1997).

Predatory "aggression" is characterised by hunting or stalking postures. Archer (1976) suggests that predatory aggression is motivated differently from any other form of aggressive behaviour, as it is not derived from intraspecific aggression; and has a different function for the animal. One prediction of this idea is that no warning should be given since this would assist the prey to escape, which differs from intraspecific forms of aggression where warnings are important to reduce the chance of overt aggressive behaviour. Predatory aggression can be directed to any living organism or inanimate object.

Territorial aggression is shown when the dog's area is invaded, with warning signs usually given before an attack. Territorial aggression can occur at home and possibly when out on walks, especially if the dog feels that the owner is part of its own territory and is regularly taken to the same area for exercise.

Possessive and protective aggression occurs in situations when a dog may appear to feel a threat to its resources (food, toy or owner) and can therefore become protective, or may also be aggressive to people that the owner tries to talk to on the street. This type of aggression may be linked to dominance aggression (Borchelt 1983; O'Farrell 1996), but is more commonly associated with a fear response as a consequence of inconsistent owner behaviour.

Fear induced aggression is hallmarked by fearful behaviour patterns (e.g., ears and tail down, snaps and withdraws; Figure 1.1) this is a normal response in an animal that feels threatened or unable to escape.

Play aggression is heavily signalled. The play bow is a typical posture seen in these cases and dogs will often perform this behaviour before launching an attack. Metacommunication, such as grinning, growling and barking is frequently displayed.

Aggressive behaviour can be directed interspecifically or intraspecifically, i.e., aggression directed towards any individual of another species, and aggression directed to members of the same species, respectively. Learning plays an important part in the development of aggressive behaviour, as on every occasion in which the display of aggressive behaviour is successful at resolving a situation, it is more likely to be selected as a behavioural strategy on future occasions.

The serious consequences of inappropriate aggression make it very relevant both to the welfare of dogs and to public safety, and therefore these shall be considered further. Animal bites were estimated to be the fourth leading cause of death in children in the USA (Underman 1987) and Beck (1975) found that there are one million dog bites every year in the USA. Dog bites directed to adults are more often directed towards males than females (Podberscek and Blackshaw 1990). Three out of forty dog bites result in death, fifty months is the median age of victims, and the face and head are the areas of the body most commonly bitten (Brogan *et al.* 1995). Aggressive dogs are more likely to be euthanised, particularly if they are aggressive to their owners (Damkjer-Lund and Brabtbo-Sorenson 1997).

1.2.6 The classification of behaviour disorders

The problems inherent in attempting to classify behaviour disorders are that they can only be characterised and understood relative to the environment in which they occur (Askew 1996).

However, numerous attempts have been made to classify canine behaviour patterns (Hart 1974; Houpt 1979; Stanford 1981; Beaver 1983; Borchelt 1983; Borchelt and Voith 1982; Blackshaw 1991; Feddersen 1991; Bebak and Beck 1993; Overall 1993; Podberscek and Serpell 1996; Serpell 1995; Polsky 1996), all of which are aimed at developing a system of defining problem behaviour that can be universally applied and assist in the application of suitable treatment protocols. Alternative methods of classifying behaviour disorders have been based on the type of treatment, the specific environmental cues, the type of training required, and the type of learning associated with the disorder (Ralston 1982; Askew 1996). In addition to the classification systems suggested in scientific papers and popular literature there are also those used by associations promoting the use of companion animal behaviour therapy such as the Association of Pet Behaviour Counsellors (APBC) and the UK Registry of Canine Behaviourists.

1.2.7 Behaviour modification

Behaviour modification is the process by which the behaviour of a pet can be manipulated to modify or eliminate a particular problematic behaviour pattern (Mugford 1981), utilising the principles of learning. Reinforcers are used to encourage or discourage particular behaviour patterns of which there are two types, positive reinforcers and negative reinforcers. A positive reinforcer is a reward given for the occurrence of the required behaviour pattern. A negative reinforcer is the removal of an unwanted factor to increase the probability of the required behavioural response (Beaver 1994). For example, aggression can be modified by learning (Barash 1982), and positive and negative reinforcement increase and inhibit aggressive behaviour patterns respectively (Berkowitz and Frodi 1977). The manner of learning can include habituation, extinction, desensitisation, counter-conditioning, flooding, and avoidance/aversive conditioning (Overall 1997). As these methods are relevant to several areas of this thesis, these procedures are summarised here.

Habituation involves repeated presentation of a low intensity or low salience stimulus until the stimulus no longer results in a response. Extinction refers to the decrease of a behaviour patterns over time due to the withdrawal of all forms of reinforcement. Desensitisation is the process by which an association is weakened and the animal becomes less behaviourally reactive due to gradual exposure to the stimulus previously eliciting the undesired behavioural response. Counter-conditioning is the method by which an animal is taught an alternative behaviour that is incompatible to the previous undesirable behaviour. Flooding overlaps with habituation in that repeated presentation of the stimulus is involved, however, flooding involves the continuous (rather than recurring) application of stimuli to an animal at such a level that it causes a behavioural response with the aim of allowing the animal to adapt to the stimulus over time. Aversive conditioning is a form of learning that leads to the avoidance of a place or object due to association with a previous aversive experience. Generally, responsible clinicians advise the use of positive reinforcement techniques in behaviour modification

as using punishment or negative reinforcement techniques can cause anxiety and has been found to be counterproductive in the training of dogs (Hiby *et al.* 2002).

Treatment for aggressive behaviour in mammals has included surgical procedures to lesion areas of the brain (Arons and Shoemaker 1992), autonomic denervation, drug therapy and hormone treatments (Moyer 1971). However, in dogs, aggressive behaviour can usually be influenced by behaviour therapy and some authors have suggested techniques involving positive reinforcement (Dodman *et al.* 1996) and punishment (Houpt 1983), which has been found to be successful. Most clinicians endeavour to take an owner's attitude into account when devising a treatment programme (O'Farrell 1997), and a full veterinary examination should be completed in order to rule out any medical causes of aggressive behaviour (Campbell 1992).

Learnt factors affect behavioural responses independently of hormonal status (Berkowitz 1962), and Scott and Frericson (1951) held that all aggression is learnt (or due to frustration activities) and that the learnt component may override any hormonal activity. For example, mice (*Mus musculus*) trained to fight, still continued to fight after castration (Beeman 1947). Although hormones do not normally initiate behaviour, hormones can create the physiological environments that influence the threshold at which particular behaviour patterns may occur or be inhibited (McFarland 1981). I shall now consider the relationship between behaviour and hormones in further depth.

1.3 Behavioural endocrinology

Behavioural endocrinology has its origins in anatomy, physiology, entomology, zoology and psychology (Beach 1975). Behavioural endocrinologists are interested in how physiological changes, and in particular hormonal fluctuations, may alter behaviour, as well as how behaviour may influence the efficacy of hormones. The link between behaviour patterns and physiology is not always obvious. For example, on exposure to a predator, sheep will adopt a rigid stance (tonic immobility). This was anecdotally considered to be disinterest toward the predator on the part of the sheep. However, on further examination and measurement of ovine cortisol it appears that the sheep is preparing itself for physical activity. Chronic exposure to the stress hormones produced from such preparation can be considered to be adverse and stressful (Archer 1976). This example emphasises the importance that physiological findings can have when used in conjunction with behavioural observations. Examining behaviour is a valuable method of studying physiological processes (Barr *et al.* 1976) as neither behavioural nor physiological investigations alone will give a full picture of the cognitive state of the animal. Additionally, behavioural parameters often have the advantage of providing non-invasive measures of stress (Beerda 2000a).

Multidisciplinary approaches that utilise endocrinological methodologies should lead to advances in the understanding of biological stress and animal well being (Matteri *et al.* 2000) as every aspect of behaviour should theoretically be resolvable into physiological, neurological and endocrinological factors (Kuo 1960).

Some endocrine hormones are known to influence mammalian behaviour directly (Carlson 1998) i.e., the sex hormones and the glucocorticoids mediated by the hypothalamic-pituitary-gonadal (HPG) axis and the hypothalamic-pituitary-adrenal (HPA) axis respectively. The HPA axis is one of the main mediators of the physiological stress response, contributing to the endocrine response to emotional arousal (Manser 1992). The activation of the HPG axis produces the sex hormones, all of which affect the sexual behaviour of mammals (Drickamer *et al.* 1996). This has been utilised in the treatment of some sexually related behaviour disorders with drugs that manipulate the balance of the sex hormones (Blackshaw 1985). Measurement of cortisol and behaviour has been found to be useful for screening rescue dogs for the likelihood of showing behaviour problems on re-homing (Hennessy *et al.* 2001).

1.3.1 Hormonal control of behaviour

In the study of mammalian behavioural endocrinology the HPG and HPA pathways are known to directly affect behaviour patterns, but there is no evidence to suggest that other hormones, including those produced by the thyroid gland, have such a direct influence on discrete patterns of behaviour (Dewsbury 1978; Leshner 1978; Hart 1985). The assumption that thyroid hormones can affect particular behaviour patterns is derived from the observation that thyroid replacement therapy has assisted in the resolution of behaviour disorders that are associated with underlying hypothyroidism, in single case studies (Dodds 1992, Dodds 1996; Dodman 1995). However, this evidence of a response to therapy, and a correlation between behaviour patterns and thyroid hormone titre, does not necessarily prove a cause and effect relationship. Therefore it is my aim to consider whether the thyroid status influences behaviour patterns, or whether behavioural change is related to changes in thyroid function. To investigate this I shall review thyroid function and consider some current causative theories/possible mechanisms for the relationship between thyroid hormone and behaviour.

1.3.2 The physiology of the mammalian thyroid gland

The mammalian thyroid is an endocrine gland comprised of two lobes situated either side of the larynx (Figure 1.2). It produces thyroxine (T4) and triiodothyronine (T3) in which iodine is covalently bound. The thyroid hormones play a role in the control of metabolism, effective growth and control of reproduction and lactation. Thyroid function is controlled by the hypothalamic-pituitary- thyroid-extrathyroid (HPT) axis (Figure 1.3), which is assumed to be similar in the dog to that in man (Chastain and Ganjan 1986). The neurones in the thyrotrophic area of the hypothalamus secrete thyrotrophin-releasing hormone (TRH). This is carried via the hypophyseal portal system to the anterior pituitary and leads to tonic production of thyroid stimulating hormone (TSH). TSH acts

on the follicular membrane producing cAMP (an intracellular messenger) that in turn acts on all stages of thyroid hormone production to increase activity. This system is controlled by a negative feedback mechanism, which is under direct control of TSH and the activation of T3 on thyrotrophin synthesis (Figure 1.3).

The thyroid gland can also be autoregulated (unrelated to TSH) by decreased iodide binding to thyroglobulins in times of excess plasma iodide; a process called the Wolff-Chaikoff Effect (Chastain 1990). Decreased iodine will cause an increase in T3 production in relation to T4. The dog thyroid gland produces the entire T4 requirement and 40-63% of the T3 requirement. Under certain conditions (Figure 1.4) reverse T3 (rT3), is also produced (Drazner 1987). The remainder of T3 and rT3 is formed from monodeiodination of T4 in the peripheral tissues.

Malfunction of the thyroid gland in mammals can result in either hypothyroidism or hyperthyroidism, due to insufficient or excess production of thyroxine respectively. However, the incidence of hyperthyroidism in the dog is extremely rare (Chastain and Ganjam 1986). In both humans and dogs there are several causes of hypothyroidism but it is most often due to malfunctions of the gland or the HPT axis. These may include inadequate secretion of TSH, decreased absorption of thyroxine, and autoimmune thyroiditis (Section 1.5.1). The types and symptoms of hypothyroidism shall be considered further as these are hypothesised as being associated with behavioural changes in both humans and dogs.

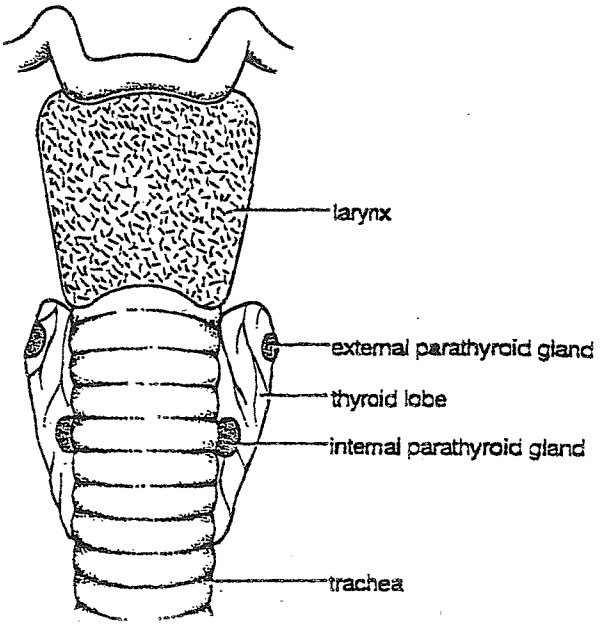

Figure 1.2 The normal canine thyroid gland (Turner 1994)

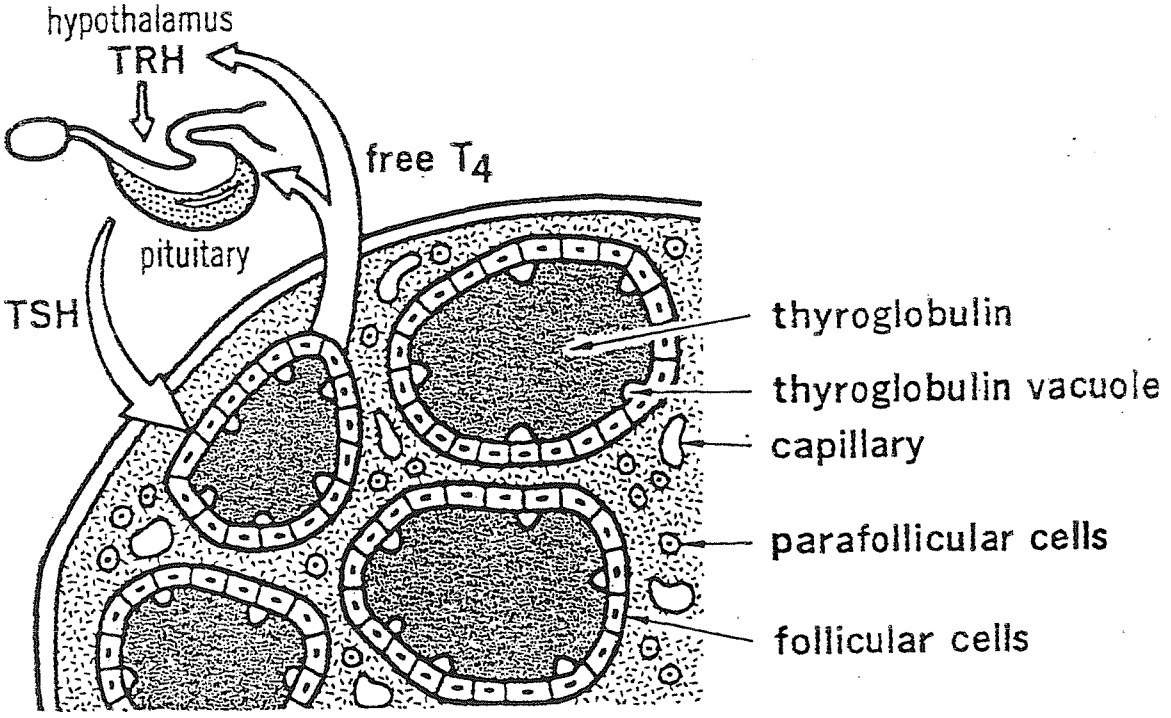

Figure 1.3 Regulation of thyroid secretion (Chastain and Ganjan 1986)

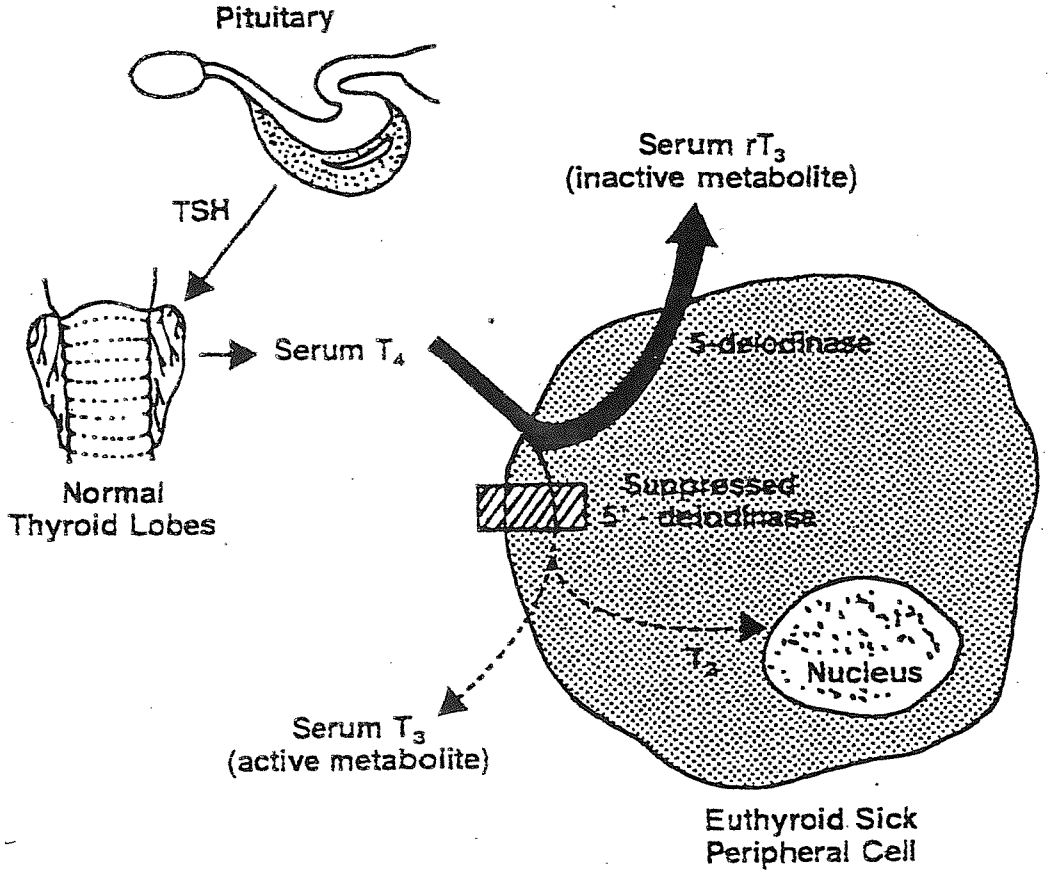

Figure 1.4 Fasting, anorexia or catabolic illness suppress 5'-deiodinase reducing serum T3 levels. The peripheral cells metabolic rate is adapted to a state that minimises the loss of protein in periods of caloric deprivation or debilitating disease. Serum rT3 concentration may be increased (as shown above), normal, or decreased, depending on the severity and duration of the calorie deprivation or disease (Chastain 1990).

1.3.3 The thyroid gland, stress and mental state in humans

For more than two centuries, relationships between the thyroid axis and abnormal behaviour have been suspected in humans and it is now evident that there is a fault with the thyroid gland in some patients with altered mental state, though the mechanism is unclear (Loosen and Prange 1982; Loosen 1986; Lesser *et al.* 1987). The link between psychological factors and the thyroid gland may be traced back at least to the seventeenth century (Mason 1968), but it was in 1949 that Asher published the original paper associating thyroid imbalance with cognitive dysfunction termed "Myxodema Madness". Since then it has generally been accepted that depression and behavioural disturbance appears to be the most frequent mental state associated with clinical and subclinical hypothyroidism in humans (Extein and Gold 1987; Lesser *et al.* 1987; Nomura 1994; Dorn *et al.* 1996; Lasser and Baldessarini 1997; Leo *et al.* 1997; Stein and Avni 1988; Denicoff *et al.* 1990; Sauvage *et al.* 1998).

Initial diagnosis of hypothyroidism may be presented with depressive mood state and in some cases hyperactivity, irritability, hallucinations, anger, fearfulness, suspicion and bouts of delusion; it has also been associated with affective disorders, attention deficit hyperactivity disorder (ADHD), impulsiveness, distractibility, inattentiveness, aggressiveness, intrusiveness and destructiveness (West *et al.* 1996). Ciaranello (1993) has questioned whether particular parts of the brain are sensitised to thyroid hormones which may cause ADHD, but Whybrow (1996) suggests that the contribution of hypothyroidism to psychiatric disability and associated behaviour patterns could be simply due to the decreased metabolic state attributable to the hypothyroidism.

An increased concentration of thyroid hormone does appear to promote the recovery of depressed patients, and thyroid hormones are known to enhance the effects of tricyclic antidepressants (TCAs; Extein and Gold 1987; Bauer *et al.* 1998; Bommer and Naber 1992). This finding is possibly due to the actions of the TCAs on altering iodine capture (Sauvage *et al.* 1998), and it is also possible that changes in pituitary - thyroid hormone production might be important in the modulation of mood, as the secretion of pituitary hormones is regulated by the same neurotransmitters involved in the aetiology of psychoses (Whalley 1989). Thyroid function is decreased by stress primarily by somatostatin-induced suppression of TRH and TSH secretion, and via the glucocorticoid induced blockade of the conversion of the inactive T4 to T3. Placidi *et al.* (1998) suggest that the co-occurrence of psychiatric and thyroid diseases may be the common result of biochemical abnormalities. Thyroid function in humans can be regulated by stimuli other than the circulating levels of thyroid hormone (Nicoloff *et al.* 1970) and changes in catecholamines, thyroid hormones and cortisol have been observed in psychiatric patients in particular cases of depression (Rao *et al.* 1995). I will review some of the main factors associating the thyroid hormones and psychoses.

(1) The implication of the neurotransmitters

An increase in T3 may help to treat depression by its effect on noradrenergic and serotonigeric neurotransmitters, which have both been implicated in the pathology of aggression (Stein and Avni 1988; Singer *et al.* 1995; Reisner 1996) and depression (Cleare *et al.* 1995). Kirkegaard and Faber (1998) hypothesised that depression causes increased levels of T3 associated with increased serotonin and therefore an elevation of the depressive symptoms. Some evidence suggests that thyroid deficiency attenuates the effects of the catecholamines and that interaction between thyroid hormones and catecholamines may underlie the therapeutic effects of combining thyroid hormones and antidepressants (Murray 1991). This is due to the interaction between catecholamine receptors and thyroid hormone and has been hypothesised as promoting the remedial effects of thyroid hormone in response to antidepressants (Denicoff *et al.* 1990). Kirkegaard and Faber (1998) proposed that changes in the HPT axis during depression might be explained by cerebral serotonin deficiency and that T3 treatment to some degree can revert this deficiency, as the thyroid hormones increase the cerebral content of serotonin (Weissel 1999).

Data derived from animal studies indicates that plasma T3 levels determine the T3 concentration in the peripheral organs whereas plasma T4 determines the intracellular content of T3 in the brain (Joffe and Singer 1990). As T3 and T4 have opposite effects in the brain it is possible that the varying levels of these hormones could have differential effects on mood. It has also been suggested that T3 may alter plasma levels of TCAs and thereby increase their therapeutic effects (Joffe and Singer 1990). T3 may have advantageous effects on the treatment of depression by enhancing noradrenergic function through increasing the activity of beta-adrenergic receptors (Joffe *et al.* 1995).

(2) The involvement of the glucocorticoids

Dorn and Chrousos (1993) and Sternberg *et al.* (1992) noted that stress increases in depression, and increased anxiety and aggression were associated with increased concentrations of cortisol. In contrast, McBurnett *et al.* (2000) found that increased aggression was associated with reduced levels of cortisol. However, hyperactivity of the HPA axis is common in cases of endogenous depression (Arque *et al.* 1987) and results in decreased thyroid hormones (Section 1.6) and several psychiatric populations have demonstrated a blunted TSH response (Gillette *et al.* 1997).

(3) Further issues

Pituitary and thyroid tumours could also influence the onset of aggressive behaviours as humans with hypothyroidism often show a reduction in their ability to reason (Ettinger 1989).

Subclinical hypothyroidism (which has very few symptoms) has been associated with depression. This association could be due to reduced thyroid hormone causing reduced serotonin, and/or

disturbances in type II deiodinase, and/or disturbances in carrier protein taking T4 to the brain (Hendrick *et al.* 1998).

T4 excess in the brain is suggested by Joffe (1990) to cause depression. He argues that as plasma T4 is the main determinant of thyroid hormone concentration in the brain, (whereas plasma T3 determines peripheral thyroid hormone concentration), T3 administration causes a lowering of T4, which produces an antidepressant effect. The brain is very sensitive to changes in thyroid hormone (Nomura 1994), but evidence that synthesis of the thyroid hormones in the brain may not parallel those in the periphery complicates assessments (Lasser and Baldessarini 1997).

The thyroid hormones, neurotransmitters and aggression pathways are linked but the detail of this association is yet undetermined. No clear relationship between thyroid hormones and noradrenergic receptor function has been described that would implicate thyroid illness in the aetiology of phobias (Lesser *et al.* 1987). Although hypothyroidism is associated with depression there is no correlation between the level of depression and the amount of thyroid malfunction (Joffe and Sokolov 1994). However, the link between aggressive behaviour, depression and abnormal thyroid function may not only be due to high cortisol levels inhibiting thyroid function (Kirkegaard and Carroll 1980). Stress and thyroid function are related, as are stress and aggressive behaviour, and Boissy (1995) suggests that animal models are useful in providing homologies.

1.3.4 The thyroid gland, stress and behavioural change in dogs

The physiological mechanisms linking the thyroid gland, stress and behavioural change in dogs is unclear. Some findings, such as Mason's (1968) research on the psychoendocrine system has, focused on the study of laboratory animals exposed to stressful situations. In rats chronic defensiveness is isomorphic to symptoms of depression (Blanchard *et al.* 1993) and work on dogs and other animals demonstrates that increased activity of the thyroid causes increased Central Nervous System (CNS) activity which can lead to either a more passive or excitable behaviour dependant on the state of the nervous activity (Krushinskii 1960).

The literature concerning the relationship between thyroid function and the behaviour of the dog is sparse. In the past 20 years there have been less than 20 papers that consider the involvement of the thyroid hormones in dog behaviour compared to over 500 papers published on the thyroid and mental state in the human scientific literature. The first paper relating hypothyroidism to the behaviour of the dog was written by Reinhard (1978). This paper was entitled 'Aggressive behaviour associated with hypothyroidism', and stated, "*hypothyroidism and other pathophysiological causes of aggressive behaviour are rare, but should be ruled out prior to treating a dog for behavioural causes of aggression*". Reinhard (1978 p. 69) also stated that, "*aggressive behaviour is not usually characteristic of this metabolic disorder*". Hypothyroidism can also cause impairment of cerebral

function that has previously been reported as a cause of "*viciousness*" in the dog (Reinhard 1978). In the early eighties, a paper concerning hypothyroidism in different breeds was published (Blake and Lapinski 1980). This paper made no direct inferences to behaviour, but noted that some breed types exhibit more hypothyroidism than others and that this is possibly hereditary.

The literature then remained sparse for over a decade until Dodds (1992) published a special feature entitled 'Thyroid can alter behaviour'. In this article the author suggested that a causal link exists between thyroid dysfunction and problem behaviour in dogs, and that irregularity in thyroid functioning causes behavioural problems. However, in response, Polsky (1993) expressed his reservations that thyroid disorder actually causes behavioural problems. He further suggested that behaviour problems co-occurring with hypothyroidism can be manifested as an increased tendency for inappropriate aggression, extreme shyness or seizure-like behaviour, but that a causal relationship was unlikely due to the lack of a direct effect of thyroid hormones on behaviour (Polsky 1993). Later, Dodds (1996) noted that dogs with thyroid disease show aberrant behaviour including aggression, anxiety, lethargy, depression and unstable temperament.

Dodman (1995) described how the use of thyroid replacement therapy produced a subsidence in aggressive behaviour (suggesting that the alleviation of the aggressive behaviour was attributable to the correction of the thyroid imbalance). However, thyroid replacement therapy has broad effects mediated through general metabolism. Gerzovich (1995) suggests that confident aggression is more likely to be associated with hypothyroidism; these findings were similar to those of Tobjorn Owren, which were never published (S. Scott pers. comm). In 1997 Gibbs discussed an association between hypothyroidism, stress and mental disorders but did not confirm how these were linked in dogs. Recently, Scott (1999) wrote an article for the Companion Animal Behaviour Therapy Study Group (CABTSG) entitled 'Hypothyroidism and behaviour, the case for further investigations'. However, as with previous articles her research is based on single case studies and the use of thyroid replacement therapy to correct behaviour problems.

Other than single case reports associating aggressive behaviour with hypothyroidism, Hamilton-Andrews (1998) noted a correlation between low thyroid hormones and aberrant behaviour (mainly due to noise sensitivity) in the Bearded Collie. However, correlation is not evidence of causality and there is no evidence to suggest that low thyroid hormone produces aggression in dogs.

It would appear that the subject of hypothyroidism in relation to dog behaviour is deserving of further investigation. The existing literature is sparse, speculative and based on single case studies. The supposition that the thyroid hormones can control behaviour is largely based upon the observation that thyroid replacement therapy assists in the correction of behaviour problems. As yet, there is no evidence to suggest that the thyroid hormones are causally linked to the behaviour of dogs. Treatment

with thyroid replacement therapy, even if successful, will affect several other hormonal pathways due to feedback mechanisms and is therefore not direct evidence of a link. However, clinical observations provide more than anecdotal evidence of a relationship between hypothyroidism and behaviour problems and in particular with aggressive behaviour. But, do the thyroid hormones control behaviour patterns? Or, does behaviour (via motivational and stress factors) affect the concentration of thyroid hormones? In order to resolve this ambiguity, it is necessary to review the physiology of the thyroid and of canine hypothyroidism, and also to consider some current causative theories pertaining to the mechanisms by which mental state/behavioural change and altered thyroid function may be related.

1.4 The physiology of stress

Stress was first defined as a non-specific response of the body to any demand made upon it (Selye 1950), but as currently used the concepts of stress have no universal definition (Moberg 2000). Toates (2000) defines stress as *"a chronic disturbance in the processes that underlie adaptive behaviour"*. Dorn and Chrousos (1993) describe stress as a state of threatened homeostasis during which the body activates adaptive mechanisms to maintain the equilibrium while the stressors are applied. There have been many other variations on such definitions (Ewbank 1973; Moberg 1985; Manser 1992). Selye, in the 1930s, coined the terms "*eustress*" to describe desirable stress and "*distress*" for undesirable stress (Chrousos and Gold 1992), to better illustrate the difference between uncontrollable and controllable stress. Mason (1968 p.791) explained, *"Selye concluded that the pituitary adrenal cortical system responds in a non-specific manner to many different stimuli or agents as part of the GAS (general adaptation syndrome)"*. It was later proposed, and is now generally appreciated, that the involvement of the HPA axis is dependent on the source of the stressor (Mason 1971). Some definitions of stress include behavioural as well as physiological adaptations, which appears reasonable as some behaviour patterns can help to restore homeostasis in stressful situations and thus reduce the requirement for physiological adaptation to stress. In addition, both the behavioural and physiological responses to stress, such as increased blood pressure and heart rate appear to be controlled at least in part by the same neuroendocrine systems (Rushen 2000). I shall now summarise the physiology of the canine CNS in order to consider the stress response further.

1.4.1 The Central Nervous System

The hypothalamus is an important part of the autonomic nervous system, which, together with the endocrine system controls the internal milieu of the body. The hypothalamus controls the release of the pituitary hormones, which act as messengers with a diversity of actions. The emotions of rage and aggression seem to originate in the hypothalamus (Carlson 1998), although they are normally inhibited by the hippocampus and the frontal lobe of the cerebral cortex (Turner 1994). Evidence

suggests that if a puppy is not adequately socialised then synaptic connections are not made between sensory input and inhibition of centres of emotion, and the range of neural plasticity is reduced in adulthood (Fox and Stelzner 1966).

1.4.2 The stress response

The stress response begins with CNS perception of a threat to homeostasis (Moberg 2000). As stress is essential to life, it is not necessarily something to be avoided (Appley and Trumbull 1986). However, distress can be considered aversive, as it is associated with the emotional response of fear (Barnard and Hurst 1996) e.g., in the immobilisation of dogs (Rothuizen *et al.*1993). Prolonged states of stress are detrimental to welfare as the stress response may lessen through adaptation (Beerda 2000). Perceived controllability is also important in influencing stress/cortisol responses (Dess *et al.*1983), as exposure to uncontrollable stress has been reported to result in increases in anxiety (File 1996).

Animals develop very similar pathologies as humans do when they suffer prolonged or uncontrollable stress (Moberg 2000). Uncontrollable stress in dogs results in increased blood cortisol (Reimers *et al.* 1990), and defeated animals are kept in a state of chronic physiological stress recognised by increased glucocorticoids (Eleftheriou and Scott 1971). Research on guinea pigs (*Cavia porcellus*) suggests that environmental stress may affect many other endocrine glands (Sachser *et al.*1994). A genetic component accounting for part of the inter-individual variability in reaction to humans has been found (Boissy 1995) in dominant dairy goats. When twin goats were assigned to a human reared group and a dam reared group the goats rank was not influenced by the group type (Lyons *et al.* 1988). Outside the laboratory it becomes almost impossible to account for inter-animal differences in the response to stress (Moberg 2000).

1.4.3 The hypothalamic-pituitary-adrenal axis

The dog has paired adrenal glands, situated at the front of the inner side of each kidney (Turner 1994; Rijnberk 1996). Each gland has two separate hormone-synthesising components, the inner medulla and outer cortex. The medulla produces adrenaline, an emergency hormone that prepares the animal physiologically to respond to flee or fight situations. The cortex produces steroid hormones (in very small quantities) regardless of sex, as well as the corticosteriods (glucocorticoids and mineralocorticoids) that are essential for the stress response.

The adrenal gland responds to internal and external environmental variables as detected by the CNS and sends this information to the hypothalamus. The hypothalamus secretes corticotrophin releasing factor (CRF; Guillemen and Schally 1963), which travels to the adenohypophysis to stimulate production of adrenocorticotrophic hormone (ACTH). This in turn stimulates the adrenal cortex to produce the glucocorticoids. The predominant glucocorticoid in the dog is cortisol and cortisol levels

have been shown to correlate with the presumed severity of a stressor by an individual (Assia *et al.* 1989; Kemppainen 1984). There is evidence that both the CNS and the pituitary may be feedback sites for adrenal corticosteriods (Levine 1972).

The function of the glucocorticoids is to prepare the body for physical activity, i.e., there is a switch from anabolic to catabolic activity and all non-essential processes are suppressed. The glucocorticoids cause an increase in the substrate for energy production, plasma glucose, and induce a release of amino acids from the skeletal muscle, which prepare for energy expenditure (Manser 1992). Such hormonal activity is essential for the maintenance of homeostasis when an individual moves into an environment that contains stimuli that may be a source of discomfort or stress.

1.4.4 *Stress, immunity and immune mediated diseases*

Stressors have been defined as stimuli that are paired with an aversive event (Maier and Watkins1999). The physiological stress response to an aversive event is a flight or fight response aimed to deal with acute emergencies. Acute stress is said to enhance certain aspects of the immune response (Maier and Watkins 1999) but both acute and chronic stress divert resources away from essential biological function (Moberg 2000).

The major role of the immune system is to recognise and eliminate foreign antibodies without harming self. However, in some cases the misdirection of humoral and cellular immune responses toward autologous antigen leads to autoimmunity (Carson 1992). Stratakis *et al.* (1995) suggest that if the immune system is compromised too much, it will bring about disease as prolonged stress results in a significant burden on the body, and the animal can enter pre-pathology or pathology state (Moberg 2000). The trigger for such a reaction is unknown. Predisposing factors that can cause an autoimmune disease can be either genetic or environmental (Kiecolt-Glaser and Glaster 1991). Environmentally, the stress of an unpleasant emotional state can cause immunosuppression (McMillian 1999). It is possible that two or more factors could occur together, e.g., drugs cross-reacting or infection by viruses (Playfair 1992); and infectious antigens have been implicated in the pathogenesis of a variety of autoimmune diseases including autoimmune thyroid disease (Section 1.5.1; Tomer and Davies 1993). Genetic make up can influence susceptibility to autoimmune disease because of the somatic generation of immune diversity; however, genetically identical individuals have different immune systems (Carson 1992).

The immune system itself can be affected by stress (Grossman 1985) as the immune cells contain receptors for glucocorticoids (Maier and Watkins. 1999), which can depress immune responses (Grossman 1986) and reduce resistance to disease (Barnard and Hurst 1996). However, it should be noted that in the absence of uncontrollable chronic stress the glucocorticoids are ultimately a valuable part of the stress response and it is only in certain adverse circumstances that there is the possibility of

the immune system reacting against itself and resulting in an autoimmune disease. Autoimmune thyroiditis (Section 1.5.1) is a very common automimmune disease, which causes hypothyroidism in dogs and is histologically comparable to Hashimoto's thyroiditis in humans (Thacker *et al.* 1995; Marshall 1996).

1.5 Canine hypothyroidism

Studies of thyroid function and iodine in the dog have indicated that the system is generally similar to humans (Belshaw *et al.* 1974). Hypothyroidism is the most common hormonal disorder diagnosed in the dog (Turner 1994, Swenson and Reece 1993; Beale *et al.* 1990; Chastain and Ganjam 1986). Haines *et al.* (1984) noted that 0.3% of dogs presented to the veterinary clinics in North America had hypothyroidism while Panciera (1994) noted a 0.2% incidence. Hypothyroidism occurs when there are incorrect concentrations of circulating thyroid hormones due to malfunctions in the gland or in the hypothalamic-pituitary- thyroid-extrathyroid axis; the causes are multiple (Hutchinson 1990; Paradis *et al.* 1991). Theoretically, hypothyroidism can also result from a defect in the mechanism of transport, plasma binding or response to precursor hormones.

1.5.1 The origin of hypothyroidism

There are several different forms of hypothyroidism, primary, secondary and tertiary. These are usually caused by either athyreosis, dishormonogenesis, thyroid tumours, inadequate secretion of TSH, idiopathic atrophy, iodine deficiency, and thyroiditis which can be autoimmune or subclinical hypothyroidism. These forms and causes shall be considered further.

Primary hypothyroidism occurs when there is an abnormality of the thyroid gland itself (Feldman and Nelson 1996). An enlarged thyroid gland (goitre) may be the result of neoplastic invasion (cancer of the thyroid which is usually malignant). Primary hypothyroidism may also occur due to a metabolic defect of hormone formation, inflammation, post operatively or as a result of exposure of the thyroid to radiation. It can be differentiated from secondary hypothyroidism by lack of response to exogenous TSH. Primary hypothyroidism accounts for 95% of hypothyroidism in dogs. However, clinical signs do not appear until three-quarters or more of the follicles have disappeared and/or have been replaced by other kinds of tissue (Chandler *et al.* 1994).

Secondary hypothyroidism is caused by pituitary dysfunction and a concomitant reduction in TSH. This is the case in less than 5% of hypothyroid dogs (Chandler *et al.* 1994). Secondary hypothyroidism is usually associated with tumours of the adenohypophysis that destroy the TSH producing cells (McDonald and Pineda 1989). A range of other processes can damage the pituitary such as inflammatory disease, although these diffuse pathologies will generally result in malfunctions

of other hormones regulated or produced by the pituitary (Chandler *et al.* 1994). This is the case in canine pituitary dwarfism in which there are congenital abnormalities in the pituitary. Secondary hypothyroidism can result from pituitary adenomas that produce excess adrenocortical stimulating hormone (ACTH) and result in hyperadrenalcorticism. The high cortisol levels depress TRH and TSH production and also inhibit peripheral conversion of T4 to T3 (Section 1.6).

Tertiary hypothyroidism is not known to occur in the dog, but in humans this is due to a lack of TRH (McDonald and Pineda 1989; Chandler *et al.* 1994; Werner and Ingbar 1978).

1. *Athyreosis* is hypothyroidism developing in utero or during the first few months prenatally. It is a type of hypothyroidism that severely affects maturation, causing physical stunting when the thyroid is either very reduced in size or absent. In humans this condition is known as Cretinism (Chandler *et al.* 1994). Puppies with athyreosis rarely survive due to developmental anomalies (West 1994).

2. *Dyshormonogenesis* is a defect in the biosynthesis of hormones because of a lack of specific enzymes. These disorders, although seen in man, have not been researched in depth for the dog (Siegel 1977)

3. *Thyroid tumours* are generally either follicular or medullary neoplasms. Some of these tumours are derived from parafollicular cells, located very closely to the thyroid, which normally secrete calcitonin. Thyroid tumours are usually malignant and may result in euthyroidism, hyperthyroidism or hypothyroidism depending on the type and the extent of the tissue damage.

4. *Inadequate secretion of TSH* may cause faults in the mechanism of trapping iodide and consequently the synthesis of thyroid hormones becomes defective (Braverman and Utiger 1996). The pituitary gland controls the secretion of several hormones. Therefore deficiency of TSH may also be associated with a deficiency in other anterior pituitary hormones such as ACTH (Siegel 1977).

5. *Idiopathic atrophy* of the follicles and their replacement by adipose tissue causes many cases of hypothyroidism (Jeffcoate 1993). Atrophy may be due to destruction of the gland by neoplasmic tumour. This does not necessarily cause hypothyroidism but suppresses TSH secretion and causes atrophy of the remaining follicles (Branam *et al.* 1982; Chandler *et al.* 1994).

6. *Iodine deficiency* will also cause hypothyroidism, as the thyroid hormones are dependent on iodine for their formation. Excess iodide causes inhibition of iodide binding to thyroglobulin. Iodine deficiency is now rare, due to the iodide salts that are added to dog food (Chandler *et al.*

1994). Goitre is a condition where the thyroid gland becomes enlarged in an attempt to compensate for iodine deficiency. Goitre results when the deficiency causes the thyroid to produce T3 instead of T4 due to feedback mechanisms and an increased secretion of TSH (West 1994, Chandler *et al.* 1994). Iodine deficiency will therefore generally lead to reduced T4:T3 ratio and increased TSH (Marshall 1996).

7. *Thyroiditis* is thyroid inflammatory disease and can be acute or chronic; however it is usually chronic in the dog (Hutchinson 1990). It has been detected in several dogs with no other signs of thyroid dysfunction. A familial chronic lymphocytic thyroiditis has been noted in some colonies of laboratory beagles, but these dogs do not develop clinical hypothyroidism or thyroid enlargement (Chastain and Ganjam 1986).

8. *Autoimmune thyroiditis* occurs when thyroglobulin autoantibodies (TgAA), and/or triiodothyronine autoantibodies (T3AA) and/or thyroxine autoantibodies (T4AA) become active against the host's own cells (Refsal and Nachreiner 1997; 1.4.4). Specific T4AA and T3AA antibodies will bind to T3 and T4 and lymphocytic infiltration of the tissue will also occur (Nemeroff 1989). Approximately half the naturally occurring cases of hypothyroidism in dogs are due to lymphocytic thyroiditis as a result of a genetic predisposition and environmental triggers (Chandler *et al.* 1994; Dixon *et al.* 1999c). It is not known exactly what starts an autoimmune disease. Some other factors cause the thyroid cells start to act as antigen presenting cells which results in the destruction of the thyroid gland itself (Ferguson 1991). Whether antibodies are the cause or effect of the disease is unclear (Ferguson 1991). Autoimmune thyroiditis can be linked to polyglandular autoimmunity, which in dogs, has also been suggested to present with aberrant behaviour patterns (Dodds 1997). Measures of autoantibodies tell us about the disease states but are not very effective for routine testing and diagnosis of hypothyroidism in the dog (Williams 1996). Primary hypothyroidism and primary adrenocortical deficiency in the dog are the same as type II polyglandular autoimmunity or Schmidt's Syndrome in humans. It has become increasingly evident that this and other combinations of endocrine end-organ failures are the result of immunological dysfunction (Kooistra *et al.* 1995). Dixon *et al.* (1999) showed that 5% of hypothyroid dogs also had diabetes mellitus and this may be consistent with the immune destruction of both endocrine glands.

9. *Subclinical hypothyroidism* in humans is most commonly caused by autoimmune thyroiditis (Joffe and Sokolov 1994). Subclinical hypothyroidism does not have the symptoms associated with the clinical form and is detectable only from laboratory findings (Hendrick *et al.* 1998; Esposito *et al.* 1997 Extein and Gold 1987). However it can lead to cognitive dysfunction (Haggerty *et al.* 1990).

1.5.2 The symptoms of hypothyroidism

Hypothyroidism can be difficult to diagnose (Happ 1995) as the signs are diverse and, therefore, can be similar to those of other diseases; they also vary from patient to patient (McDonald and Pineda 1989). Typical clinical signs of hypothyroidism include obesity (not always), lethargy and behavioural changes as well as bilateral symmetrical alopecia which is non-puritic, i.e., the dog does not scratch. In this case the hairs die in the follicles and fall out in the areas of greatest friction, e.g., the tail, leading to conditions such as 'rats' tail' (Chandler *et al.* 1994) and 'carpet coat' (Turner 1994). There are also dermatological abnormalities (Jeffers 1990; Rosychuck 1997). Melanin is deposited and the follicles become plugged with keratin, which causes the skin to feel rough and flaky. Myxoedema, puffy thickened skin, produces folds of skin on the neck and forehead (Ramsey 1997). There is also often muscle wastage, particularly the gluteal and shoulder muscles, and dogs show intolerance to the cold. Low plasma thyroid hormones may also cause constipation; mild anaemia, lack of libido, abnormal lactation, infertility and the absence of an oestrous cycle. They may also suffer some pain due to impaired joint function. In severe cases corneal ulceration (dry eye), diarrhoea and/or vomiting occur, bradycardia, muscle atrophy and peripheral neuropathies may also be evident. Occasionally, there is a low body temperature and myxoedematous coma (Chandler *et al.* 1994).

Behavioural abnormalities which are symptoms of hypothyroidism (Dixon *et al.* 1999c) include lethargy (Dunn 1989), mental dullness, impassive behaviour and reduced ability to exercise as a result of muscle wastage (Pearson and McGinn 1998; Chandler *et al.* 1994). Other clinical signs can include an abnormal gait (treading softly) due to excess wear of the dorsal anterior surface of the toenails on the front feet, and a change in voice as the vocal chords thicken (West 1994).

With respect to behaviour disorders associated with hypothyroidism, case histories indicate a gradual onset of an irritable or "crabby" disposition, with resentment to handling or attention in which, "the pet may not want to share the sofa; it may bite if the owner walks through the door at the same time" which suggests that confident aggression is more likely to be associated with hypothyroidism (Gerzovich 1995; Section 1.3.4). Both dogs and cats have been presented with aggression as the only symptom of hypothyroidism (Beaver 1994; Dodman and Shuster 1998). Dodman (1995) and Dodds (1992) discuss a decrease in canine aberrant behaviour following hormone replacement for hypothyroidism. However, McDonald and Pineda (1989) describe the effected animal as "dull" and "less aggressive" than normal. As hypothyroidism is a multisystemic disease, treatment with replacement thyroid hormones will affect several feedback mechanisms, which in turn may affect behaviour.

1.5.3 The signalment of hypothyroidism in dogs

The signalment refers to the age, breed and sex of dogs most commonly diagnosed with hypothyroidism.

Age: Hypothyroidism occurs most often in dogs aged six to ten years (Turner 1994; Eckersall and Williams 1983; Dixon 2001; Kaelin *et al.* 1989) and is unlikely to occur in dogs less than two years of age (Dixon 2001; Reinhard 1978; Dodman and Shuster 1998). Refsal and Nachreiner (1997) observed that autoantibodies are more prevalent in young to middle-aged dogs and T4 titres were found to decrease with increasing age (Wolford *et al.* 1988). Neonatal dogs (up to 100 days of age) have higher concentrations and old dogs have lower concentrations of T4 than do healthy adults.

Breed: Large breeds aged three to six years and small breeds aged six to nine years are most commonly affected (Dixon *et al.* 1999c, Simko 1992). McDonald and Pineda (1989) stated that there are marked differences in the thyroid morphology and function between breeds of European and African origin. There is also a higher prevalence for hypothyroidism in medium to large breeds (Reinhard 1978; Dodman and Shuster 1998; Turner 1994; Dixon 2001) giant breeds (Eckersall and Williams 1983), and large to giant breeds (Tuckova *et al.* 1995). Breeds that have been found to be at high risk were Golden Retrievers (Nelson and Ihle 1987; Milne and Howard 1981; Panciera 1994), Doberman Pinschers (Nesbitt *et al.* 1980; Nelson and Ihle 1987; Milne and Howard 1981), Beagles, Samoyeds, Huskies and Malamutes (Blake and Lapinski 1980). The American Kennel Club has described hypothyroidism as a primary concern, and the predisposition to develop the disease appears to be an inheritable trait (Dodman and Shuster 1998). Larsson (1986) suggests heredity to be of great importance in the development of primary hypothyroidism; Haines *et al.* (1984) noted a familial tendency in a group of Great Danes, and Conaway *et al.* (1985) confirm familial occurrence of lymphocytic thyroiditis in a group of related Borzoi dogs.

Sex: The majority of incidence studies of hypothyroidism show no evidence that either sex is at greater risk of developing hypothyroidism (Eckersall and Williams 1983; Jaggy *et al.* 1994) neither is there a difference with neuter status (Dixon *et al.* 1999c; Dixon 2001). However, Haines *et al.* (1984) noticed that hypothyroidism was predominant in female dogs, and spayed females had an even greater risk of developing the disease.

1.5.4 The treatment of hypothyroidism

Synthetic levothyroxine (L-thyroxine) is the preferred treatment for virtually all cases of hypothyroidism (Panciera 1997). There are five major types of preparations used (Siegel 1977). These are desiccated thyroid preparations from cows, thyroglobulin, sodium levothyroxine (T4), sodium liothyronine (T3), and preparations with a combination of T3 and T4. Historically,

preparations made from extractions of animals' thyroid gland were used for the treatment of hypothyroidism, but due to the spread of Bovine Spongiform Encephalopathy (BSE) the use of desiccated thyroid from cows is no longer practiced (Ramsey and Herrtage 1997). Administration of exogenous L-thyroxine will result in an increase in plasma thyroxine (T4), an increase in T3 (via deiodination of T4) and a decrease in thyrotrophin (TSH) through negative feedback inhibition of the pituitary gland. Treatments of thyroid conditions are generally long-term due to the irreversible nature of the disease.

1.5.5 Factors affecting thyroid function

Dehydration and fasting: The ratio of free: bound thyroid hormone in the blood can be affected by dehydration and fasting. An increase in unsaturated binding sites on the thyroxine binding proteins and a decrease in free thyroxine in the circulation is common. Effects of starvation in dog and man are the same; rT3 is increased (De Brunjne *et al.* 1981).

Pregnancy and the oestrous cycle: Result in a change in the oestrogen concentration and fluctuations in thyroxine and thyroid binding globulin synthesis (Chastain and Ganjam 1986).

Inherent rhythms: These are believed by some authors to affect the thyroid gland. Chastain and Ganjam (1986) note that there is no evidence of a circadian rhythmic secretion of thyroid hormones in dogs, but Hutchinson (1990) describes the time of day as having an effect on thyroid hormone concentrations. Drazner (1987) holds that although these rhythms exist they have no effect on thyroid function, and Bruner *et al.* (1998) notes that whatever time of day a blood sample is taken from a dog there is no affect on thyroid hormone measures.

Illness: Disease and malnutrition are associated with a lower extrathyriodal conversion of T4 to T3 or rT3, as the animal is stressed and the release of cortisol inhibits the production of active thyroid hormones. The reduction in T3 conserves the body protein during illness and is called Euthyroid Sick Syndrome. Non-thyroidal illness (NTI) results in elevated rT3 in the plasma and a decreased conversion of T4 to T3 (Kaplan *et al.* 1977; Ferguson 1984; Kaptein 1988). In addition to NTI, short and long-term glucocorticoid treatment decreases the serum thyroxine binding protein to levels below the normal range (Duick *et al.* 1974), which in dogs can suppress T4 (Kemppainen and Behrend 1998).

Glucocorticoid concentrations: Little work has been done on the effects of stress on thyroid function in the dog, therefore where appropriate the human medical literature is reviewed. Pharmacological as well as physiological doses of glucocorticoids may reduce TSH secretion both in humans and animals.

Pharmacological doses of glucocorticoids have a suppressive influence on the endogenous secretion of TSH (Brabant *et al.* 1989) but the mechanism is unclear (Brabant *et al.* 1987). Dexamethasone (an oral glucocorticoid) causes a decrease in T4, T3 and TSH and an increase in rT3 (Mitsuma and Nogimori 1982; Laurberg and Boye 1984; Brabant *et al.* 1987). Glucocorticoids also decrease the available amount of thyroid-binding globulin in the plasma (Gambert 1996).

Physiological doses of glucocorticoids are produced during chronic stress, influencing the HPT axis (Esposito *et al.* 1997), but the reaction of the endocrine system is likely to be dependant on the duration and type of the stressor. The primary action of the glucocorticoids is to decrease T4 to T3 conversion, and decrease the rate of rT3 breakdown (Chastain 1990). The rise in rT3 correlates with the fall in corticosteroid binding globulin during times of stress and sickness (Burr *et al.* 1976; Azukizawa *et al.* 1979; Gamstedt *et al.* 1979; Cavalieri and Pitt-Rivers 1981; Feldman 1987 cited in Beale *et al.* 1990; Bartalena *et al.* 1990; Kemppainen and Behrend 1998) and has been found to be higher in depressed patients (Zach and Ackerman 1988; Bauer and Whybrow 1988). Animal studies have shown that antidepressants affect deiodinase activity, which could be due to stress (Baumgartner *et al.* 1992) or due to the Euthyroid Sick Syndrome (Besser and Thorner 2002).

The main cause of the increase in rT3 in the event of increased pharmacological as well as physiological levels of glucocorticoids is due to the inhibition of peripheral 5'monodeiodination which converts T4 to T3 in dogs (Silva and Leonard 1985; Ferguson 1988; Kaptein 1988; Benker *et al.* 1990). A separate 5'-deiodinase catalyses conversion of T4 to rT3 (Wartofsky and Burman 1982). Hedge *et al.* (1987) states that the activity of 5'-monodeiodinase falls as does rT3 clearance, but the reaction rate is dependent on T4 concentration (Kaplan *et al.* 1977).

1.6 Physiological mechanisms associating the stress hormones and hypothyroidism

The mechanism by which reduced thyroid function influences canine behaviour is uncertain (Aronson and Dodman 1997). Dodman and Shuster (1998) also state that the mechanisms by which low thyroid levels are associated with aggression are unclear, but could be due to serotonin sensitivity to dopamine (which has received little research in dogs and therefore has been reviewed in Section 1.3.3), the rate of cortisol clearance causing stress, and/or the inhibitory effect of elevated glucocorticoids on TSH, which in animals exerts behavioural effects (Loosen and Prange 1982). These latter issues shall be considered further.

Activation of the HPA axis is a vital part of the physiological response to stress that can, in turn, affect basal hormone concentrations (Dodman *et al.* 1995). Both natural and synthetic glucocorticoids inhibit various parameters of thyroid function in both man and laboratory animals (Wilber and Utiger

1969) and in general, most stressful situations tend to decrease the release of thyroid hormones from the gland (Wartofsky and Burman 1982). Hyperactivity of the HPA axis occurs in depression (Extein and Gold 1987; Lesser *et al.* 1987; Nomura 1994; Dorn *et al.* 1996; Lasser and Baldessarini 1997; Leo *et al.* 1997; Stein and Avni 1988; Denicoff *et al.* 1990; Sauvage *et al.* 1998), and the adrenocorticotrophic hormones influence the secretion of hormones of other endocrine systems. This then influences physiological processes and behavioural patterns. Under conditions of physical or emotional stress corticosteroid levels are increased to promote the release of glucose from non-carbohydrate precursors to provide a source of energy for muscular activity. Corticosteroids also appear to have a modulatory effect on the immune system (Hart 1985; Maier *et al.*1994). Even if the stressful encounter were to have periods of respite, it should be adaptive to continue the anti-inflammatory response of stress-induced immunosuppression. Cortisol is the principal endogenous glucocorticoid of the canine adrenal cortex and acts as a negative feedback regulator in the HPA axis to reduce ACTH.

There are several mechanisms by which glucocorticoids lower baseline levels of the principal thyroid hormones. Glucocorticoids inhibit TSH release in response to TRH (Otsuki *et al.* 1973; Section 1.6.1;), decrease the amount of conversion of T4 to T3 (Chopra *et al* 1975; Section 1.6.2) and can have some direct effect on the gland itself (Section 1.6.2; Kemppainen *et al.*1983).

1.6.1 Glucocorticoids and the hypothalamic pituitary thyroidal axis

Glucocorticoids can suppress thyroid function by inhibiting the secretion of TRH from the hypothalamus, thus reducing TSH concentration (Ferguson 1984; Sowers *et al.* 1977; Hess and Ward 1998). This is also evident in patients receiving glucocorticoids for long periods or in high doses (Re *et al.* 1976) and chronic pharmacological treatment with glucocorticoids may have a suppressive effect on TRH (Woolf *et al.* 1973). In the dog, glucocorticoids decrease the basal TSH response to TRH (Woltz *et al.*1983). It can been concluded therefore, that large doses of glucocorticoids inhibit TSH secretion from the anterior pituitary (Corticogenic Hypothyroidism; Otsuki *et al.*1973). A reduction in TSH secretion is also seen during periods of stress in both humans and animals (Wartofsky 1974; Pamenter and Hedge 1980). Rubello *et al.* (1992) found a highly significant inverse correlation between urinary cortisol levels and TSH response to TRH, indicating inhibition at the pituitary. Morley (1981) also suggested that cortisol appears to have a direct inhibitory effect on TSH secretion from the pituitary.

In humans, the data suggests that high physiological levels of cortisol may suppress the TSH response to TRH (Loosen *et al.*1978; Dam *et al.*1986) as numerous psychiatric populations demonstrate blunted TSH response to TRH (Bunnevicius *et al.*1994; Gillette *et al.*1997), and glucocorticoids decrease TRH in normal and hypothyroid patients (Faglia *et al.*1973). Dexamethasone (oral

glucocorticoids) affects either the release of TRH from the hypothalamic cells or the release of TSH (Haigler *et al.* 1971) or both (Ranta 1974; Mitsuma and Nogimori. 1982), and also decreases plasma thyroid hormone concentrations (Duick *et al.* 1974). However, Dussault (1974) suggests that dexamethasone has no effect when administered to hypothyroid patients.

1.6.2 Glucocorticoids and thyroid hormone conversion

Glucocorticoids have also been suggested to decrease thyroid hormone concentration by causing TSH deficiency, changes in thyroid hormone binding in the plasma, and alterations in peripheral metabolism of hormones. These mechanisms may be operative in both humans and dogs. However, as the concentrations of binding proteins, binding affinity and peripheral metabolism differ in dogs and humans it should not be assumed that the mechanisms are identical (Peterson *et al.* 1984). Kemppainen (1984) has described the inhibition of peripheral conversion of T4 to T3 in the dog, and Chopra *et al.* (1975) found that in rats glucocorticoids reduce the overall peripheral deiodination of T4 to T3.

In addition, administered corticosteroids such as dexamethasone have been found to cause a reduction in the peripheral conversion of T4 to T3 in dogs and humans (De Groot and Hoye 1976; Woltz *et al.* 1983; Kemppainen 1984; Moriello *et al.* 1987; Flow and Jaques 1997; Dluhy 1996). Serum T4 and T3 titres are significantly decreased in dogs with hyperadrenocorticism (Peterson *et al.* 1984). This suppressive effect of the glucocorticoids on the conversion of T4 to T3 may act as a protective or adaptive cellular response, which can be associated with acute or chronic states of glucocorticoid excess. As well as causing in a decrease in plasma T3 high levels of glucocorticoids also result in an increase in rT3 (Gamstedt *et al.* 1979).

1.6.3 Factors influencing the thyroid gland itself

Glucocorticoids can also have an effect on the thyroid gland as a result of physical and/or mental stress (Kemppainen 1984). One mechanism by which chronic stress potentially influences thyroid function is through its modulatory effect on the immune system (Maier and Watkins 1999). The immune system is capable of producing products that can signal to the CNS, via the vagus nerve (Carlson 1998), altering neural activity and thereby behaviour. The immune response communicates with the brain in order to initiate the stress response. Stimulation of the immune cells is important in the development of autoimmune thyroiditis. Therefore the stress hormones may contribute to the occurrence of autoimmune thyroiditis in dogs with a predisposition for the disease (Section 1.5.1).

Kumar *et al.* (1968) observed that glucocorticoids such as prednisolone cause a decrease in thyroid binding globulin (TBG; also noted by Dluhy 1996), but Oppenheimer and Werner (1966) found no evidence that glucocorticoids have an effect on the binding capacities of thyroxine binding proteins. In the dog however, Woltz *et al.* (1983) noted that glucocorticoids affect TBG and increase rT3 by

inhibiting 5'-deiodinase. The glucocorticoids are also known to inhibit iodine uptake by the thyroid gland (Ingbar and Freinkel 1955; Re *et al.* 1976; Dluhy 1996).

1.7 Hypothesised relationship associating canine behaviour and hypothyroidism

The first description of hypothyroidism associated with depression in humans appeared in 1888 (Pitts and Guze 1961). In 1951 Ham *et al.* recorded that significant external events produce a chronic emotional response, which via the cortico-thalamic pituitary axis, leads to changes in thyroid function. Later, Chrousos and Gold (1992) reported the association of stress with reduced thyroid hormone production, and speculated that the mechanism is probably due to the actions of the glucocorticoids. Aggressive behaviour is associated with hypothyroidism in humans (Section 1.4.4) and aggressive behaviour can be regarded as a response to a stressor (Barash 1982). Glucocorticoids are produced as a result of the stress response mediated by the General Adaptation Syndrome (GAS; Selye 1932) as an individual prepares for 'fight or flight' action (Cannon 1927) glucocorticoids are produced which cause a decrease in thyroid hormone concentration (Section 1.5.5). Wilson *et al.* (1983) in a study on Beagle dogs describe a method appropriate for demonstrating thyroid responses to changes in stress, and since minor changes in thyroid homeostasis may lead to major changes in metabolism and behaviour (Whybrow 1996) in humans, it is feasible that an association between stress glucocorticoids and thyroid hormone may be operative in other mammals. In 1972 Leppaluoto described a change in thyroid function which may be induced by stress in the rabbit (*Oryctolagus cuniculus*) and Gregerman and Davis (1996 p.233) noted, *"in a number of extensive studies in rabbits, rats and guinea pigs, various stresses were found to produce decreased thyroid function as a result of decreased TSH release."*

Endocrine dysfunctions are one of the main causes of abnormal behaviour in the dog (Owren and Matre 1994), and veterinarians and animal owners frequently use changes in behaviour as an indicator of sickness and disease (Hart 1985). Aggression is one such non-specific sign of various pathological conditions (Ettinger 1989). However, glucocorticoids increase under conditions where aggression occurs which is related to fear and anger (Blauvelt 1964), and these can affect thyroid function.

Therefore, considering all the evidence relating to stress, thyroid function and behaviour, it is possible to suggest an alternative hypothesis to that previously suggested, where thyroid hormones control behaviour directly. In this alternative hypothesis behaviour could be affected by a factor that also influences thyroid titre, i.e., the glucocorticoids. In other words, it is the individual's perception of its environment as stressful causes stimulation of the HPA axis and a change in the individual's behaviour. This in turn has an inhibitory affect on thyroid hormone concentration.

The association between environmental stimuli and increased stress hormones is well established in humans and dogs, but the association between environmental stressors and thyroid function is less well understood. Nevertheless, on the basis of the physiological association between the thyroid hormones and the glucocorticoids as well as the association between stressors and the HPA axis, I hypothesise that in the dog the environmental stimuli that are experienced as stressful will cause production of cortisol, which in turn inhibits thyroid hormone production. Additionally, the perception of the stressor also causes behavioural change, and specifically, behavioural disorders such as inappropriate aggression (Figure 1.5).

Additionally, this hypothesis predicts that aggression may not be the only behaviour problem associated with hypothyroidism, as the expression of behaviour is determined by genetic make up and other physiological factors as well as previous learning experiences. For example Hamilton-Andrews (1998) found an association with noise sensitivity. My initial aim is to examine the incidence of different types of behaviour disorders and their consistent behaviour patterns, and how these are related to thyroid function and physiological indicators of stress.

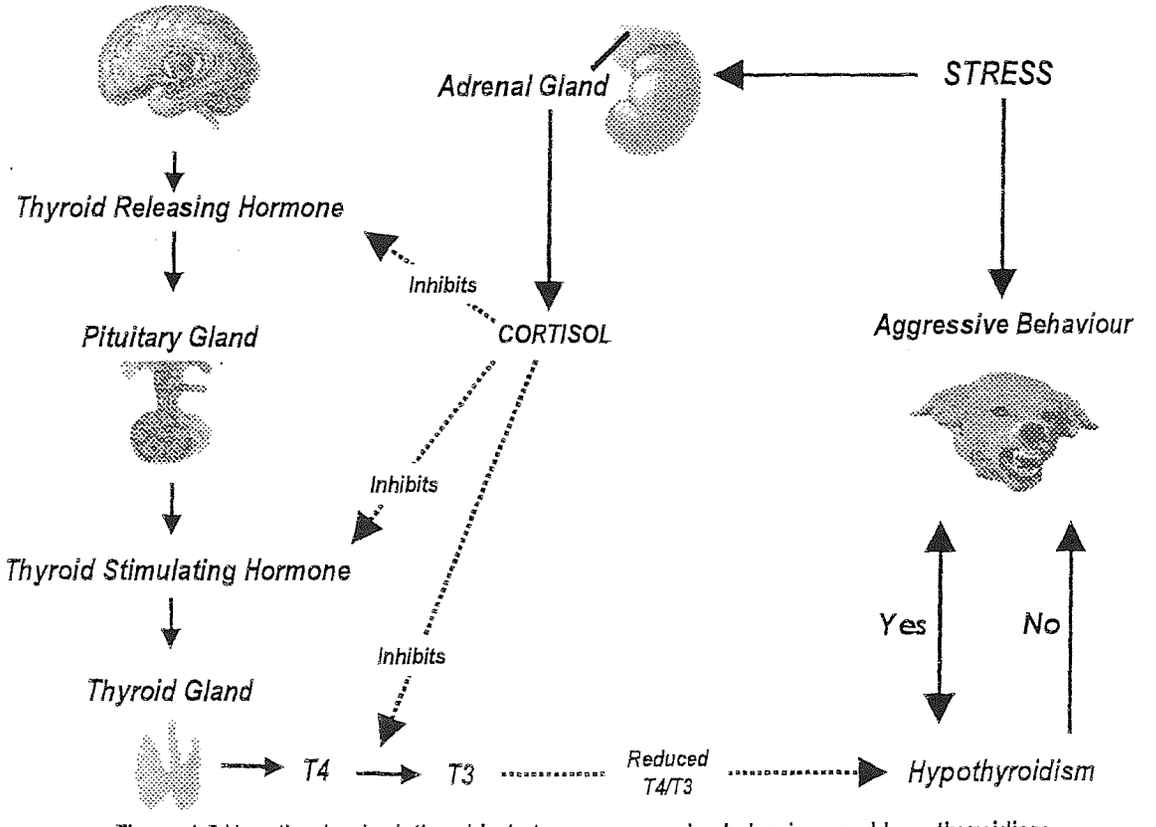

Figure 1.5 Hypothesised relationship between aggressive behaviour and hypothyroidism

1.8 Welfare implications of canine hypothyroidism

Welfare has been defined as encompassing all aspects of animal well-being including disease prevention, treatment and responsible care (Appleby 1999), but a more succinct definition is, "*the welfare of an animal is its state as it regards itself to cope with its environment*" (Broom 1986 p524), which can be measured by physiological (Baldock and Silby 1990; Wartofsky and Burman 1982) as well as behavioural correlates of the stress response.

I predict that situations which induce fear, or in which established rules are suddenly changed, or in which behaviour that was previously effective no longer accomplishes its goal are likely to result in an increased production of glucocorticoids (which reduce thyroid hormone concentrations) as well as changes in behaviour (possibly perceived as a behaviour disorder). Such stress-induced disorders are, by definition, decrements in welfare.

As most common signs of hypothyroidism in both humans and dogs are metabolic and behavioural changes, having reached a diagnosis we should assume that dogs also experience emotional components similar to those experienced by humans with hypothyroidism. This will draw attention to any undue suffering related to the onset and development of hypothyroidism such as those related to mental state, that dogs may have difficulty in conveying, and may ultimately lead to ways in which psychological as well as physiological effects of hypothyroidism can be associated.

However, some clinicians have noted the simultaneous onset of behavioural change (principally aggression) with hypothyroidism and have found that the use of thyroid replacement therapy was effective in treating both hypothyroidism and the aggressive behaviour (Dodds 1996 and Dodman 1995). However, the administration of thyroid replacement therapy without confirmation of hypothyroidism (by diagnostic tests) can have a harmful effect on the welfare of dog, as in cases where dogs have a behaviour disorder and are not hypothyroid such inappropriate treatment can increase catabolism, accentuate cardiac dysfunction and potentially increase morbidity and mortality (Kaptein 1988).

Additionally, if behaviour modification results in reduced stress (Moberg 2000), for those physiologically hypothyroid (non-pathological hypothyroidism; Wartofsky and Burman 1982) dogs treatment with thyroid replacement therapy would not be required because the condition reverses (Ferguson 1988; Ferguson 1984; Peterson *et al.* 1984) when the elevated glucocorticoids are reduced.

1.9 Synopsis, aims and objectives

1.9.1 Synopsis

Hypothyroidism occurs in both humans and dogs and presents similar diagnostic symptoms in both species. While the association between mental state and hypothyroidism is well established in humans, very little is known about the mechanism by which these are associated, although it would appear likely that the glucocorticoids which inhibit the functioning of the HPT axis, or have a inhibitory effect on the pituitary gland, are involved. Little is known about the relationship between hypothyroidism and canine behaviour. This may be due to the difficulty of collecting accurate life history information for dogs, as any current dog behaviour is likely to be influenced by past experience as well as current physiological factors.

I hypothesise that the behavioural response to environmental stimuli affects the HPA axis and this in turn affects the measurable plasma thyroid hormone concentration. So, rather than the concentration of thyroid hormones controlling the behaviour of the individual, the behaviour could be a reflection of stress, resulting in the production of glucocorticoids which cause a decrease in thyroid titre. By establishing the mechanism by which thyroid hormones and dog behaviour are related, a greater understanding of this disease can be obtained and the results can then be applied to improving the welfare of Britain's most popular urban pet, the dog.

1.9.2 Aims

This project will attempt to explore the relationship between behaviour disorders and hypothyroidism in the domestic dog, *Canis familiaris* Linnaeus (Mammalia; Canidae), making comparisons with the links between thyroid function and emotion. In this thesis I test the general hypothesis that there is a link between hypothyroidism in the dog, and aggression and other behavioural disorders.

1.9.3 Objectives

The following objectives will be considered in this thesis:

(i) To determine whether aggressive behaviour is related to altered thyroid state.

(ii) To explore the relationship between stress, thyroid hormones and dog behaviour.

(iii) To assess the association between dog behaviour and hypothyroidism by

a) Investigating the incidence of hypothyroidism in dogs with behaviour disorders,

b) Determining what behaviour disorders / patterns are related to altered thyroid state and

c) Examining the types of behavioural abnormalities in dogs with different types of hypothyroidism.

Chapter 2

General Methods

2.1 Introduction

The investigation of the relationship between altered thyroid function and behaviour patterns required the use of both ethological and endocrinological methods. Due to the recurring use of several methods, this chapter contains the entire methodology utilised in this research project.

Ethology and psychology are the originating fields from which methods are derived for the study of animal behaviour (Martin and Bateson 1993); in this study, behavioural data was gathered both indirectly, through questionnaires completed by owners, and directly, through the observation of animal behaviour patterns either from direct observation or from video recordings (Section 2.4).

Several endocrinological methods were used. For the determination of hormone titre, plasma samples were collected. Radioimmunoassay was the preferred method for the measurement of T4, T3, TSH, rT3 and plasma cortisol, whilst ELISA was used for the measurement of TgAA. The technical version of each method is presented (Section 2.5) as supplied by the manufacturer of the reagents.

Both exploratory and confirmatory statistical analysis of the behavioural and hormonal data was required. As the physiological data was normally distributed and the behavioural data was not normally distributed, both parametric and non-parametric statistical tests were used. The relevance, methodology and application of each test are discussed (Section 2.6).

2.2 Canine ethogram

2.2.1 Normal canine behaviour patterns

The categorisation of normal dog behaviour in the literature is inconsistent; therefore I have considered normal dog behaviour patterns as those that appear to be functional and not indicative of illness or fatigue. In order to draw an ethogram of normal dog behaviour patterns I conducted 6 hours *ad libitum* sampling per dog with intermittent continuous bouts of observation on five different dogs in their own homes (in and around Southeast England; Table 2.1) and a total of five hours *ad libitum* sampling with intermittent continuous bouts of observation of 30 dogs in a local park (Southampton Common, Southampton, UK, 50.486N 1.178W; Table 2.2). No quantitative measures were made; I simply noted the behaviour patterns exhibited by dogs.

Table 2.1 Dog signalment information for dogs observed in their home environment (n=5).

Breed	Sex	Age (years)
1. German Shepherd x Husky	Entire male	10
2. Labrador x Collie	Neutered Male	9
3. Rottweiler x Doberman	Entire Female	4
4. Jack Russell Terrier	Entire male	13
5. German Shepherd x Labrador	Neutered Female	3

Table 2.2 Signalment of dog breeds observed in Southampton Common (n=30), *sex is not divided into neuter status as it was not always possible to determine from observation.

Breed Group	No. of dogs	*Sex	No. of dogs	Age (years)	No. of dogs
Working	7	Male	14	0–18 months	6
Toy	3	Female	16	19 months–6 years	11
Gun	10			>6 years	13
Terrier	5				
Cross	5				

2.2.2 Inappropriate canine behaviour patterns

Inappropriate behaviour can be considered as containing behaviour patterns that are the result of inappropriate associative learning, operant reinforcement, caused by a physiological or pathological change and not generally part of a dogs usual behavioural repertoire. However, I additionally considered normal canine behaviour patterns to include any normal behaviour which was inappropriately placed as inappropriate behaviour, as these dogs are often considered by their owners to have behaviour disorders. In order to observe these behaviours it was necessary to include inappropriate dog behaviour patterns in the ethogram. Therefore I conducted a total of five hours *ad libitum* observation (one hour per dog) in intermittent continuous bouts of five dogs considered by their owners to have [1]behaviour disorders in their own homes (in and around Southeast England Table 2.3). Additionally, I conducted five hours *ad libitum* observation (one hour per dog) of 30 different dogs at an animal shelter (Blue Cross Animal Shelter, Burford, Oxfordshore, UK, 51.486N 1.378W; Table 2.4). In observing the behaviour of dogs in both animal shelters and during home visits I recorded both normal and inappropriate behaviour patterns. No quantitative measures were made; I simply noted the behaviour patterns exhibited by the dogs.

[1] I categorised the behaviour disorders listed in Table 2.2 based on the information given to me by the owners about the onset and development of the behaviour as well as direct observation of the dog's behaviour. I used the APBC (http:www.apbc.org.uk/) method of classifying behaviour disorders to categorise the behaviour disorders I observed.

Table 2.3 Dog signalment information for dogs observed in their home environment and considered to have a behaviour disorder (n=5).

Breed	Behaviour disorder	Sex	Age (years)
1. English Bull Terrier	Stereotypy	Entire male	3
2. Belgian Shepherd	Inappropriate aggression	Neutered Female	6
3. West Highland Terrier	Fear of people	Neutered Male	10
4. Greyhound	Separation related disorder	Entire male	8
5. Cross	Inappropriate aggression	Neutered Male	6

Table 2.4 Signalment of dog breeds observed at Burford Rescue Shelter (n=30), *sex is not divided into neuter status as this information was not always available.

Breed Group	No. of dogs	*Sex	No. of dogs	Age (years)	No. of dogs
Working	5	Male	19	0–18 months	5
Toy	1	Female	11	19 months–6 years	15
Gun	2			>6 years	10
Terrier	7				
Cross	15				

2.2.3 Construction of canine ethogram

The ethogram was constructed based on the preliminary observations made of dogs exhibiting both normal and inappropriate behaviour patterns (Table 2.5).

Table 2.5 Canine ethogram (although "Proximity" is not a behaviour pattern and therefore not usually included in an ethogram, it was included here as it was considered to be relevant in a clinical behaviour setting).

BEHAVIOUR		DESCRIPTION
Ear Position	Up	Tip of pinna pointing above head
	Down	Tip of pinna pointing toward the back of the head
Tail Position	Tucked	Tail located between the back legs
	Relaxed	Tail limp and hung loosely to the rear of dog
	Flag	Tail upright with the tip facing above the head or slightly curled at the end, may or may not be moving.
Tail Movement	Moving	Tail in motion in any direction
Head Posture	Up	Head raised up above the withers
	Level	Head in line with the withers
	Down	Head hung below the level of the withers
Body Posture	Stand	Body in a stationary upright position with weight distributed between four feet
	Sit	Body in an upright position with the dog's weight placed upon the joint of the hind leg between the knee and the fetlock.
	Lie	Body deposited ventrally or laterally on a surface
Movement	Walk	All gaits where at least one foot is in contact with the ground at any one time
	Run	Dog moves with speed and smooth motion
	Jump	Dog leaps from the ground with a sudden start, either front two, or all four feet leave the ground.
Proximity	Near Familiar	Dog orientates its head (nose) toward a familiar person, dog or toy.
	Near Unfamiliar	Dog orientates its head (nose) toward an unfamiliar person, dog or toy.
Vocalisations	Bark	Sharp explosive cry
	Growl	Guttural murmur of antagonism
	Whimper	Crying querulously or to whine softly, can be a high or soft pitched vocalisation.
Elimination	Urinate	Discharge of urine from body
	Defecate	Discharge of faeces from body

2.3 The Animal Behaviour Clinic

The Animal Behaviour Clinic (ABC) at the University of Southampton, Hampshire (50.486N 1.178W) was used for teaching and research as well as providing service to the general public. Every dog was referred to the clinic from veterinary surgeries situated in and around Hampshire. There were two behaviour counsellors at the clinic who saw clients either individually or together. Owners willing to participate in research projects were asked to give written authorisation for the consultation to be recorded and a blood sample to be taken from their dog.

2.3.1 The behaviour consultation

On arrival at the ABC the clients and their dogs were seated in the consultation room. The room used for the consultation was west facing, carpeted, and had neutral décor with large windows. The room measured 3.7 metres by 2.6 metres and contained soft furnishings, a table, and a water bowl (Figure 2.1). All items placed in the consultation room remained consistent for the duration of the study. The technical equipment used to film the consultation was discreet so that the owners did not feel that they were obviously being recorded (Figure 2.1). Four closed circuit television cameras (ESP Witness quad kit CCD 370) and boundary microphones (Electret BE90X) responding to 70-15kHz were used. All cameras and microphones were connected to a quad observation system (Elite security), which allowed four pictures to be displayed simultaneously (Figure 2.2). This equipment was tested for effectiveness during a pilot study. The recording equipment and observer were located in an adjacent room as in some cases dogs were being treated for aggression related disorders and this arrangement complied with health and safety regulations.

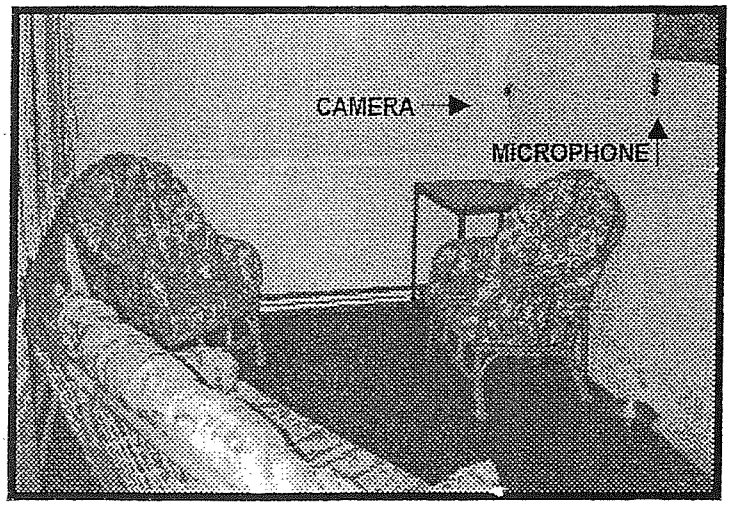

Figure 2.1 Consultation room at the ABC.

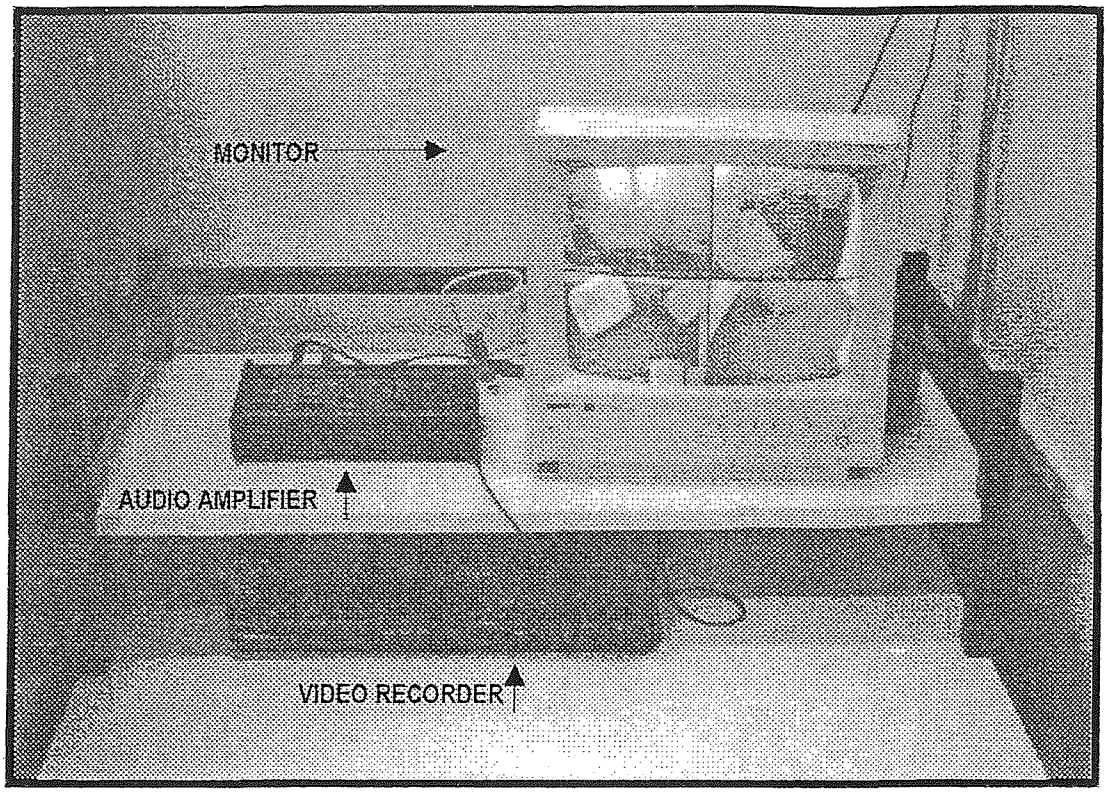

Figure 2.2 Quad observation system at the ABC.

Once the owners were seated, the dog was allowed off its lead and the owner(s) asked to ignore their dogs' behaviour. The consultation lasted for two hours; in the first hour the counsellor asked the owners about the history of the dog and the development of the disorder. The discussion centred on a questionnaire that had been previously completed by the owner (Section 2.4.1). The second hour was aimed at explaining to the owner why their dog behaved as it did and how best to treat the behaviour disorder using the principles of behaviour modification (Section 1.2.7). The behaviour consultation was sometimes subject to interruptions if the dog, owner or counsellor needed to leave the room or in the event of a fire drill. After the consultation a blood sample was taken by a veterinary surgeon in order to obtain physiological data to support the diagnosis (2.5.1). A proportion of each sample was made available for this research project, as permitted by the Code of Practice for Veterinary Surgeons (Anon. 2000). The treatment protocol was reinforced by a report, which was sent out two weeks post consultation, and if necessary followed up by telephone advice. The frequencies of behavioural disorders are shown in Figure 2.3.

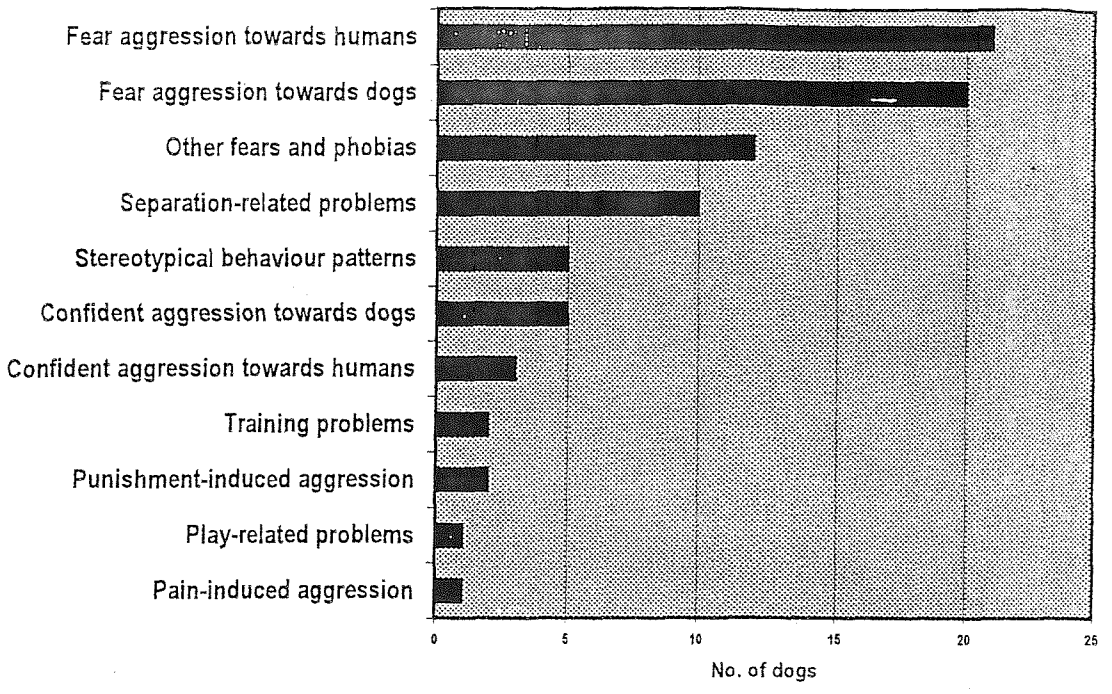

Figure 2.3 Distribution of behavioural disorders in the sample population of dogs seen at the ABC (n=82). The system of classification used was by Borchelth and Voith 1982, see page 47.

2.3.2 Recruitment and subjects

From 14 March 1999 to 15 December 2000 dogs were recruited from the ABC for use in the studies described in Chapters 3, 4 and 5. All the dogs attending the ABC were considered by their owners to have a behaviour problem for which they required help via a behaviour modification programme. Prior to attending the behaviour consultation the owners of all dogs aged at least 18 months were asked if they would be willing to participate in the research project. A minimum age of 18 months was selected, as clinical hypothyroidism is rare in dogs aged less than two years (Dixon 2001). The sample of dogs used in Chapters 6 and 7 were recruited separately to those recruited in Chapters 3,4 and 5. Therefore the recruitment and signalment of the samples used in Chapter 6 and 7 are introduced in Sections 6.2 and 7.2 respectively.

The dogs used in the studies comprising Chapters 3 and 5 (n=82), and Chapter 4 (n=81) was of mixed signalment. The breed groups represented were gun dog, hound, terrier, utility and working. All dogs that were not purebred were placed in a sixth group called 'mixed' shown in Figure 2.4. The sex distribution is shown in Figure 2.5.

Age

Dogs aged less than 18 months were not included in the study. Since in several cases the exact age of the dog was unknown ages were classified as 18 months-6 years (n=65) and 7 years or more (n=17).

Breed

Numerous breeds were used and in order to have a group of dogs large enough to enable statistical analysis, each dog was categorised into a breed groups according to the [2]UK Kennel Club. The most common breed group was the working group (n=27) and the least common was the hound group (n=3).

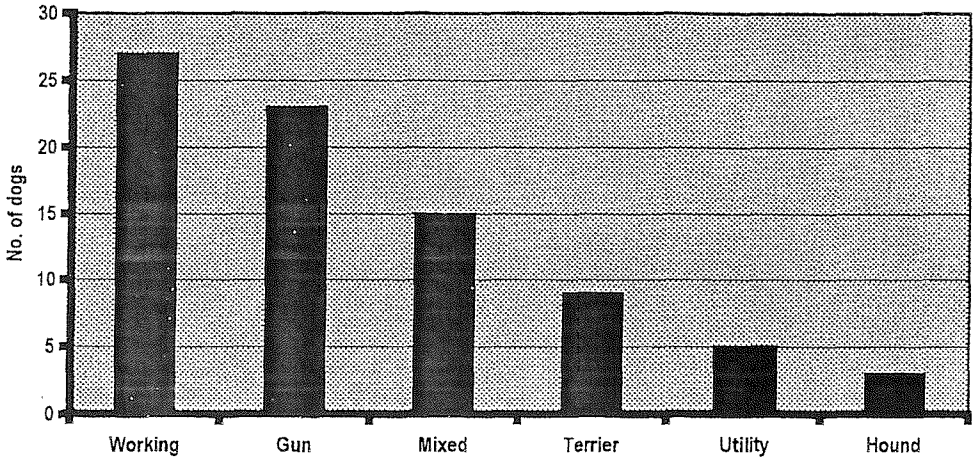

Figure 2.4 Distribution of breed groups (n=82)

Sex

The dogs were also categorised into male entire (n=9), male neutered (n=35); female entire (n=6) and female neutered (n=30). In two cases females could not be categorised, as it was unknown if they were neutered and their owners and veterinary surgeons were unaware whether they were having or previously had, oestrus cycles.

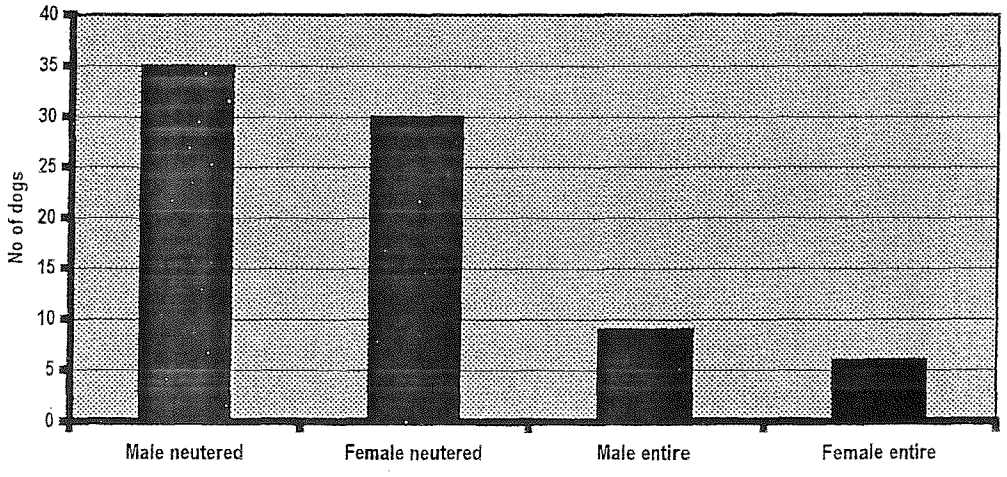

Figure 2.5 Distribution of sex and neuter status (n=80)

[2] The UK Kennel Club (http://www.the-kennel-club.org.uk/).

2.4 Behavioural data collection

Questionnaires were composed of closed as well as open-ended questions. With the exception of the counsellor progression questionnaire, which included answers on a Likert scale (2.4.3), the answers to all other questions were categorical. The questionnaires are described generally, as each questionnaire was used in different Chapters for various investigations.

2.4.1 Animal Behaviour Clinic questionnaires

The clinic questionnaire (Casey 1997, Unpublished behaviour questionnaire, included with the permission of author; Appendix 2) was compiled to collect information about the dog's behavioural disorder and was therefore completed by the owners prior to admission to the clinic. This process enabled the behaviour counsellor to have some information about the case and therefore comply with the necessary health and safety procedures prior to entrance to the clinic. The questionnaire was divided into sections entitled; background information (owners and dogs basic details), early history, diet, exercise, housing, training history, family members, medical history, the main problem, other problems and rehabilitation. The ABC questionnaire was also used to collect data for Chapters 5 and Chapter 7.

2.4.2 Owner Interaction questionnaire

An Owner Interaction questionnaire (Appendix 3) was completed by the owner of every dog, prior to attending the ABC for a behavioural consultation, based on a test procedure used by Rooney and Bradshaw (submitted to the *Journal of Applied Animal Welfare Science*) and was aimed at determining how the dog behaved when it was with a familiar person in a familiar environment. A grid format was used to ask the owner about the dog's actual response to their actions (Table 2.6). Information about the dog's behaviour was obtained, rather than the owner's subjective interpretation of their dog's behaviour, but as this technique relies on owners being able to accurately report their dog's behaviour, it was not possible to test for inter-observer reliability. For an entirely objective method I could have measured every dog's behaviour in a familiar environment; however, my presence might have influenced their behaviour and it would also have been impractical to devote enough hours to each dog to record its entire behavioural repertoire. This method was used to collect data for Chapter 6.

Table 2.6 Behaviour grid. The dogs' behavioural responses (top row) and the actions carried out by the owners (left hand column). The equivalent grid (Appendix 3) was adapted from a behavioural test developed by Rooney and Bradshaw (paper submitted to the *Journal of Applied Animal Welfare Science*).

When you:	Tail wag	Nuzzle	Growl	Bark	Whimper	Lick lips or you	Climb or jump up	Sit	Lay	Avoid gaze	Roll	Paw	Obey
Approach it	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pet it	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ignore it	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Return after leaving it	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Call it to you	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ask it to sit	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ask it to lie down	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Raise voice and point finger at it	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Take its food away	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Take its toy away	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Move it from its resting area	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lie on the floor with it	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Groom it	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

2.4.3 Assessment questionnaire

Assessment questionnaires were used in Chapter 6 to monitor the progress of behaviour modification at 6-week intervals for 12 weeks after the initial consultation. Owners were asked to complete the Owner Interaction questionnaire (Appendix 3) and a Symptoms of Hypothyroidism questionnaire (Appendix 4). This questionnaire was delivered verbally, by the visiting counsellor during follow up visits; the owner was asked if they had noticed the presence or absence of several behavioural and physiological symptoms indicative of hypothyroidism. In addition to this, the counsellor was asked to complete a Behaviour Modification questionnaire (Appendix 5) about how effective the programme was in altering their dog's behaviour patterns.

2.4.4 Hypothyroidism and behaviour questionnaire

The Hypothyroidism and Behaviour questionnaire (Appendix 6) was used to collect data on the behaviour patterns and behaviour disorders in dogs at different stages and states of hypothyroidism (Chapter 7). The questionnaire, which was completed by the dog's owner, was composed of the ABC questionnaire and an additional section that questioned the owner about the presence or absence of specific behavioural disorders. The study was conducted with the assistance of The College of Veterinary Medicine, Animal Health Diagnostic Laboratory, Michigan State University, USA, as they have access to a large population of dogs at different stages of hypothyroidism. The dog population, described in Chapter 7, was comprised of four groups with different categories of abnormal thyroid status as well as one euthyroid group.

2.4.5 Videotaped behaviour

The investigation of thyroid status and dog behaviour patterns (Chapter 3) required the quantification of dog behaviour from direct observation. From preliminary observations and a pilot study it was concluded that the measurement of dog behaviour was best achieved by the direct observation of recorded footage of the behaviour consultation. This enabled observation of spontaneous dog behaviour whilst in a novel environment. Preliminary observations suggested that the dogs could take up to 15 minutes to habituate to the new environment, and the 60-120 minute period often involved training-based interaction with the dog on the part of the behaviour counsellor, so the 15-45 minute period was regarded as the most useful. Instantaneous time sampling was used every 20 seconds of the 30 minute observation period to determine the behaviour of the [3]focal dog. Behaviour patterns as defined from the ethogram (Table 2.5) were noted on a check sheet (Appendix 7). The behaviour patterns were then analysed quantitatively (Section 2.6).

2.4.6 Diagnoses of disorders

Classification of canine behaviour disorders was required for use in Chapter 5. All behaviour disorders were classified by the ABC behaviour counsellors using a modified version of Borchelt and Voith's (1982) classification and the reliability of their diagnoses was tested (Chapter 5). Diagnosis was made from direct observation of the dog's behaviour and the information given by the owner during the consultation, as well as the information provided in the ABC questionnaire.

[3] In cases where there was more than one dog present the behaviour of the referred dog(s) was studied.

2.5 Hormonal data collection

Blood samples were routinely taken to screen dogs for other illnesses prior to behavioural treatment.

2.5.1 Collection of plasma sample

With a pair of blunt rounded scissors a small area of hair was clipped away from the dogs front right leg in the area of the cephalic vein. The exposed skin was then cleaned with surgical spirit. The blood sample was taken using a 5ml syringe and either a $21GA^{5/8}$ needle, for dogs measuring up to 60cm at the shoulder, or a $23GA^{5/8}$ needle for dogs measuring more than 60cm at the shoulder. The dog was held in the standard position for the collection of a blood sample from the front right leg. The procedure requires two people, a veterinary surgeon and an assistant (myself). The assistant bent down on one knee at the left side of the dog with her right arm placed round the body of the dog and her right hand holding up the front right leg of the dog. The thumb of the right hand drew the skin in order to expose the cephalic vein. The assistant's left arm was positioned around the dog's neck to hold the head in a position facing away from the vet who extracted a sample of blood.

The blood sampling procedure can be considered as stressful (Egwu 1990). Venipuncture in sheep, for example, causes an increase in plasma cortisol (Falconer 1976). All blood samples intended to be used for the determination of cortisol concentrations were collected within 3 minutes of handling the dog, based on evidence that in rodents glucocorticoid measures taken within this time frame were reflective of baseline stress state and not affected by the process of the handling procedure and venipuncture (Tuber *et al.* 1996; 4 minutes according to Hennessy *et al.* 2001). All dogs were muzzled as a precautionary measure and as change in dress can affect some subjects (Gay 1968), the style of clothing worn by the veterinary surgeon and assistant was the same as in the consultation, and neither white coats nor overalls were worn in case these triggered aversive behaviour learned during previous veterinary procedures.

The blood was then immediately transferred into a 10ml lithium heparin tube and a lid placed on top. The tube was gently manually rotated in order that the blood adhered to and mixed with the heparin coating on the side of the tube. The tube was then placed into an ultracentrifuge (Heraeus Sepateh Suprafuge 22) and spun at 3000rpm (1630*G*) for six minutes. With a disposable pipette, the plasma was separated from the red blood cells and equal amounts of the plasma were aliquoted into cryogenic vials. Sealed lids were placed on the vials, which were labelled with the date and the owners' name. The samples were frozen in an upright position at -20°C. Once frozen these samples were placed in labelled bags and stored for subsequent and appropriate bioassay. The plasma was frozen while in an upright position and repeated freeze/thaw cycles were avoided. Samples that were haemolysed, where

the integrity of the red blood cells was lost with the resulting release of haemoglobin, or grossly lipaemic, where excess fatty acid was present in the sample, were rejected.

2.5.2 Which hormones to measure and why?

Several authors have reviewed the advantages and disadvantages associated with different methods of measuring the thyroid hormones (Appendix 8). These methods can be specific measures of the thyroid gland or thyroid hormones, or, as hypothyroidism is a multisystemic disorder (Hutchinson 1990), indirect non-specific measures may also support a clinical diagnosis. Non-specific tests include radiology, electrocardiogram measures, haematology changes, biochemical changes, urine changes and skin biopsy, however, due to the subtle onset of the disease, specific tests of thyroid function are more accurate. Before I consider the specific methods for assessing thyroid function I will recap on the clinical significance of the thyroid hormones.

TRH is produced by the hypothalamus, which stimulates the pituitary gland to secrete TSH. TSH in turn acts on the thyroid gland to release predominantly T4, which is converted into T3. The release of TSH and TRH is controlled by negative feedback from T4 and T3. The circulating T4 is almost entirely bound to serum proteins and only a small amount is present as free T4, although it is widely considered that only the free fraction is biologically active (Chastain and Ganjan 1986). rT3 is formed from monodeiodination of T4 in the peripheral tissues and rT3 titres increase during periods of non-thyroidal illness (NTI) and in the presence of glucocorticoids.

Specific tests of thyroid function include the detection of low circulating T4 or T3, but as a number of factors other than inappropriate thyroid function can affect the plasma concentration of the thyroid hormones, specific tests for individual thyroid hormones are not always the best indicator of thyroid damage. The TSH stimulation test has been recommended as a more effective way of diagnosing hypothyroidism (Eckersall and Williams 1983; Heripret 1997; Ferguson 1991), in which basal serum T4 is measured before and after TSH stimulation of the thyroid gland. However, due to a shortage of bovine TSH (Ferguson 1994; Eckersall and Williams 1983) and the high cost of the pituitary hormone (Eckersall & Williams 1983) the TSH stimulation test is not always feasible. In addition the TSH simulation test may give a normal result in the early stages of autoimmune thyroiditis (Johnstone 1988).

Before hypothyroidism can be detected 75% of thyroid follicular tissue must be destroyed (Ferguson 1991). The onset of autoimmune thyroiditis is subtle whereby the destruction of thyroid tissue takes up to several years to occur and clinical symptoms only occur once thyroid glandular reserves are depleted (Dodds 1997). Therefore, it is possible to test for the presence of thyroglobulin antibodies (TgAA) and/ or to antibodies to the thyroid hormones (T4AA and T3AA). However, the presence of these antibodies does not definitely diagnose hypothyroidism (Merchant and Taboada 1997) as

thyroglobulin leakage from the thyroid gland is a normal occurrence and increases the tendency for normal animals to develop TgAA and T3AA (Ferguson 1991).

T4 and TSH concentrations are useful for the diagnosis of hypothyroidism in dogs; however, some dogs with clinical hypothyroidism will have serum TSH concentrations within reference limits (Peterson *et al.* 997) and 18-38% of dogs with hypothyroidism have a TSH measurement in the reference range consistent with euthyroidism (Bruner *et al.* 1998). In addition, elevated TSH is not uncommon in euthyroid dogs (Dixon *et al.* 1997). A false positive can be avoided by measuring T4 as well as TSH (Dixon *et al.* 1999). Dixon and Mooney (1999a) noted that T4 and TSH measurement in a single sample is a valuable tool for diagnosis of hypothyroidism.

T4, T3 and rT3 measures may help to confirm a decrease in T3 associated with non-thyroidal illness (Ferguson 1984; Section 1.5.5). Ferguson (1988) noted that measures of rT3 are best interpreted with measures of T4 and T3 because all rT3 is derived from T4 in which the 5'deiodination has been inhibited.

Other combinations for the diagnosis of hypothyroidism include a measure of T4 and cholesterol concentrations (abnormally high cholesterol levels are associated with hypothyroidism), radionucleotide studies, thyroid biopsy and the TRH stimulation test, (Hutchinson 1990). However, Frank (1996) states that the TRH stimulation test is not a useful means of diagnosing hypothyroidism in dogs. It has been suggested that T3 measures can be unreliable, but Marquez *et al.* (1986) notes that T3 measures are reliable if the plasma is extracted within 24 hours of sampling.

Commercial kits are available and commonly used for the assessment of thyroid function in dogs. For the purposes of this study kits were used for the measurement of T4, T3, rT3 and TSH, which was measured for all dogs by radioimmunoassay (RIA; Appendix 8 for justification of RIA for the determination of thyroid hormones), as this is effective for the measurement of the thyroid hormones and cortisol (Reimers *et al.* 1982). In some cases TgAA was also measured and this was done by indirect enzyme immunoassay (ELISA).

2.5.3 Measurement of hormones

As there were differences in amount of blood collected from each dog, the order of priority for thyroid testing was T4, T3 and TSH, and there was insufficient plasma to test six dogs for TSH. The analysis of plasma was carried out at a commercial laboratory (Cambridge Specialist Laboratory Services, Sawston, Cambridge, UK; CSLS); therefore it was necessary to determine the reliability and specificity of the assay procedure. Thus, the assays were tested for precision and quality control data, which is available for T4, T3, TSH and cortisol and is presented in Appendix 9. I piloted the kit used for T4 at a University of Southampton laboratory (Appendix 10) and I observed and assisted in the

analyses of T4, T3 and TSH at CSLS. The measurement of rT3 by RIA and TgAA by ELISA were carried out in my absence. However for completeness I have included the technical methods for all procedures (Appendix 11).

Kelley and Oehme (1974) was the first to put forward normal thyroid hormone concentrations for dogs. However, Kemppainen and Behrend (1998) suggested that the deduction of normal ranges from the measurement of hormones in healthy dogs could be misleading, as euthyroid dogs can give false positive readings for TgAA. However, it is usual to refer to the laboratory reference ranges for the interpretation of concentrations of hormones. Normal reference ranges, as supplied by CSLS, are given in Table 2.7. These were used in Chapters 3,4 and 5. In Chapter 7, different assay methods were used and the reference ranges were supplied by The Endocrine Diagnostic Section, The College of Veterinary Medicine, Animal Health Diagnostic Laboratory (AHDL), Michigan State University (MSU).

Table 2.7 Reference ranges for the thyroid hormones, TgAA and cortisol in dog plasma, supplied by CSLS

Hormone or antibody	Normal range
T4	13 – 52 nmol/l
T3	0.3 - 2.5 nmol/l
TSH	< 0.41 ng/ml
TgAA	<120% Negative
	(121%-200% Borderline positive)
rT3	0.27-0.61 ng/ml
Cortisol	Up to 250nmol/l

2.6 Statistical methods

2.6.1 Preliminaries to inferential statistical testing

Prior to hypothesis testing, the data was explored using descriptive statistics. The primary reason for exploratory data analysis was to examine the distribution of the data in order to select parametric or non-parametric tests. Histograms, means, medians, standard deviations and ranges were used to produce a summary of the data prior to more detailed statistical analysis. Categorical, interval, ordinal and continuous data had all been collected and appropriate statistical tests were selected for each (Table 2.8).

In some cases it was necessary to reduce large quantities of behavioural measures into composite variables, which could then be used for inferential analysis. Principal components analysis (PCA)

was selected as a data reduction technique suitable for combining variables into underlying Factors for use in parametric tests. The test makes some assumptions that all data is continuous and normally distributed, but it is generally accepted that in practice a normal distribution is not essential for all variables. PCA is used on data for which there are at least two variables measured on the same individuals, converted into z scores (mean=0, S.D.=1) prior to analysis. The analysis can be conceived of as plotting all the points and then drawing lines of best fit through the points. The first line accounts for the most variation in the sample and a line at right angles to the first line forms the second component, and so on (Dytham 1999).

The importance of the Factors is given as Eigenvalues (an Eigenvalue of 1 indicates equivalent variation to one original variable), which can be converted into a percentage of variation. The cumulative variation indicates how much of the variation in the data set is accounted for by each stage of the PCA. The Eigenvalues can be used to generate a Scree diagram from which an abrupt break in slope of the line indicates a cut off point, below which Factors are not usually considered to be informative, whereas the Factors above this point are considered useful and can be used as variables in further analysis. The original variables are loaded on each factor and each can usually be allocated to a single Factor, although occasionally an original variable can be strongly loaded on more than one factor. PCA was used in Chapters 3 and 4. In some cases a Varimax rotation was used, this is a method rotation that reduces the number of variables that have high loadings on each factor and therefore simplifies the interpretation of the factors. Transformation was required to normalise data for use in parametric tests, by eliminating the dependence of the variance on the mean. The square root method of transformation was used as the variation in the data was proportional to the mean; 0.5 was added to the sample before square root transformation, as some of the data set contained samples measuring less than one. Square root transformation was used in Chapter 6.

The Kappa statistic was used to measure the levels of agreement between the two independent classifications by measuring the amount of agreement between multiple values, this test is suitable for cases where several subjects have to assign to different classes. The inter-counsellor reliability was tested in Chapter 5 using the Kappa statistic.

The Cronbach alpha coefficient of consistency was used in Chapter 6 in order to determine the degree of association between five questions compiled in a questionnaire.

2.6.2 Parametric and non-parametric tests

Parametric tests require that the test variable is measured on an interval or ratio scale and both the dependant and independent variables were normally distributed. Inferential data analysis involved the use of both parametric and non-parametric tests, as the use of non-parametric statistics is suitable for measuring the relationship between thyroid hormones and psychosomatics (Mason 1968). Due to the

lack of research in this particular area of behavioural endocrinology no predictions were made about the results prior to the commencement of each project. Therefore all statistical tests were two tailed. The significance levels for all tests was $p<0.05$.

Table 2.8 Utilised statistical tests

Test	Statistic	Parametric / Non parametric	Appropriate data	Used in Chapter(s)	Reference
Spearman's rank correlation	r_s	Non-parametric	Ordinal / Interval	3 4 6	Fowler and Cohen (1990)
Cronbachs alpha	α	Non-parametric	Interval	6	Zar (1996)
Analysis of covariance	F	Parametric	Interval	6	Roberts and Russo (1999)
ANOVA	F	Parametric	Interval	3 4	Roberts and Russo (1999)
Mann-Whitney U-test	U	Non-parametric	Ordinal / interval	3 7	Martin and Bateson (1993)
Kappa statistic	K	Non-parametric	Nominal /Categorical	5	Siegel and Castellan (1988)
Kruskall Wallis test	k	Non-parametric	Ordinal	7	Dytham (1999)
Chi squared test	χ^2	Non-parametric	Nominal / categorical	7	Russell and Roberts (2001)

2.6.3 Statistical programs

All analysis was done using SPSS for Windows release 10.0 standard version, Excel 2000 for Windows NT and Lotus 123 for Windows NT.

Chapter 3

Behaviour of dogs with behavioural disorders, recorded in an unfamiliar environment: relationship to thyroid hormone titres

Extracts of results presented at the British Small Animal Veterinary Association Annual Congress, International Convention Centre, Birmingham, England, 5-8 April 2001 and as a poster at the Third International Congress on Behavioural Medicine, Vancouver, BC, Canada, 7-8 August 2001.

3.1 Introduction

The veterinary literature reveals that hypothyroidism is the most commonly diagnosed endocrine disease in dogs (Swenson & Reece 1993, Behrend *et al.* 1998) for which lethargy and heat seeking behaviour are the usual behavioural symptoms. However in the past 20 years, veterinarians and behaviourists have suggested that hypothyroidism could also be a cause of canine aggression (Reinhard 1978; Dodds 1992; Gerzovich 1995; Dodman & Shuster 1998; Scott 1999). One mechanism by which hypothyroidism and aggressive behaviour patterns has been proposed to occur is that low concentrations of thyroid hormone can cause impairment of cerebral function, which has previously been reported as a cause of aggressiveness in the dog (Reinhard 1978). Dodman *et al.* (1995) describes two dogs presented with aggression disorders that were diagnosed with autoimmune thyroiditis. Dodds (1992) also describes a decrease in aberrant behaviour patterns when dogs were given thyroid replacement therapy. The relationship between thyroid dysfunction and dog behaviour is not well established and for mammals in general there is no indication that thyroid hormones directly influence discrete behavioural responses (Dewsbury 1978; Leshner 1978). However, stress hormones such as the glucocorticoids produced by the adrenal gland have been shown to directly influence behaviour in mammals (Carlson 1998), as well as suppressing thyroid hormone production in dogs and humans (Section 1.6). Stress responses can be associated with aggressive and hyperactive behaviour patterns, and also result in cortisol production which in turn can cause a reduction in circulating plasma thyroid hormone concentration (Section 1.6).

Therefore, rather than the concentration of thyroid hormones controlling the behaviour of the individual, aggressive behaviour patterns could be a reflection of a chronic stress response reflected in glucocorticoid production which subsequently exerts an inhibitory effect on thyroid hormone production. In order to determine the relationship between stress, behaviour patterns and thyroid titre this study examined the types of behaviour patterns exhibited by dogs in a clinical population many of which were considered to have a stress-related behavioural disorder including dogs with stress related aggressive behaviour. Each dog's behaviour was then compared to its thyroid status. Emphasis was placed on determining whether dogs exhibiting aggressive behaviour patterns have lowered thyroid titre, and whether dogs with lowered thyroid hormone titre exhibit the typical symptoms associated with hypothyroidism such as inactivity.

3.1.1 Outline and aims of chapter

This chapter describes an exploratory study that aims to investigate empirically the possibility that lowered thyroid hormone titre may be associated with inappropriate aggression. The methodology is summarised in Chapter 2, to which reference is made where appropriate. The results of inferential statistical testing are presented and the discussion critically considers the probable relationship

between the dog thyroid hormone titre and behaviour patterns. The relationships between the individual thyroid hormones within this population are also considered.

This is a survey study aimed to test the general hypothesis that there is a correlation between thyroid hormone levels and the behaviour of dogs. To investigate this hypothesis one approach would have been to test whether there is a difference in the thyroid hormone titres of dogs with and without behaviour disorders. However, this would have entailed assembling a matched control group of dogs without behaviour disorders and testing their thyroid hormone titres. As there is no established definition of a behaviour problem/disorder and because dog owners have different considerations as to what is inappropriate dog behaviour (Rooney 1999), recruiting a matched control group was infeasible. Additionally, due to the requirement of restraining and muzzling dogs for the blood sampling procedure, it was not considered ethical to test seemingly healthy dogs and this would also have required a Home Office licence. Therefore, I chose to explore the relationship between hormone levels and behaviour solely within a clinical population of dogs with a range of behaviour disorders.

3.1.2 Rationale

Deduction of this relationship may highlight behavioural symptoms indicating the onset of canine hypothyroidism and determine if dogs with behaviour disorders have altered thyroid function. Such clarification will assist in the prompt diagnosis and appropriate treatment of hypothyroidism and behaviour disorders in dogs. Fundamentally, the mental and physical health of affected dogs can thereby be improved, enhancing the welfare of such dogs.

3.1.3 Survey of dog behaviour patterns and thyroid status

Although aggressive behaviour is not typically associated with clinical hypothyroidism (Section 1.5.2) it is possible that the transition from euthyroidism to hypothyroidism may alter an individual's perception of their environment, lower thresholds for anxiety, and result in conflicting motivational states. Any of these changes could possibly manifest as an increased tendency for the exhibition of inappropriate aggressive behaviour, and/or other behaviour disorders, (the individual's breed, adequacy of socialisation and lifetime experiences will also influence the probability of developing a behaviour disorder; Section 1.2). Thus, I examined the distribution of thyroid hormone titres in dogs presented with behaviour disorders.

There is no unanimously favoured test for clinical hypothyroidism in dogs (Section 2.5.2), but lower than normal plasma levels of T4 and/or T3, combined with an increased plasma level of TSH are generally indicative of a hypothyroid state (Section 1.5). Whilst it is not known if individual hormones comprising the HPT axis exert behavioural effects, the physiological role of each thyroid hormone is well established, and relative concentrations of the thyroid hormones are known to be associated with the degree and type of hypothyroidism (Section 1.5).

This study was conducted at the Animal Behaviour Clinic (ABC; Section 2.3) in which several dogs attending the clinic for behaviour modification had blood samples taken for the measurement of T4, T3 and TSH. To objectively examine the dogs' behaviour, I measured their behaviour patterns in a novel environment. An alternative method would have been to interview the owner about their dog's behaviour and ask them open-ended questions; however, this method may introduce inconsistencies and anthropomorphism due to the subjectivity of the owner's descriptions and the dissimilarities in dog-owner relationships.

3.2 Methods

The methods are described in summary as a full detailed description of each procedure can be found in the General Methods, Chapter 2; reference is made to the relevant sections.

3.2.1 Subjects and recruitment

The dogs used in this study (n=82) were of mixed signalment and recruited from the Animal Behaviour Clinic, University of Southampton, UK (Section 2.3). All the dogs were considered by their owners to have a behaviour disorder, for which they required a behaviour modification programme. Each dog was referred by its veterinary surgeon for treatment. The most commonly referred behaviour disorder was fearful aggression towards humans and the least common behaviour disorder was pain-induced aggression (Section 2.3.2).

3.2.2 The consultation

Behavioural measures

With the informed consent of the owner, the behaviour of the dog was recorded during the behavioural consultation (Section 2.3). A canine behaviour ethogram was used (Section 2.2) to measure the behaviour patterns observed. Instantaneous time sampling was used on the subject dog every 20 seconds for the 15-45 minute period mid-consultation (Section 2.4.5). Nine mutually exclusive behaviour patterns were noted on check sheets (Section 2.4.5).

Thyroid measures

At the end of the consultation a blood sample was taken (Section 2.5.1) and analysed for T4, T3 and TSH concentrations by radioimmunoassay (Appendix 11). Due to differences in the amount of blood collected from each dog it was not always possible to test for all three hormones (T4, T3 and TSH), so the order of priority for thyroid testing was T4, T3 and TSH. The normal ranges for the thyroid hormones T3, T4 and TSH are shown in Table 2.7. Whilst T4 and T3 had a minimum and maximum value defining the euthyroid range, the normal range for TSH was defined as any value <0.41mg/ml.

However, the radioimmunoassay for TSH was sensitive to 0.1ng/ml and several dogs had a TSH reading below this, so the precise concentration of TSH for these dogs is unknown. TSH titre for these dogs was recorded as 0.1ng/ml for use in statistical analysis as omitting these dogs would have reduced the sample size substantially.

Several confounding factors are known to influence thyroid hormone titre, which may cause false results (Section 1.5.5). These include: vaccination of dogs in the two weeks preceding blood sampling, illness, whether or not dogs were on any current medication, and time of oestrus in entire females. In total, 11 dogs presented with confounding factors and no individual dog presented with more than one confounding factor.

3.2.3 Statistical analysis

Behavioural variables

Graded behaviour patterns were rated on a three-point scale (scored as -1, 0 or +1), as measuring the presence or absence of such behaviour would not sufficiently account for the variations of the behaviour patterns observed (Table 3.1), and several behaviour patterns were combined into one behavioural variable. For example, the variable "tail position" was comprised of the behaviour patterns tail flag, tail relax and tail tuck. In the case of measuring Proximity a value of one indicates that the dog was located near (10cm) from a familiar person, object or dog (Section 2.2.3).

As behaviour consultations were subject to interruptions and dogs were sometimes out of camera shot, there were differences in the total observation period for each dog (Section 2.2.2.2), however, 50 dogs were observed for an entire 30 minutes and the minimum time observed for any dog was 7 minutes. Therefore, the rate was calculated for each behaviour variable (as defined in Table 3.1) by dividing the total score by the total number of observations.

Table 3.1 Behaviour patterns comprising behavioural names and score

Variable name	*Behaviour patterns*	*Score*
Body Position	Stand	1
	Sit	0
	Lay	-1
Head position	Up	1
	Level	0
	Down	-1
Ear Position	Up	1
	Down	0
Tail position	Flag	1
	Relax	0
	Tuck	-1
Tail movement	Wag	1 / 0
Bark	Barking	1 / 0
Growl	Growling	1 / 0
Whimper	Whimpering	1 / 0
Proximity	Near familiar	1 / 0

The behavioural variables were then tested for correlations using Spearman's rank order correlation (Section 2.6.2). Rotated PCA with Kaiser normalisation (Section *2.6.1*) was used to identify behavioural Factors. The Scree diagram method was used to indicate interpretable Factors and those with an Eigenvalue below 1.0 were not retained. In order to deduce if there were any trends between signalment and the Factors, ANOVA was used to test for relationships between the factors and the dogs' age (grouped into 18 months-6 years and ≥7 years), breed group (Working, Gun, Terrier, Hound, Utility and mixed) and sex (female neutered, female entire, male neutered and male entire).

Thyroidal measures

The relationship between the concentrations of the thyroid hormones was examined using Spearman's rank correlation in order that any abnormal associations could be explored. As the presence of confounding factors was uncommon a new binary variable was constructed for the presence or absence of any confounding factors and the independent Student's unpaired *t*-test was used to test the overall effects of confounding factors on the thyroid hormone titres.

Comparative analysis of behavioural and thyroidal measures

After independent examination, the behavioural and thyroidal data was analysed collectively (Section 3.3.3) and Spearman's rank correlation was used to examine the relationship between the thyroid hormones (T4, T3 and TSH) and each of the behavioural factors produced from the PCA. As 48% of the test population had a TSH titre ⩽0.1ng/ml Mann Whitney U test, (Section 2.3.2) were used to determine whether behavioural factors differed between dogs with a TSH titre ⩽0.1ng/ml and those with TSH titre >0.1ng/ml.

3.3 Results

3.3.1 Behavioural data analysis

All nine behavioural variables were correlated and of the 36 possible combinations, 33% were significant ($p<0.05$; Table 3.2). PCA with a varimax rotation and Kaiser normalisation (Section 2.6.1) on the behavioural variables produced three factors accounting for 57% of the cumulative variance. These factors were named; Alertness (Factor 1), Activity (Factor 2) and Aggressivity (Factor 3) and included all 9 behavioural variables were strongly weighted on a single Factor. Alertness comprised the behaviour patterns Tail Position, Tail Movement and Barking. Activity comprised the behaviour patterns Body Posture, Head Position and Whimper, whilst Aggressivity included the remaining behaviour patterns measured which were Ear Position, Growling and Proximity (which described the dog's location relative to a familiar object or person) (Table 3.3).

Table 3.2 Significant correlations between behaviour patterns

Associated behaviour pattern	Spearman's rank order correlation	2-Tailed significance level
Head position and body posture	$r_s = 0.508$	$p<0.0001$
Tail position and body posture	$r_s = 0.518$	$p<0.0001$
Tail movement and body posture	$r_s = 0.464$	$p<0.0001$
Proximity and body posture	$r_s = -0.236$	$p<0.05$
Tail movement and head position	$r_s = 0.238$	$p<0.05$
Bark and ear position	$r_s = 0.287$	$p<0.01$
Tail movement and tail position	$r_s = 0.690$	$p<0.0001$
Bark and tail position	$r_s = 0.255$	$p<0.01$
Bark and tail movement	$r_s = 0.317$	$p<0.01$
Whimper and tail movement	$r_s = 0.231$	$p<0.05$
Proximity and tail movement	$r_s = -0.238$	$p<0.05$
Proximity and bark	$r_s = -0.235$	$p<0.05$

Table 3.3 The behaviour patterns and scores comprising each of the three factors produced by PCA.

	Factor 1	Factor 2	Factor 3
% of variance explained	**31%**	**14%**	**12%**
Behaviour pattern	**Alertness**	**Activity**	**Aggressivity**
Bark	**0.816**	-0.172	0.038
Tail Position	**0.810**	0.174	0.001
Tail movement	**0.752**	0.374	-0.113
Head position	0.074	**0.749**	0.148
Whimper	0.005	**0.695**	-0.145
Body Position	0.577	**0.596**	-0.078
Growl	0.000	-0.046	**0.693**
Proximity	-0.321	0.055	**0.595**
Ear Position	0.331	-0.043	**0.482**

There were no significant associations between the Factors and the dogs' age class, (Alertness F=1.87; Activity F=1.46; Aggressivity F=0.79: all 1 d.f.) breed (Alertness F=0.22; Activity F=1.58; Aggressivity F=1.53: all 5 d.f.) and sex (Alertness F=0.90; Activity F=0.73; Aggressivity F=0.12: all 3 d.f.).

3.3.2 Thyroidal data analysis

The distribution of the thyroid hormones T4, T3 and TSH are shown in Figures 3.1, 3.2 and 3.3 respectively. Comparing this sample to the normal hormone titres for T4, T3 and TSH (as supplied by CSLS; Table 2.7) indicates that 10 dogs had either a T4, T3 or TSH titre outside of the normal range. One dog had both T4 and T3, and another both T3 and TSH outside of the normal range, and their owners were advised to have the dogs retested for clinical disease. One dog (Figure 3.1) had a T4 titre marginally above the euthyroid range, which is unusual in dogs (Chastain and Ganjam 1986). This dog did not have any behavioural or anatomical symptoms of hyperthyroidism; it is possible however, that this could have been indicative of the early stages of canine autoimmune thyroiditis as fluctuations in circulating T4 are evident in humans developing Hashimoto's thyroiditis (Volupe 1996). Fluctuations are thought to be occur due to homeostatic feedback loops acting to increase levels of plasma T4 in response to the binding of T4 to autoantibodes in the early stages of autoimmune disease.

Student's unpaired *t* test was used to examine the effect of the confounding factors on T4, T3 and TSH; no significant associations were found. Those dogs with confounding variables showed no significant difference to those without confounding variables in thyroid hormone concentrations (T4, t=0.9, d.f =80, $p>0.05$; T3, t=1.7, d.f =79, $p>0.05$; TSH, t=1.0, d.f =74, $p>0.05$).

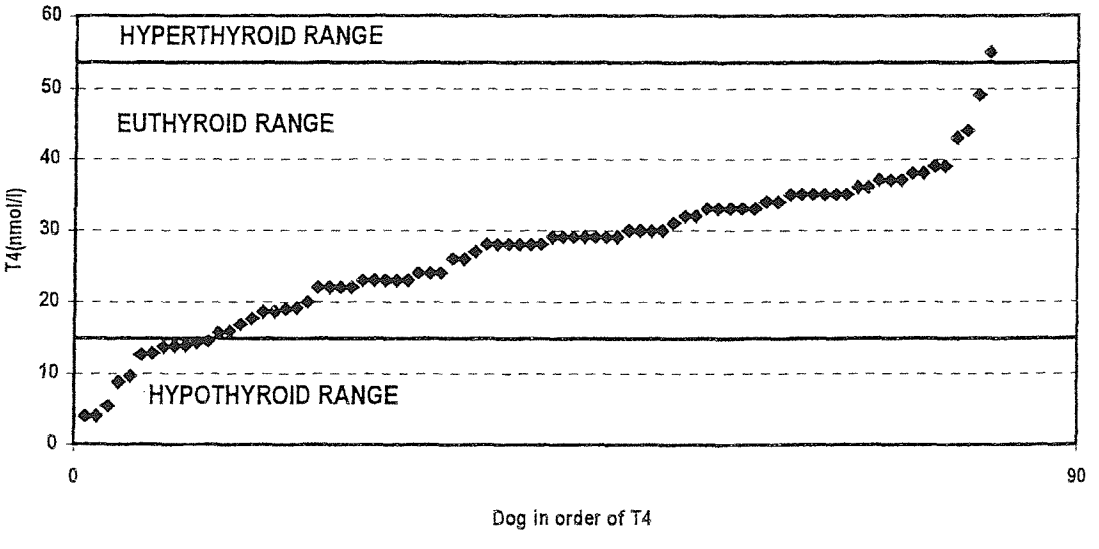

Figure 3.1 Cumulative distribution of T4 (nmol/l) for all the dogs in the population. The area between the solid lines indicates the euthyroid range.

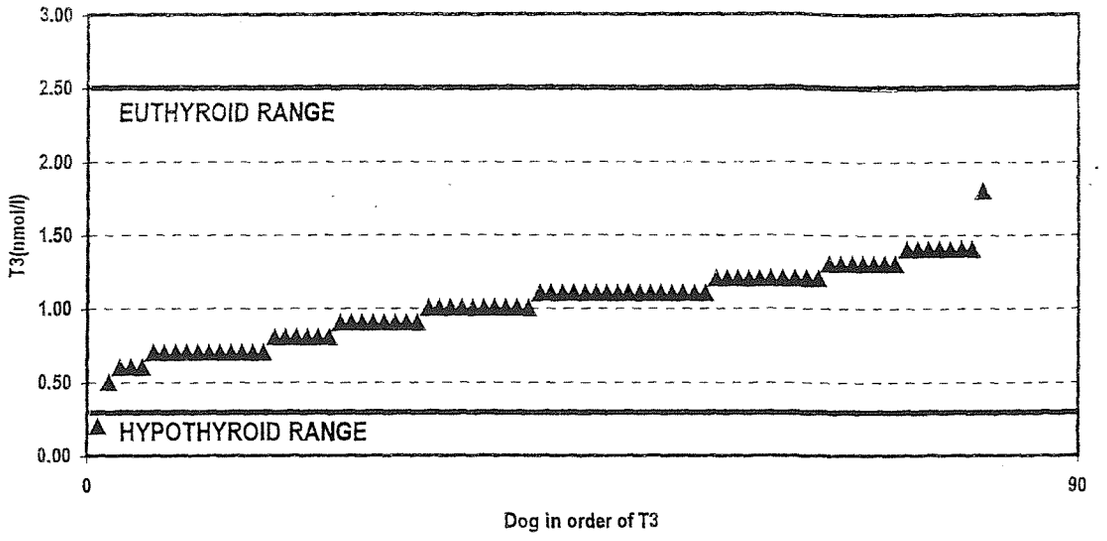

Figure 3.2 Cumulative distribution of T3 (nmol/l) for all the dogs in the population. The area between the solid lines indicates the euthyroid range.

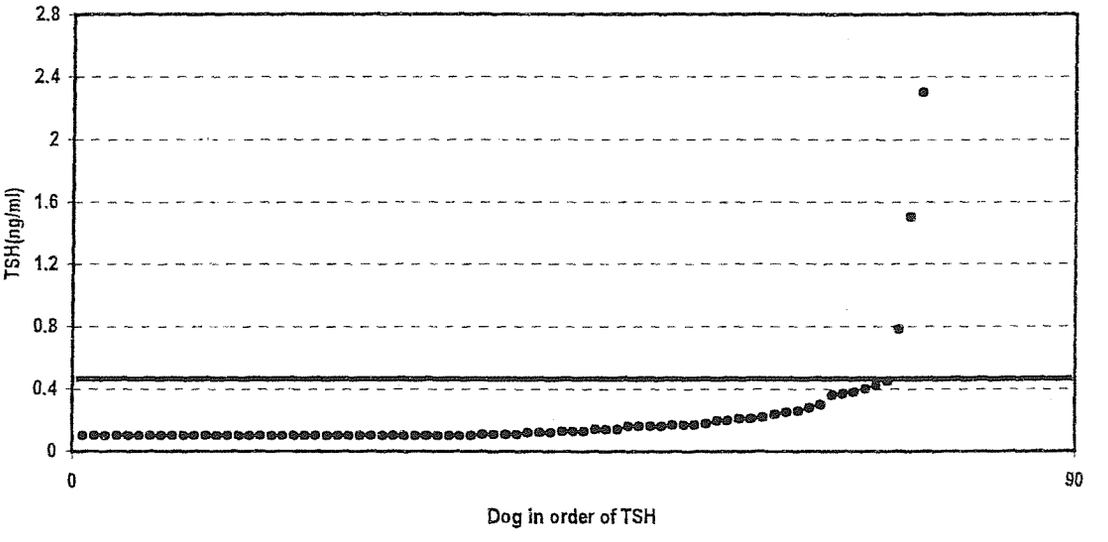

Figure 3.3 Cumulative distribution of TSH (ng/ml) for all the dogs in the population. The area below the solid line indicates the euthyroid range.

The relationship between the thyroid hormones was explored and is shown in figure 3.4. Spearman's rank order correlation was used to test for relationships between the thyroid hormones. There was a significant positive correlation between T4 and T3 and a non-significant positive correlation between T3 and TSH (Table 3.4).

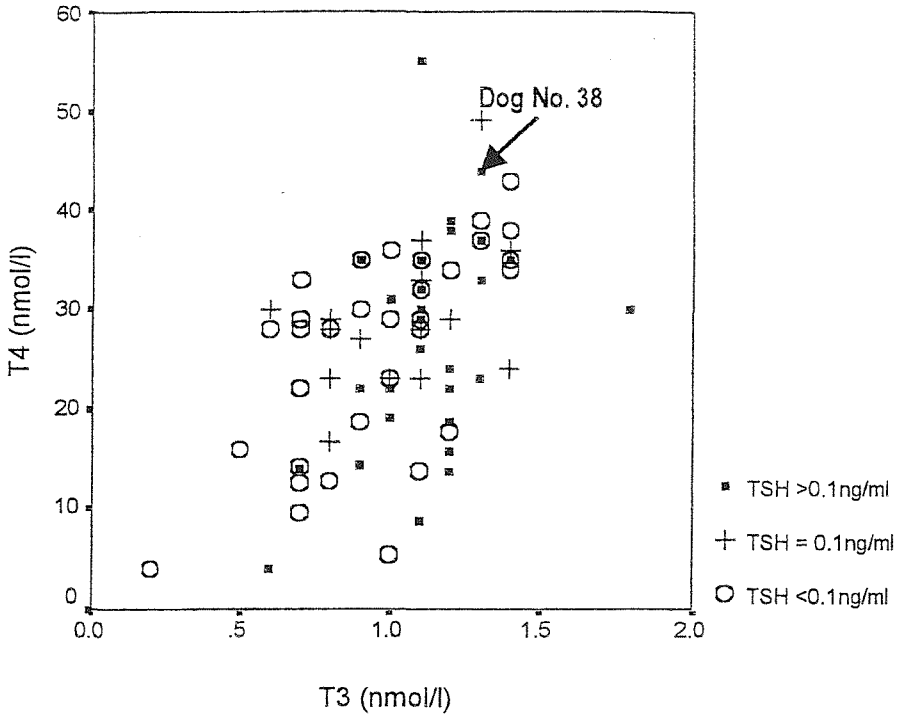

Figure 3.4 Association between T4, T3 and TSH (The position of dog number 38 is highlighted as this individual scored very high on Aggressivity and is relevant to discussion in Section 3.3.3)

Table 3.4 Correlation between thyroid hormone titre

	T4	T3
T3	r_s = 0.496 *p*<0.0001 n=81	
TSH	r_s = - 0.101 *p*>0.05 n=76	r_s = 0.208 *p*=0.07 n=76

The nonsignificant trend between TSH and T3 was explored further as half of the dogs in the sample had a TSH titre of ≤0.1ng/ml (Figure 3.3). This is attributable to the sensitivity of the radioimmunoassy assay technique for the detection of TSH titres from canine plasma, which was sensitive to a minimum value of 0.1ng/ml (Section *2.5.3.3*). Results indicate a larger range of T3 values in dogs with TSH titres ≤0.1ng/ml (Figure 3.5), while the median value of T3 for dogs with TSH titres of ≤0.1ng/ml and >0.1ng/ml was 1.0 nmol/l and 1.1 nmol/l respectively.

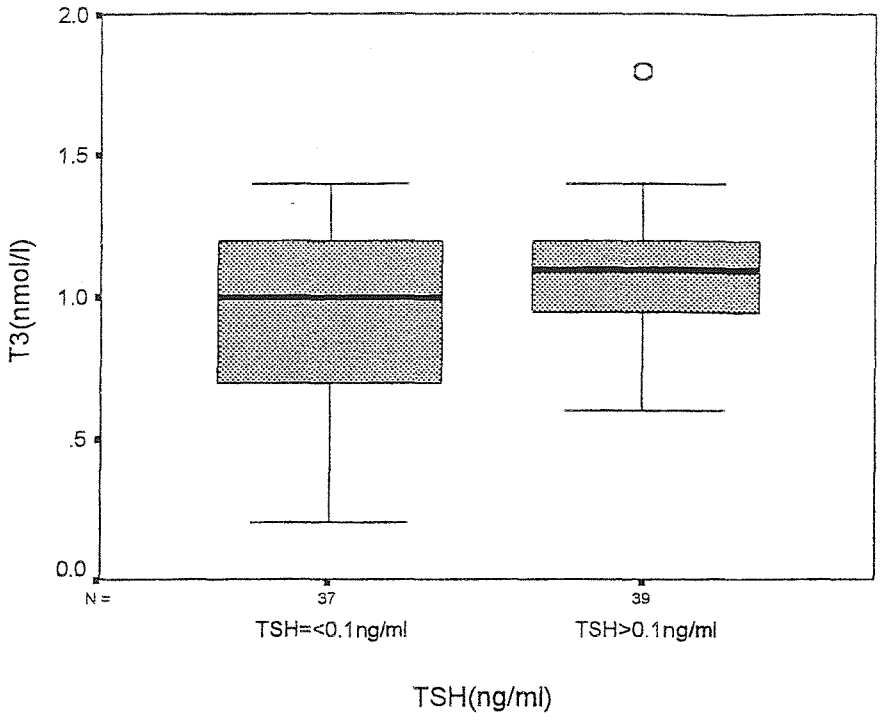

Figure 3.5 Box-and-whisker plot of the range of T3 for dogs with TSH titres of ≤ 0.1ng/ml and >0.1ng/ml. Solid lines indicate medians; the box extends from the 25th to the 75th percentile, and the four horizontal lines the minimum and maximum values, except for the single point at T3=1.8nmol/l which is an outlier (dog no. 49).

3.3.3 Comparing behavioural and hormonal data

Spearman's rank order correlation was used to test for correlations between the behavioural factors and the thyroid hormone concentrations T4, T3 and TSH in each dog (Table 3.5).

Table 3.5 Spearman's rank order correlation coefficients (r_s) between the behavioural factors and the thyroid hormones.

Factors	T4	T3	TSH
Alertness	r_s = 0.076	r_s = -0.003	r_s = -0.033
Activity	r_s = 0.120	r_s = 0.223	r_s = 0.386
		p<0.05	p=0.001
		n=80	n=75
Aggressivity	r_s = 0.237	r_s = 0.058	r_s = -0.151
	p<0.05		
	n=81		

The association between Activity, T3 and TSH

Low scores for Activity were generally associated with low titres of T3 (r_s = 0.233; p<0.05) (Figure 3.6) and with low titres of TSH (r_s = 0.386 p=0.001) (Figure 3.7).

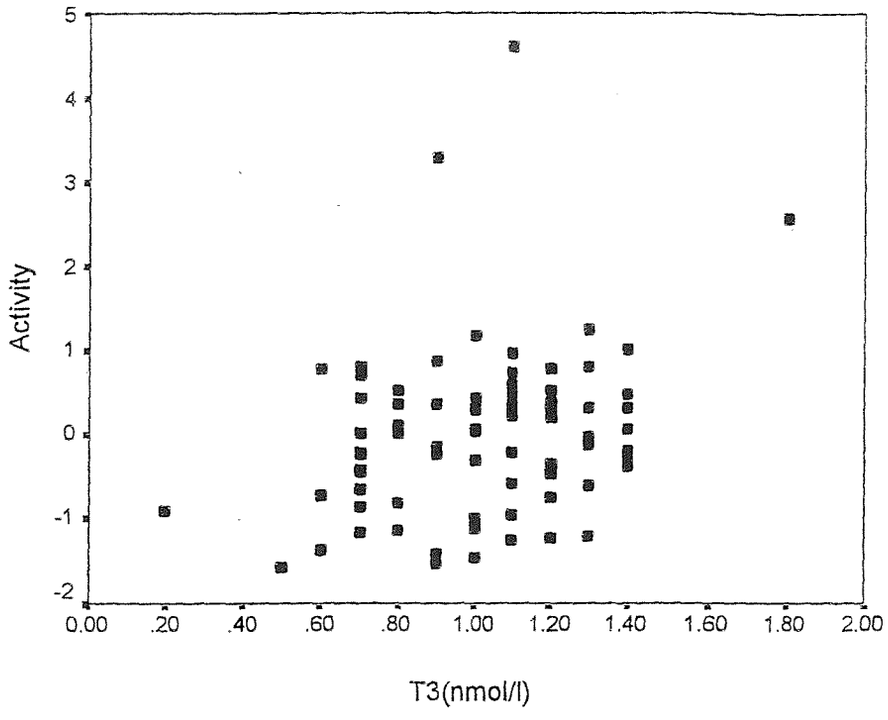

Figure 3.6 The relationship between T3 concentration (nmol/l) and score for Activity (rs = 0.233, $p<0.05$; measures are at 0.1 intervals as this is the accuracy of the results generated by the assay procedure).

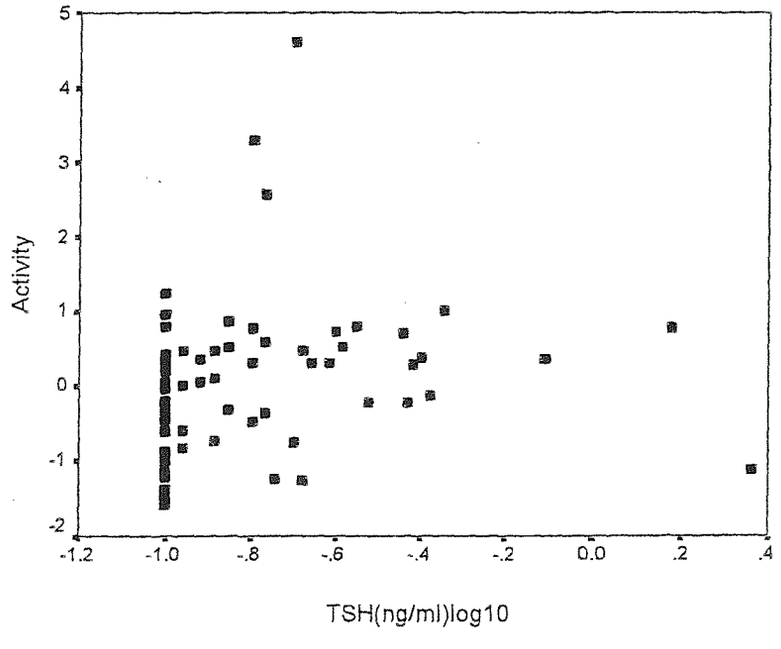

Figure 3.7 The relationship between TSH concentration (ng/ml) expressed as $\log_{10}$ and score for Activity (r_s = 0.386, p=0.001).

Since TSH titres were not continuously distributed, the relationship with Activity was also tested by Mann-Whitney, comparing dogs with titres ≤ 0.1ng/ml and >0.1ng/ml. The median Activity values for dogs with a TSH titre ≤0.1ng/ml and >0.1ng/ml groups was -0.28 and 0.34 respectively; Mann-Whitney test, U=389, p=0.001; Figure 3.8) confirming the result of the correlation that lower values of TSH were associated with lower levels of Activity.

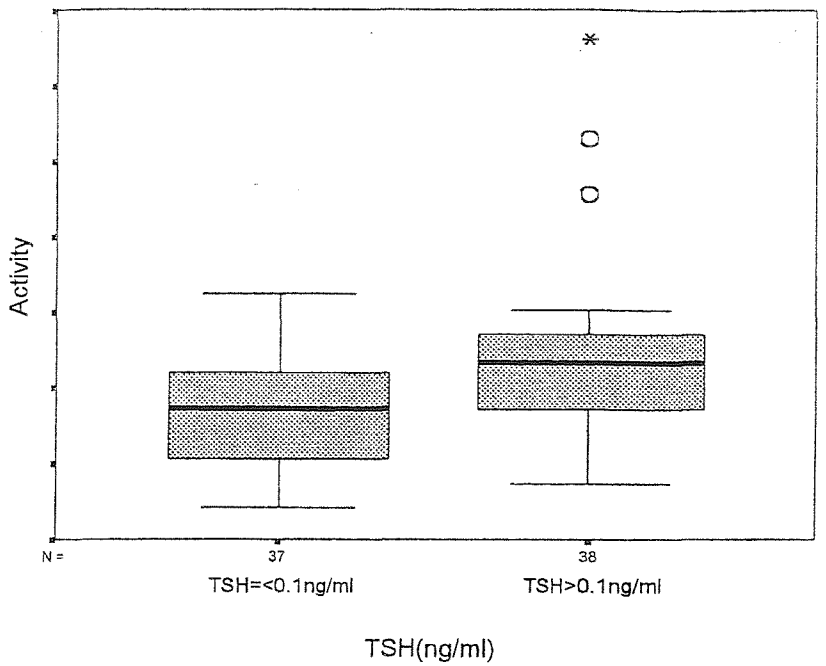

Figure 3.8 The relationship between Activity and TSH for dogs with TSH titres of ≤ 0.1ng/ml and 0.1ng/ml.

I further considered the possibility that the associations between Activity and T3 and Activity and TSH may have been due to the nonsignificant association between T3 and TSH (Table 3.3). Ideally, carrying out a partial correlation would usually test such an association; however as partial correlation is based on a Pearson's correlation coefficient (and thus a normal distribution) this test was not suitable for TSH, which had a skewed distribution.

Therefore, I divided the subjects into two groups, one with TSH titres of ≤ 0.1ng/ml and the other with TSH titres of >0.1ng/ml, and examined the relationships between T3 and Activity separately with Spearman's rank correlations. The relationships between Activity and T3 titres in the group of dogs with TSH titres of ≤ 0.1ng/ml and in the group of dogs with TSH titres >0.1ng/ml were not significant at p=0.05 but the coefficient for dogs with TSH ≤ 0.1ng/ml was higher than that obtained when the two groups were combined (Table 3.6), confirming that T3 is linked to Activity in those dogs with detectable levels of TSH (when TSH levels were >0.1ng/ml).

In order to determine if Activity and TSH was associated in those dogs with detectable levels of TSH I examined the relationship between Activity and TSH in those dogs with TSH>0.1ng/ml with Spearman's rank correlation, the association was non-significant (r_s = 0.18, p=0.28).

Table 3.6 Association between Activity and T3. The correlation coefficients and *p* values for a) all dogs for which TSH was measured, b) dogs with a TSH titre >0.1ng/ml and c) dogs with a TSH titre =<0.1ng/ml

	Association between Activity and T3		
	a) All dogs	b) Dogs with TSH titres >0.1ng/ml	c) Dogs with TSH titres ≤0.1ng/ml
N=	80	37	38
Correlation coefficient	0.22	0.29	0.09
p value	<0.05	>0.05	>0.05

The association between Aggressivity and T4

High scores for Aggressivity were associated with high T4 titres ($r_s = 0.237$ $p<0.05$; Figure 3.9).

Dog number 38 had a very high aggressivity score (Figure 3.9) and therefore it was possible that inclusion of this atypical individual could have biased the results. Therefore the analysis was repeated omitting this individual from the sample, however, the results (Table 3.7) remained similar to those presented in Table 3.5.

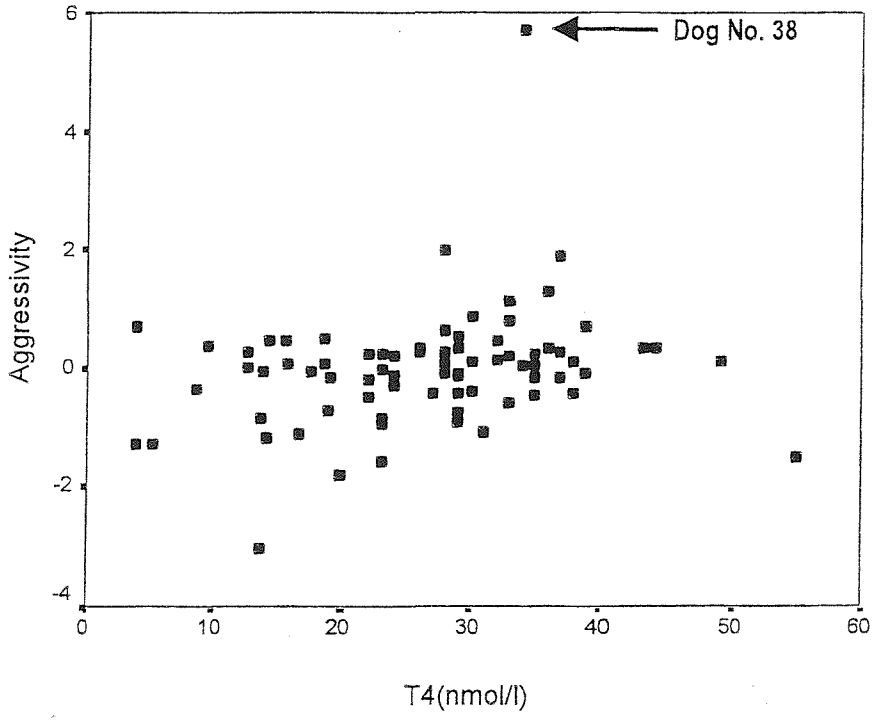

Figure 3.9 The relationship between T4 (nmol/l) and score for Aggressivity ($r_s = 0.237$, $p<0.05$).

Table 3.7 Spearman's rank order correlation coefficients (r_s) between the behavioural factors and the thyroid hormones having omitted dog number 38.

Factors	T4	T3	TSH
Alertness	$r_s = 0.085$	$r_s = 0.014$	$r_s = -0.044$
Activity	$r_s = 0.126$	$r_s = 0.237$	$r_s = 0.382$
		$p<0.05$	$p=0.001$
		n=79	n=74
Aggressivity	$r_s = 0.224$	$r_s = 0.025$	$r_s = -0.132$
	$p<0.05$		
	n=80		

Dog number 38 was a female neutered Springer Spaniel attending the ABC as a result of developing a separation related disorder, however, during the course of the consultation it became evident that this dog also had a fear aggression behaviour disorder. The heavily signalled entire range of agonistic behavioural patterns shown prior to biting the behaviour counsellor may have contributed to its high scoring for Aggressivity. This dog had an elevated T3 titre relative to other dogs in the population, and a TSH titre of<0.1ng/ml (Figure 3.4).

3.3.4 Summary of results

High scores for aggressivity were associated with high T4 titres. Low scores for activity were associated with low titres of T3 and titres of TSH <0.1ng/ml. The associations between T3 and Activity and TSH and Activity are largely independent of the relationship between T3 and TSH, but may apply to different dogs; low Activity is associated with very low TSH (independent of T3) but when TSH is detectable, low activity is more closely associated with low T3.

3.4 Discussion

3.4.1 Initial findings

The sample of dogs used in this study was of mixed composition; 79% of the dogs were aged between 18 months and 6 years. The under representation of older dogs is as would be expected in a referred behavioural clinical population, possibly because owners tend to be more accepting of the behaviour of older dogs and often accept that "you can't teach an old dog new tricks" and because many behaviour disorders tend to be more obvious in younger dogs, which are generally more energetic and are display more active behaviour patterns. Only 18% of the dogs were mixed breed; this is not unusual in a referred population as owners who purchase their dogs from breeders often expect higher standards of performance from them and are more likely to seek advice for inappropriate behaviour, hence, crossbreeds are under represented. Additionally, a proportion of purebred dogs are shown at

dog shows where they are required to be well behaved and tolerate handling by judges and close contact with other dogs. Most of the dogs (79%) were neutered irrespective of sex. Neutering is often considered a panacea for behaviour disorders by veterinarians and is therefore often the first step owners often take in an attempt to resolve a behaviour disorder, thus the proportion of neutered dogs in this population might be expected to be higher than that in the general population.

3.4.2 Evaluation of the relationships between thyroid hormones

Levels of the thyroid hormones T4 and T3 were significantly correlated and there was also a weak positive association between T3 and TSH. These correlations were expected, since the regulation of all the thyroid hormones is controlled by the hypothalamic-pituitary- thyroid-extrathyriod axis, (HPA axis). TSH acts on the follicular membrane of the thyroid producing cAMP (intracellular messenger), which in turn stimulates T4 production and subsequently T3 production. However, this does not explain why there was no correlation between T4 and TSH. I suggest that it is possible that T4 fluctuates in order to maintain T3 at a constant level based on titres of TSH, as one third of plasma T4 is converted to T3, which is the more potent and active form of the thyroid hormone. TSH levels are also controlled by a negative feedback mechanism; whereby levels of T4 and T3 influence the release of TSH from the pituitary. TSH is therefore produced in greater amounts when T4 is low, and there is a subsequent time delay before T4 levels increase. At this point TSH production is switched off. Hence at any one point in time one would not expect levels of T4 and TSH to be correlated. The finding that there is a greater range in T3 values when TSH titres are at low levels can also be explained by considering the feedback mechanism of the HPT axis. Higher TSH concentrations are likely to occur after stimulation of the pituitary gland, when T3 is low, and result in rise in T4 and T3 titres.

3.4.3 The relationship between thyroid hormones and behaviour

The results of this study do not support the theory that dogs with lowered T4 titres display aggressive behaviour patterns, as a positive correlation was found between Aggressivity and levels of T4. This finding suggests that when T4 is decreased during ill health, or in times of stress, the dog, is less likely to display aggression due to its generally lethargic state. Thus, no evidence was found to suggest the idea that the dog may exhibit aggressive behaviour patterns as a primary symptom of hypothyroidism.

The deduced relationship between low activity with low T3 is as expected. T3 is the most physiologically active form of thyroid hormone and is of primary importance in the control of metabolic function. Therefore, when T3 is reduced, metabolic rate is lowered and this results in inactivity. Lethargy and inactivity is a major symptom of reduced T3. However, all but one of the T3 titre measures were within the accepted euthyroid range and thus in this population no conclusions

can be made about levels of activity associated with clinically low levels of T3. Reduced activity was therefore largely associated with sub clinical hypothyroidism.

The relationship between low activity and very low TSH can be explained by considering the feedback mechanism of T3 and T4 on TSH. When T4 and T3 are elevated the thyroid gland does not need to produce further thyroid hormones, negative feedback occurs, and TSH titre is reduced. When titres of T4 and T3 are low, TSH increases to increase T4 production, and physical activity increases, so the relationship between low activity and low TSH initially appears contradictory. The relationship may indicate a direct effect of TSH or TRH on the emotional or motor centres of the brain, it is also possible that destruction of the thyroid gland itself may affect the speed of response of the thyroid feedback mechanism. However, as thyroglobulin autoantibodies were not measured in this population this theory cannot be confirmed.

3.5 Concluding comments

Hypothyroidism is the most commonly referred endocrine disorder in dogs and (due to the seriousness of the consequences), inappropriate aggression is the most commonly referred behaviour disorder. However, the results of this study suggest that dogs referred for inappropriate aggression do not appear to have thyroid hormone titres below the normal range. The finding that dogs with lowered T3 appeared to be more inactive is consistent with the generally accepted view that lethargy is a key symptom in dogs with low thyroid function (Section 1.5.2). Dogs with low levels of T4 (marginally hypothyroid) did not show elevated aggressive behaviour patterns contrary to the suggestions by Dodds 1992, Dodman *et al.* (1995) and Scott 1999. Although in individual cases clinical hypothyroidism may contribute to behavioural changes within dogs, the findings of this study do not support the hypothesis that lowered thyroid hormones is a major contributory factor to aggression in the domestic dog.

These results are based on measures of dog behaviour when placed in an unfamiliar environment. Therefore subsequent studies were aimed at correlating the behaviour of dogs in a familiar environment with their thyroid hormone titre (Chapter 4) for which comparable results would be expected if the current findings were a genuine reflection of the relationship between dog behaviour and lowered thyroid titre.

Chapter 4

Reported behaviour patterns in dogs with problematic behaviour in a familiar environment and related thyroid status

4.1 Introduction

4.1.1 Owners' reports of dog behaviour patterns and thyroid status

The comparison between observed behaviour patterns and thyroid hormone titres supported the acknowledged relationship between lethargy and hypothyroidism (Section 1.5.2). However, the hypothesised relationship between lowered thyroid hormone titre and behaviour disorders such as inappropriate aggression was not confirmed (Section 1.3.4). The behaviour recorded in Chapter 3 was performed in an unfamiliar environment. As dog behaviour can be context specific (Overall 1997), owners' reports were obtained for the behaviour patterns exhibited by the same population of dogs whilst in their familiar home environment. If thyroid hormone titre is related to canine behaviour patterns as found in Chapter 3, similar results should be obtained in this study.

The methods of behavioural data collection used in Chapter 3 were not appropriate for this study as this required novel equipment and/or people to be placed in the dog's environment, which would then have contained unfamiliar elements. Therefore the assessment of behaviour was achieved from questionnaires designed for completion by the owner about their dog's behaviour at home. Owners were asked to categorise their dog's behaviour towards them in specific situations.

4.1.2 Outline and aims of chapter

As the population of dogs used in the study is identical to those used in Chapter 3, reference is made to the relevant sections for data on group composition and thyroid hormone titres. Details of the methodology are contained in Chapter 2; however a summary of the methods is included here. The discussion critically considers similarities to previous findings and the conclusion includes a summary of results and suggestions for further work.

4.2 Methods

4.2.1 Recruitment of sample and collection of thyroidal and behavioural data

The dogs used in this study (n=81) were the same population as Chapter 3 apart from one dog for which the owner did not complete a questionnaire; therefore the recruitment of subjects and collection of thyroidal data was identical (Chapter 2). Behavioural data (Section 2.4) was collected using a questionnaire (Section 2.4.2), which was in the format of a grid (Appendix 3). The questionnaire was completed by the owner on arrival to the ABC but prior to their consultation. The owner was required to tick one or more of 12 box(es) that best described their dog's behavioural response (see table 4.1) to each of 13 actions performed by them.

These were: Approach it, Pet it, Ignore it, Return after leaving it, Call it to you, Ask it to sit, Ask it to lie down, Raise voice and point finger at it, Take its food away, Take its toy away, Move it from its resting area, Lie on the floor with it and Groom it. Space was available for the owner to answer, "don't know" or to add a description in their own words, but this qualitative data was not analysed because only few owners provided further details.

4.2.2 Compilation and analysis of behavioural and thyroidal data

The blood samples taken at the clinic (Section 2.5.1) were used for the measurement of T4, T3 and TSH (Section 2.5.3) and were compared with the behavioural data provided by the owner's questionnaire. The number of situations the owner reported their dog displaying each behavioural pattern was totalled, and then a varimax rotated PCA with Kaiser normalisation (Section *2.6.1.3*) was used to identify the underlying behavioural factors. In order to deduce if there were any underlying trends between signalment and the factors, ANOVA was used to test for relationships between the factors and the dogs' age (grouped into 18 months-6 years and ≥7 years), breed group (Working, Gun, Terrier, Hound, Utility and mixed) and sex (female neutered, female entire, male neutered and male entire). The behavioural factors and thyroid titre were explored and analysed using a Spearman's rank order correlation, 2-tailed test (Section 2.6.2) and $p<0.05$ was considered a significant association.

4.3 Results

4.3.1 Behavioural data analysis

Summary of observed behaviour patterns: The most frequently reported behaviour pattern was tail wagging and the least commonly reported behaviour patterns were barking, growling and whimpering (Table 4.1)

The reduction of the behavioural variables: The PCA produced 5 factors (Table 4.2) accounting for 64% of the cumulative variance. The lowest retained factor was at/above the break point on the Scree diagram (Section 2.6.1). The factors were named; Appeasement (Factor 1), Attention Seeking (Factor 2), Sedentary Postures (Factor 3), Agonistic Vocal (Factor 4) and Submission (Factor 5); these included all 13 behavioural responses recorded by the owners in the questionnaire. The behaviour patterns comprising the factors are shown in Table 4.2.

Table 4.1 The median values for the number of situations each owner reported each of the behaviour patterns.

Behaviour pattern	Median value
Tail wag	5
Sit	2
Nuzzle	2
Lay	2
Climb or jump up	2
Roll	1
Paws	1
Other	1
Lick lips or licks owner	1
Avoid gaze	1
Whimper	0
Growl	0
Bark	0

Table 4.2 The behaviour patterns and scores comprising each of the 5 factors produced by PCA.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 4
% of variance explained	19%	13%	11%	11%	10%
Behaviour pattern	Appeasement	Attention Seeking	Sedentary Postures	Agonistic Vocal	Submission
Licks lips	**0.764**	0.221	0.152	-0.043	-0.189
Climb / Jump	**0.703**	0.252	-0.180	0.113	-0.008
Avoid gaze	**0.528**	-0.166	0.302	0.090	0.380
Bark	**0.436**	-0.175	0.295	0.489	-0.071
Paw	0.013	**0.734**	-0.116	0.333	0.242
Nuzzle	0.294	**0.717**	0.117	-0.075	-0.109
Tail Wag	0.062	**0.650**	0.175	-0.337	-0.188
Lay	-0.042	0.056	**0.776**	-0.012	0.020
Sit	0.348	0.010	**0.725**	-0.064	-0.034
Roll	-0.245	0.027	**0.528**	0.445	-0.075
Growl	0.078	-0.023	-0.080	**0.889**	-0.080
Obedient	-0.042	-0.222	0.010	0.000	**0.787**
Whimper	-0.041	0.174	-0.052	-0.092	**0.757**

The association between signalment and the factors: There were no significant associations between any of the five factors and the signalment of the population. There were no significant associations between the dogs' age, (Appeasement F=3.73; Attention seeking F=1.67; Sedentary Postures F=2.55: Agonistic vocal F=0.02; Submission F=1.45; all 1 d.f.) breed (Appeasement F=0.54; Attention seeking F=0.90; Sedentary Postures F=0.85: Agonistic vocal F=0.37; Submission F=1.90; all 5 d.f) and sex (Appeasement F=0.40; Attention seeking F=1.82; Sedentary Postures F=1.31: Agonistic vocal F=0.71; Submission F=1.24; all 3 d.f.) with the factors.

4.3.2 Correlation with thyroid hormones

Spearman's rank order correlation was used to test for correlations between the behavioural factors (Appeasement, Attention Seeking, Sedentary Posturing, Agonistic Vocalisations and Submission) and the thyroid hormone titres for T4, T3 and TSH for each dog (Table 4.3). A significant negative correlation was found between Sedentary Posturing and T3 titre ($p<0.05$) in that dogs that have low T3 titres tended to show more laying, sitting and rolling over (Figure 4.1). Although not significant at $p<0.05$, there was a tendency for dogs with low T4 to score high on Sedentary Posturing but low for Appeasement (Figures 4.2 and 4.3 respectively).

Table 4.3 The correlation between the behavioural factors and thyroid hormones, r_s =Spearman's rank order coefficient, ns=not significantly associated. *ns but shows a trend.

Factors	T4	T3	TSH
Appeasement	$r_s = 0.201$ $*p = 0.07$ n=81	$r_s = 0.157$	$r_s = 0.058$
Attention Seeking	$r_s = 0.109$	$r_s = -0.108$	$r_s = -0.152$
Sedentary Postures	$r_s = -0.211$ $*p = 0.06$ n=81	$r_s = -0.267$ $p = 0.02$ n=80	$r_s = -0.067$
Agonistic Vocal	$r_s = 0.034$	$r_s = 0.115$	$r_s = 0.019$
Submission	$r_s = 0.038$	$r_s = 0.012$	$r_s = -0.027$

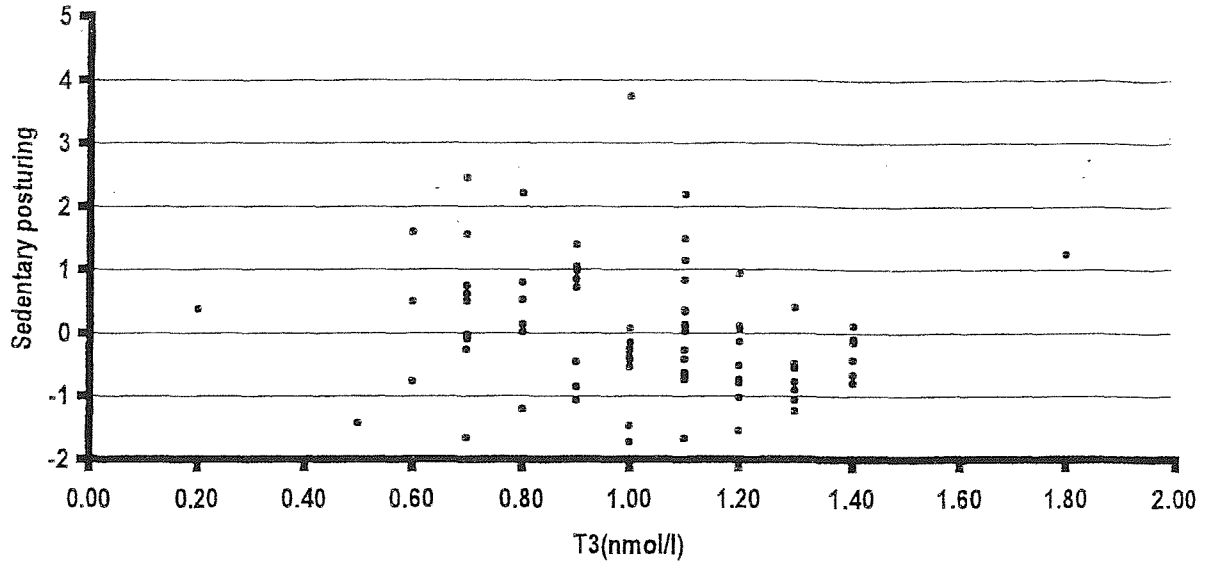

Figure 4.1 Scatter graph showing the significant negative correlation (r_s = - 0.267, p<0.05) between T3(nmol/l) and Sedentary posturing (Factor 3).

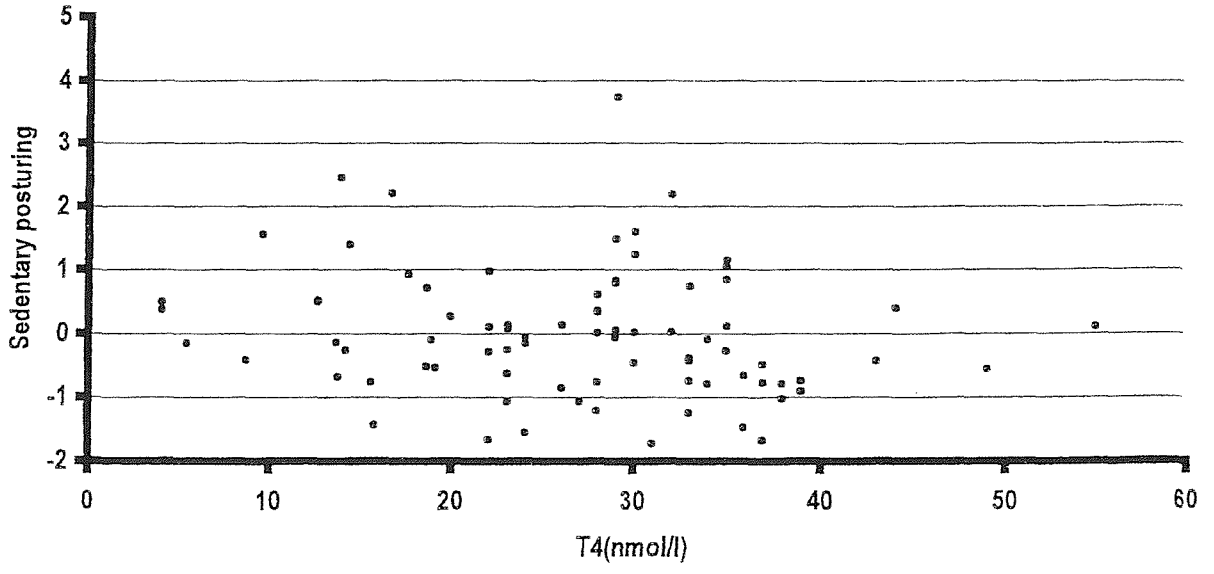

Figure 4.2 Scatter graph showing the trend towards a negative association (r_s= - 0.211, p=0.06) between T4(nmol/l) and Sedentary Postures (Factor 3).

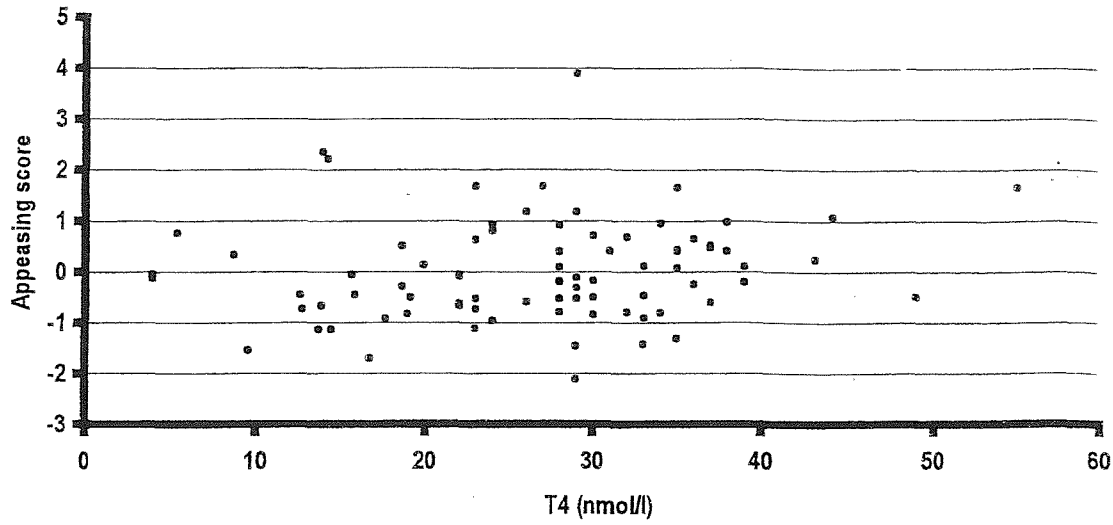

Figure 4.3 Scatter graph showing the trend towards a positive association (r_s= 0.201, p=0.07) between T4(nmol/l) and Appeasing (Factor 1).

4.4 Discussion

4.4.1 Behavioural data analysis

As the most commonly referred behavioural disorder to the ABC is inappropriate aggression I expected aggressive behaviour patterns such as growling to be more prevalently reported by owners. This may not have occurred for two reasons. Firstly, owners are often reluctant to admit that their dog behaves aggressively toward them as they feel it may reflect negatively upon them. There have been several cases at the ABC when the preliminary information given by the owner suggested that their dog had a training or obedience problem and in the resulting consultation the dog was classified as showing inappropriate aggression directed toward the owner. Secondly, aggressive behaviours directed towards other dogs are the second most common reason for referral to the ABC (5.3.1), and the questionnaire did not collect information on dog - dog aggression. Thus, some aggressive behaviour patterns may have been under represented in this analysis.

Barking was loaded on the Appeasement behaviour factor rather than with agonistic vocal. This suggests that barking is not purely an agonistic or territorial behaviour but is more likely to be a non-specific vocalisation; occurring as a result of several motivational different drives, in response to a variety of stimuli. It is likely that it is not barking *per se* that reflects the motivational state, but the intensity and duration of barking and the context in which it occurs that contains the accurate information about the subjective mental state of the dog and thus to which behavioural components it is more likely to be related to.

4.4.2 Associations with hormone titres

The significant relationship between low T3 concentration and a high score for Sedentary Posturing is consistent with the findings of Chapter 3 in which low concentrations of T3 were correlated with low scores for Activity. The active form of the hormone produced by the hypothalamic pituitary thyroidal axis is T3 (Section 1.3.2) and minor changes in thyroid homeostasis may lead to major changes in metabolism and behaviour (Whybrow 1996), lethargy and inactivity are the most frequently confirmed signs of lowered thyroid hormone concentration. Thus, lowered T3 titres parallel inactivity and confirm the acknowledged association between lethargy and hypothyroidism (Section 1.5.2), even though in this sample all but one of the dogs were within the euthyroid range for T3, the majority of dogs had T3 titres in the lower end of the euthyroid range (Figure 3.5). The trend associating high T4 concentration and Inactive Posture suggests that low T4 may also be related to the behavioural symptoms of hypothyroidism such as lethargy. The weak trend associating low T4 concentration and low levels of Appeasement suggests that there might be an association between lowered T4 and a tendency not to avoid conflict. This could be consistent with the theory that low T4 is associated with aggression, but it is equally possible that sub clinically hypothyroid dogs are unlikely to jump or bark (both behaviour patterns highly weighted on Appeasement) due to overall lethargy.

4.5 Concluding comments

This study was aimed at measuring the behavioural responses of a dog to a familiar person. All the dogs had behaviour disorders for which their owners required help in attaining a behaviour modification programme. As inappropriate aggression is the most frequently treated behaviour disorder in the ABC population, I expected signs of agonistic behaviour to be more prevalently reported by owners than were described. It is possible that some owners underrepresented aggressive behaviour patterns as owners' reports of their dog's behaviour patterns are not always reliable (Rooney 1999). Due to the acknowledged association between lethargy and hypothyroidism the correlation between inactivity and thyroid hormone titres was expected. The results confirmed the relationship between lowered thyroid hormone and inactivity as found in Chapter 3, which is reflective of the acknowledged association between lethargic behaviour and lowered thyroid hormone. Aggressive behaviour patterns were not associated with lowered T4, there was a suggestion however, that dogs with low T4 might be less likely to perform appeasement behaviour patterns. As a result of this study further work was conducted which was aimed at direct measurement of dog behaviour when in a familiar environment and relating this to thyroid hormone concentrations (Chapter 6).

Chapter 5

Dog behaviour disorders and related thyroid status

5.1 Introduction

Although some clinicians have noted an association between behaviour disorders and hypothyroidism in dogs (Reinhard 1978; Mills 1991; Dodds 1992; Beaver 1994; Gerzovich 1995; Dodman & Shuster 1998; Scott 1999), empirical research provides no evidence that mammalian thyroid hormones directly influence individual behaviour patterns (Dewsbury 1978; Leshner 1978; Hart 1985). The existing literature detailing the relationship between canine hypothyroidism and behaviour disorders is sparse, exploratory and based on single case studies. The supposition that thyroid hormones can control behaviour is derived from the observation that thyroid replacement therapy can sometimes assist in the correction of behaviour disorders, with underlying hypothyroidism, in single cases (Dodds 1992, Dodds 1996; Dodman 1995). Although this provides evidence of an association between behaviour patterns and thyroid hormone levels, it does not necessarily indicate a direct cause and effect relationship (Section 1.7). The relationship between behavioural change and thyroid hormone titre may possibly be mediated by the actions of the glucocorticoids, which are known to affect mammalian behaviour patterns directly (Carlson 1998), in addition to exerting an inhibitory effect on the HPA axis (Section 1.6). In Chapters 3 and 4, I have shown that some aspects of the behaviour of dogs presented at a behavioural clinic are correlated to their thyroid hormone levels. This was true for both behaviour observed in a clinic setting and those described by owners in their own home.

The causes and forms of canine behaviour disorders are multiple (Section 1.2). The most frequent behaviour disorders referred to behaviour counsellors are inappropriate aggression and separation related disorders (Section 1.2.5). Aggressive behaviour can be regarded as a response to a stressor (Barash 1982) and separation anxiety is also considered to occur as a result of stress (Overall 1997). Thus, it is possible that the attempt to cope with a stressor may simultaneously lead to cortisol-induced lowering of plasma thyroid hormones, and the development of a behavioural response such as separation related behaviours or aggression (Section 1.2). In Chapter 3 I correlated directly observed dog behaviour patterns with thyroid hormone titre, whereas in Chapter 4 I correlated dog behaviour patterns observed by owners with thyroid hormone titre. The aim of the investigation described in this Chapter was to explore the relationship between different types of dog behavioural disorders and thyroid hormone titres, by surveying the thyroid status of dogs presented at the ABC exhibiting inappropriate behaviour.

5.1.1 Survey of dog behaviour disorders and thyroid status

In order to ascertain the relationship between behaviour disorders and hypothyroidism, it is necessary both to consistently categorise behaviour disorders and to establish a diagnosis of hypothyroidism. To obtain such information a detailed history about the development of the disorder and measurement

of several thyroidal hormones is required. By taking this approach the behavioural and hormonal measures of the entire sample group may be compared and the subtleties of this unknown relationship can be examined. This investigation attempts to answer two questions. Firstly, do dogs with behaviour disorders have altered thyroid function? Secondly, are particular behaviour disorders associated with a non-euthyroid state?

Do dogs with behaviour disorders have altered thyroid function?

To date there have been no systematic investigations to ascertain whether there is an association between the occurrence of dog behaviour disorders in dogs and hypothyroidism, (the principal non-euthyroid state found the dog; Section 1.5). Although some authors have suggested the contrary, their assumptions are often based on subjective observations (Mills 1991; Dodds 1992; Beaver 1994; Dodman & Shuster 1998; Scott 1999), and they do not present empirical data. Whether dogs with hypothyroidism develop behaviour disorders or dogs with behaviour disorders develop subsequent changes in thyroid status is yet undetermined. In order to investigate this "chicken and egg" scenario, in this chapter I compare thyroid titre between dogs presented with different behaviour disorders. In Chapter 7 I then explore the inverse relationship, that is, the behaviour patterns exhibited by dogs with altered thyroid status.

Are particular behaviour disorders associated with a non-euthyroid state?

Several authors have suggested that particular behaviour disorders are related to canine hypothyroidism. These include Reinhard (1978 p.70) who believes that "*Hypothyroidism and other pathophysiological causes of aggressive behaviour are rare, but should be ruled out prior to treating a dog for behavioural causes of aggression*", Beaver (1994 p142) who describes a form of aggression as "*Hypothyroid Aggression*" and suggests that aggression can be the only sign of a hypothyroid condition and Gerzovich (1995) who noted that it can be very difficult to establish a differential diagnosis between dominance aggression and hypothyroidism. But the relationship between thyroid dysfunction and dog behaviour disorders is not fully established, as Dodman and Shuster (1998) note that hypothyroid dogs may present with aggression, and Hart (1985) states that hormones from the thyroid gland have minor behavioural influences but are affected by behavioural events.

The difficulties in establishing the relationship between canine hypothyroidism and behaviour disorders are two-fold. Firstly, because there are several types of hypothyroidism (Section 1.5) there remains much controversy over which test is the best for effective diagnosis (Section 5.2.2). Secondly, there is no universal classification system for behaviour disorders. There is contradictory terminology and flawed definitions are used in the behavioural literature (Section 1.2.6), making it difficult to classify cases of undesirable behaviour.

5.1.2 Limitations in the classification of behaviour disorders

The classification of behaviour patterns is useful not only for the understanding and treatment of behaviour disorders but also assists in the appreciation of how behaviour relates to physiology. This is hampered in the clinical field of companion animal behaviour therapy, since there remain inconsistencies in the use of terminology and there are no universally accepted definitions of behaviour problems. Although, several authors have suggested a range of classification systems (Section 1.2.6), these are incompatible with one another.

There are several paradigms used in the classification of behaviour disorders and this is likely to be the primary reason for the delay in the emergence of a universal classification system. Several distinct approaches have been used. These include the animal's behavioural response to particular stimuli, the physiological changes associated with different behaviour patterns, the environmental situation in which the particular behaviour pattern occurs, as well as the recommended treatment programme. In the case of inappropriate aggression, the bite scenario has also been used (Borchelt 1985). Many of these methods rely on identifying cause and motivation of behaviour disorders; however, a classification system based on directly observable behaviour is more objective (Appleby *et al.* 2002) and thus will be used in this study.

The Borchelt and Voith (1982; Appendix 12) classification system was selected for the classification of behaviour disorders, as it was the most comprehensive published system, and classified behaviour disorders according to the behavioural signs and circumstances in which the disorder occurred. However, there are three disadvantages associated with this system. Firstly, the classification of disorders was not straightforward when a dog was presented with more than one behaviour disorder, or the precise circumstances in which the inappropriate behaviour occurred were unknown. Secondly, there was much overlap in the categories suggested; for example, fearful behaviour was relevant to several categories and was therefore complicated to classify. Additionally, there is no category specifically for stereotypical behaviour patterns in this system; this category was therefore added.

5.1.3 Why is the relationship between thyroid hormone titre and specific dog behaviour disorders important?

The deduction of the relationship between behaviour disorders and thyroid hormone alteration is advantageous as both behavioural and physiological cues may then be identified as early indicators of ill health. Thus the correct treatments can be given, such that (i) the risks associated with the inappropriate treatment of hypothyroidism from thyroid replacement therapy can be prevented, (ii) behavioural therapy may become a valuable alternative treatment for corticogenic hypothyroidism (Otsuki *et al.* 1973; Section 1.6.1) and (iii) thyroid replacement therapy may be an important element in resolving a behavioural disorder.

5.1.4 Outline and aims of chapter

This chapter aims to determine whether there is an association between particular behavioural disorders and hypothyroidism. Several of the methods used for this study are similar to previous studies and are therefore contained in the General Methods; reference is made to the various sections where appropriate. However, a methods section is included as some procedures are only applicable to this study, such as the determination of inter-counsellor reliability for the diagnosis of behaviour disorders. The population of dogs used in this study is the same as that used in Chapters 3 and 4; so reference is made to Chapter 3 for the signalment composition of the group. The results are discussed with reference to earlier and forthcoming chapters and followed by concluding comments.

5.2 Methods

5.2.1 Recruitment of subjects

A population of dogs with behaviour disorders of (n=82) was recruited from the ABC University of Southampton, UK (Section 2.3). This group was of mixed composition (Section 2.3.2).

5.2.2 Establishing a diagnosis

All dogs attending the ABC for behaviour therapy was seen by one of two experienced behaviour counsellors, one qualified to PhD standard in biology and psychology, and the other a veterinary surgeon specialising in behavioural medicine. Prior to the collection of data, they had agreed a consensus view on theories of behaviour counselling and recommended behaviour modification programmes (Section 2.3).

Each counsellor saw no more than two cases in one day. A typical session lasted for approximately two hours and comprised the following:

i. *The introduction* lasted for 15 minutes in which counsellor and client became acquainted, the format for the consultation was explained, consent forms were obtained from the dog owners so that the consultation could be filmed, and any other required questionnaires and paperwork were completed (Section 2.1).
ii. *The history* lasted for 45 minutes in which the owner(s) asked questions aimed at gathering the information to diagnose the behaviour disorder (Section 2.1).
iii. *The treatment* lasted for 60 minutes in which the counsellor explained the behaviour modification programme (Section 2.1).

After each consultation the counsellors conferred to establish a diagnosis according to the system proposed by Borchelt and Voith (1982). In addition to the 18 categories proposed by Borchelt and

Voith (1982) I added another category "Other" to encompass unforeseen behaviour disorders and also to include those behaviour disorders not adequately defined in the classification system, such as Stereotypies. In order to establish a unanimous diagnosis, if necessary, the recorded footage was reviewed to re-examine the behaviour of the dogs and the information given by the owner(s). In the cases where dogs presented with more than one behaviour disorder, classification of the disorder was based on the predominant problem as defined by the behaviour counsellors.

5.2.3 Determination of the inter-counsellor reliability

The classification made by the two ABC counsellors (Section 5.2.2) was compared to that of another experienced APBC behaviour counsellor, in order to test that the diagnoses and classification of behaviour disorders were reliable and consistent. This also ensured that, in cases where dogs presented with more than one behaviour disorder, the predominant disorder had been classified. This study utilised a volunteer member of the APBC (a science graduate with an MSc in behavioural counselling practising behaviour therapy for over two years) who observed the video and audio record of the entire consultation for 15 randomly chosen cases seen by the ABC behaviour counsellors, which were not used as part of the sample group used in Chapter 3, 4 or this chapter. The principal reason for the exclusion of these from the test sample was due to lack of owner's full consent and willingness to participate in the study. The footage was edited to exclude sections in which the diagnosis reached by the ABC behaviour counsellors was mentioned directly. The APBC counsellor then classified the behaviour disorder according to the Borchelt and Voith (1982) method.

The cases in which diagnosis agreed and disagreed were recorded (Table 5.2), and the inter-counsellor reliability was analysed using the Kappa statistic (Section 2.6). The Kappa statistic measures the levels of agreement between the two independent classifications and results in a figure between 1 (which indicates complete agreement), and 0 (where there is no agreement other than what would be expected to occur by chance; Siegel and Castellan 1988; Section 2.6).

5.2.4 Statistical analysis

A predominance of fear related behaviour disorders were recorded indicating a relatively homogenous population. Subsequent inferential statistical tests were therefore not feasible and the results are largely considered descriptively rather than quantitatively.

5.3 Results

5.3.1 Review of the behaviour disorders and thyroid status in the population

Of the 18 categories of behaviour disorders suggested by Borchelt and Voith (1982) only 11 were used in the classification of the 82 cases seen at the ABC. Fear-related disorders accounted for 69%

of the total disorders (Figure 5.1 presented in a different format in Figure 2.3) and the least common behaviour disorders were play related problems. Cumulatively 91% of dogs had a behaviour disorder that could be considered linked to the stress response via activation of the fight or flight mechanism as a result of stress, i.e., fear related and separation related disorders, or arousal, i.e., confident aggression to humans and confident aggression towards dogs. Therefore 9% of behaviour disorders were made up of the remaining behaviour disorders; Other, (which included stereotypical behaviour), Training Disorders and Play Related Disorders; (Figure 5.1). In classifying behaviour disorders according to the system proposed by Borchelt and Voith (1982) several categories were not used which included Maternal Aggression, Housebreaking, Marking, Submissive Urination, Excitement Urination, Fear Induced Elimination, Chewing, Mouthing and Nipping, (the latter three behaviours were incorporated into one category).

5.3.2 Inter-counsellor reliability

Preliminary examination of the ABC and APBC classification of cases indicated a 60% level of agreement (Table 5.1). The calculation of the kappa statistic indicates moderate agreement between the ABC diagnosis and APBC diagnosis (Kappa statistic, Two-tailed, κ=0.52, z=4.27, p<0.0001). Diagnoses made by the ABC counsellors have been used in all subsequent analysis of the data.

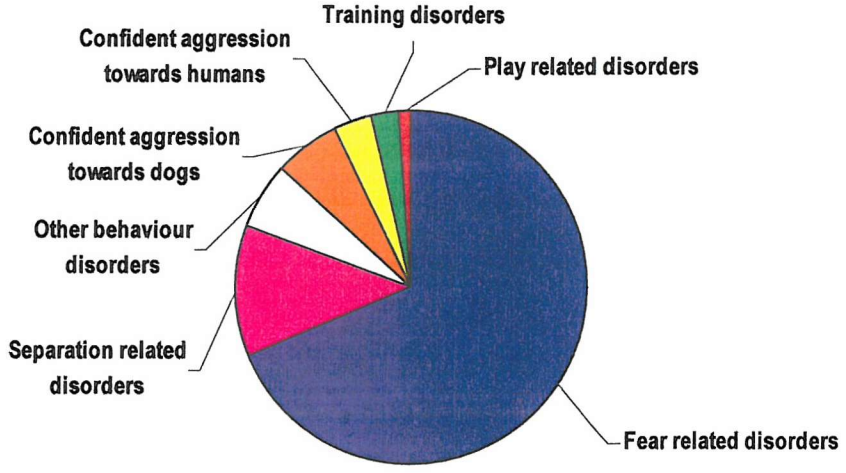

Figure 5.1 Distribution of behaviour disorders in the sample population of dogs seen at the ABC (n=82). This is the same data presented in Figure 2.3, however, this data is presented in a format to show the proportion of dogs presented with a fear related behaviour disorder; Fear related behaviour disorders include fear aggression towards humans, fear aggression towards dogs, fears and phobias, punishment induced aggression and pain induced aggression.

Table 5.1 Outcome of the classification of cases (n=15) by ABC counsellors and an APBC counsellor.

Case	ABC counsellors classification	APBC counsellor classification	Agree / Disagree
A	Training problem	Fear aggression towards humans	Disagree
B	Fear aggression towards humans	Confident aggression towards humans	Disagree
C	Fear aggression towards humans	Fear aggression towards humans	Agree
D	Re-directed aggression	Re-directed aggression	Agree
E	Fear aggression towards humans	Fear aggression towards humans	Agree
F	Fear aggression towards humans	Fear aggression towards humans	Agree
G	Fear aggression towards dogs	Fear aggression towards dogs	Agree
H	Fear aggression towards dogs	Predatory aggression	Disagree
I	Fear aggression towards dogs	Confident aggression towards dogs	Disagree
J	Confident aggression towards dogs	Confident aggression towards dogs	Agree
K	Separation related disorder	Separation related disorder	Agree
L	Punishment induced aggression	Fear aggression towards humans	Disagree
M	Fear aggression towards humans	Fear aggression towards humans	Agree
N	Play related problem	Training problem	Disagree
O	Fear / Phobia	Fear / Phobia	Agree

5.3.3 Assessment of behaviour disorders and thyroid status

Seven dogs had T4 titres in the hypothyroid range, four of these dogs were presented with a fear related disorder, one with a separation-related disorder, one with confident aggression towards dogs and one with a training problem (Table 5.2). Dog number one had a T4 reading suggestive of marginal hyperthyroidism and presented with fear related dog-dog aggression. This dog was referred back to its veterinary surgeon for re-testing of thyroid status. The results of T3 indicate that one dog had a titre below the normal range; this dog, a seven year old, male neutered Border Collie also had a T4 titre below the normal range and presented with a fear-related disorder. Four dogs had high TSH titres, suggestive of hypothyroidism; three of these presented with a fear related disorder and the other presented with a training problem. One dog, a 4 year old, female neutered Retriever had titres of T4 and TSH suggestive of hypothyroidism.

Table 5.2 Thyroid status and related disorders

Hypothyroid T4	
Case Number	*Diagnosis*
24	Fear / phobia,
29	Fear aggression to humans
33	Training problem
41	Fear / phobia
52	Confident aggression towards dogs
55	Separation related problem
59	Fear aggression towards dogs
Hyperthyroid T4	
Case Number	*Diagnosis*
1	Fear aggression toward dogs
Hypothyroid T3	
Case Number	*Diagnosis*
41	Fear/Phobia
Hypothyroid TSH	
Case Number	*Diagnosis*
4	Fear Aggression towards dogs
33	Training problem.
38	Fear aggression towards humans
58	Fear aggression towards dogs
62	Fear aggression towards people

The behaviour disorders of the dogs with at least one thyroid hormone (either T4 and/or T3 and/or TSH) within the hypothyroid range (n=12) were examined and 83% were classified as having a fear related disorder. However, the proportion of fear related behaviour disorders between the hypothyroid and euthyroid dogs (61% of euthyroid dogs presented with a fear related disorder) was not significantly different (Fisher's exact test, p=0.198).

In order to determine if particular behaviour disorders associated with a non-euthyroid state I explored the difference between the incidence of each behaviour disorder between the Hypothyroid (n=12) and Euthyroid (n=70) groups. However, due to the imbalance between the sample sizes for these groups in addition to the over represented incidence of fear related disorders statistical analysis was not appropriate, and no obvious differences were found.

5.4 Discussion

5.4.1 Behaviour disorders and thyroid status

The finding that dogs with at least one thyroid hormone outside the normal range presented with largely fear related behaviour disorders supports the hypothesis that, behaviour disorders indicative of stress are related to hypothyroidism, as the cortisol produced as a result of stress has an inhibitory effect on thyroid hormone titres (Section 1.7). The dogs with lowered thyroid titres presented with fear aggression towards dogs and fear aggression towards humans, fears and phobias, confident aggression towards dogs, separation related disorders and training problems. With the exception of training disorders (which could be linked to stress if the owner and dog are frequently engaging in conflict situations) all the presenting disorders can be considered linked to the stress response via activation of the fight (via confident behaviour disorders) or flight (via fear related behaviour disorders) responses. It should also be noted that in some cases a confident behaviour pattern is not always reflective of the underlying motivation, e.g., a dog may be motivated by fear but learns that displaying an aggressive pattern of behaviour results in its desired outcome (i.e. making the fearful stimulus go away) and therefore repeats the behaviour pattern and becomes progressively more confident in doing so. The use of both behavioural and physiological measures is therefore valuable in the understanding of behaviour patterns in the dog.

5.4.2 Classification of behaviour disorders

The inter-observer reliability between the ABC and the APBC diagnoses using the Borchelt and Voith (1982) classification system of behaviour disorders indicated a level of agreement, which is better than expected by chance. However 2 out of every 5 cases were disputed and this needs further consideration. The uncertainty between the ABC and APBC systems is likely to have been introduced at the stage of classifying cases e.g., the APBC counsellor classified a case of punishment induced aggression as fear aggression. This is likely to have occurred because the method suggested by Borchelt and Voith (1982) for classifying punishment, fear and confidence aggression have some overlapping criteria. Therefore a case of punishment induced aggression could be classified as fear induced aggression or confidence aggression ('dominance aggression') as well as punishment induced aggression. As the ABC and APBC behaviour counsellors utilise the same theories of behaviour counselling I suggest that the inconsistencies between their classifications is wholly due to the method by which they interpreted the events of the behavioural consultations and how they applied this information to the classification of cases according to Borchelt and Voith (1982).

5.4.3 Categories of behavioural disorder not represented in the sample

Several categories within the utilised classification system were not used, a-g listed below.

a. Maternal Aggression.

It is commonly recognised amongst dog breeders that formal treatment is inappropriate for Maternal Aggression and no form of treatment should be given to a bitch post natally, as both interspecific or intraspecific aggression at this time is due to an innate behaviour pattern that occurs at times when a bitch feels that her offspring are endangered. Pseudopregnancies can also result in aggression; however, this is predominantly a physiological condition and requires veterinary intervention. During the time of this study Maternal Aggression or pseudocyesis did not form the primary reason for referral for any bitches to the ABC.

b. Chewing, mouthing and nipping.

These are non-specific behaviour patterns that can occur with all types of behaviour disorders and are therefore difficult to classify.

c. Housebreaking.

Urination or defecation inside the house can be caused by several factors but the primary reasons are lack of training or elimination due to stress. The treatment of housebreaking was mentioned in some behaviour consultations, but this was never a primary reason for conducting a behaviour consultation.

d. Marking.

Marking in companion animals usually refers to urination within the home, but this was not the primary reason for a behaviour consultation at the ABC during the time of this study.

e. Submissive urination.

Borchelt and Voith (1982) describe this as urination accompanied by submissive postures when an owner or stranger approaches. Submissive urination was only seen in dogs as part of active submission and therefore appeared in several dogs referred for different reasons, e.g., fear related aggression and separation related disorders.

f. Excitement urination.

Borchelt and Voith (1982) describe this as urination accompanied by an excited greeting. The differentiation of excitement and other motivations such as restlessness was difficult and therefore this category was not utilised by the behaviour counsellors.

g. Fear induced elimination.

Borchelt and Voith (1982) describe this as occurring in fearful situations, which is difficult to define unless it is possible to see the dog within the situation that causes the fearful behaviour. Additionally fear induced elimination, where seen, is largely due to the occurrence of other, primary behaviour disorders e.g., fear related aggression and separation related disorders. The main difficulty in the classification of Housebreaking, Marking, Submissive urination, Excitement urination and Fear induced elimination was due to the overlap in the definition of each categories.

5.4.4 Suggestions for further work

If the relationship between cortisol, thyroid hormones and stress (Section 1.7) are as postulated, I would have expected an association between altered thyroid state and fear related behaviour patterns. Although the majority of dogs with hypothyroidism presented with a fear related disorder this was not significantly different from the euthyroid group because of the high population of this diagnostic category in the whole sample. Therefore, I would suggest that additional research is required, preferably comparing an equal group of hypothyroid and euthyroid dogs with and without fear related behaviour disorders.

In addition to the problems associated with the classification of behaviour disorders, the test referral population consists of dogs attending the ABC principally because their owner finds their behaviour objectionable. As there is much variation in owner's expectations of their pet it is likely that unless the disorder is causing harm to the dog or to people or property, it may not be referred for behaviour modification. Hence the most popular referred cases are those due to inappropriate aggression or separation related anxiety, even though other disorders such as the fear of noises (Hamilton-Andrews 1998), or the fear of unfamiliar people, may be more common in the general population. Behaviour disorders are therefore not equally represented in the ABC sample, and it is possible that underrepresented disorders could be more closely associated within hypothyroidism.

This study has provided a preliminary analysis of the relationships between thyroid hormone levels and specific behaviour disorders in a clinical population. However, in order to fully deduce if there is a relationship between specific behaviour disorders and altered thyroid state further work needs to be done. For future work I would suggest that:

i. Precise definitions of behaviour disorders and behaviour problems are established.

ii. An effective classification system that considers the commencement, motivation, development, presentation and advancement of behaviour disorders is produced to subdivide the large 'fear-based' category within this study. A validated ethogram incorporating the behaviour patterns of dogs with and without behaviour disorders is compiled.

iii. Inter counsellor reliability with a Kappa statistic of k>0.8 (excellent strength of agreement) is attained, which should be possible with effective definitions and a comprehensive classification system.

iv. Longitudinal studies are conducted that include the measurement of thyroid titre every six months from eighteen months of age and onwards in matched breed groups. Examination of behavioural development with measures of conditions the dogs were exposed to during their socialisation as well as aversive experiences during their development could be compared with long-term changes in thyroid hormone status.

v. As well as considering if dogs with behaviour disorders have hypothyroidism it is also necessary to consider if dogs with hypothyroidism have behaviour disorders as investigated in Chapter 7.

vi. The physical health of the dogs is monitored to account for those dogs that may have thyroid conditions (euthyroid sick syndrome; Section 1.5.5) resulting in elevated cortisol, which is unrelated to emotional stress.

5.5 Concluding comments

Due to the limitations of the existing classification systems for behaviour disorders and a non-representative sample of disorders, it has not been possible to confirm if dogs with particular behaviour disorders have altered thyroid function. Additional research is recommended.

Chapter 6

Behavioural and hormonal changes associated with behaviour modification

6.1 Introduction

6.1.1 Aim of study

The aim of this study was to examine the effect of a behaviour modification programme on the hormonal indicators of stress and thyroid hormone concentration. Dogs with stress related behaviour disorders may have increased levels of plasma cortisol (Section 1.7) which could lead to a reduction in the conversion of T4 to T3 and an increase in the production of rT3 (Section 1.5.5), i.e., subclinical corticogenic hypothyroidism might occur (Otsuki *et al.* 1973). Whilst clinical hypothyroidism has specific behavioural and physiological signs (Section 1.5.2) subclinical hypothyroidism in humans is often without observable symptoms (Section 1.3.3) and has not been fully investigated for dogs. However, corticogenic subclinical hypothyroidism would be reversible if the source of cortisol was a stress response that was resolved (Section 1.5.5). Therefore, I would expect a successful behaviour modification programme that focused on reducing fear and anxiety in dogs (Section 1.8) to result in a decreased stress response and remove the inhibition on thyroid hormone production.

6.1.2 Rationale

The detrimental effects of excessive stress on health are constantly reviewed in human medicine, and reducing stress is an important factor in the prevention and resolution of physical and mental illnesses where a link has been found with a stressful lifestyle. Similarly, there should be a benefit to welfare if ill health as a result of excessive stress can be prevented in dogs. Hypothyroidism is the most common endocrine disease in dogs (Turner 1994, Swenson and Reece 1993; Beale *et al.*1990; Chastain and Ganjam 1986), and is one of several diseases that may be associated with excessive stress (Section 1.3). As the dog is the second most popular pet in the UK (Anon. 2002) and the species most commonly referred to behaviour counsellors, the relationship between thyroid hormone concentration, stress and behavioural change may be of great consequence in terms of canine welfare.

6.1.3 Hypothesis and predictions

In order to measure the association between behavioural change, stress and thyroid function a study that examined hormonal changes in parallel to the development of stressful conditions would have been ideal. However, the recruitment of such a sample is not feasible, and creating such a study sample ethically objectionable. Alternatively, a retrospective study could have been carried out, but this was inappropriate, as the accurate and therefore direct observation of dog behaviour patterns was required. Therefore, I measured behavioural changes and thyroid and stress hormones concurrently during behaviour modification programmes used to treat a sample of dogs presented clinically with behavioural disorders. Several hypotheses were tested and predictions were made (Table 6.1).

Table 6.1 Null hypotheses and corresponding predictions

Number	Hypothesis	Prediction of alternative hypothesis
1	H_0 *"There is no association between the change in observed behaviour patterns (due to a behaviour modification programme) and thyroid hormone titre (T4, T3, TSH)".*	*Low thyroid hormone titres are associated with inactive behaviour patterns.*
2	H_0 *"There is no association between the change in observed behaviour patterns (due to a behaviour modification programme) and stress hormone titre (cortisol and rT3)".*	*If the behaviour modification programme is adhered to and successful, then a reduction in stress hormone titre is expected.*
3	H_0 *"There is no association between stress hormone titre (cortisol and rT3) and thyroid hormone titre (T4, T3, TSH)".*	*Since cortisol is known to have a direct influence on thyroid hormone concentration, and because rT3 is a metabolite of T4 and T3, then it is likely that there will be an association between the stress and thyroid hormone titres.*
4	H_0 *"There is no association between the behaviour counsellors assessment of the dogs behavioural change over time and stress hormone titre (cortisol and rT3)".*	*If the behaviour modification programme is adhered to then a reduction in stress hormone titre is expected.*
5	H_0 *"There is no association between the behaviour counsellors assessment of the dogs behavioural change over time and thyroid hormone titre (T4, T3, TSH)".*	*If the behaviour modification programme results in a decreased stress response then the inhibitory effect of cortisol on the thyroid hormones would be removed. The T4 and T3 titres would be expected to increase and the TSH titre decrease (due to feedback from increased levels of T4 and T3; Section 1.3.2).*

[1] rT3 titres are increased during periods of stress (Section 1.3.4).

6.2 Methods

This study required the measurement of dog behaviour (by direct observation and with the use of questionnaires) concurrent to the measurement of thyroid and cortisol hormone concentrations. To ensure that the chosen methods were reliable and valid for this investigation, a pilot study was carried out.

6.2.1 Pilot study

6.2.1.1 Introduction

The pilot study was aimed at determining the feasibility of using new data collection techniques as well as those previously used under controlled conditions in Chapters 3-5. The pilot study involved visiting five households to conduct an initial behaviour consultation and two follow up consultations for individual dogs (Table 6.2). The consultations were conducted by one of the two behaviour counsellors introduced in Chapter 2 (a veterinarian experienced in behaviour counselling for dogs). Two out of the five households visited were multi-dog occupancies, but only one dog per multi-dog household was treated for a behaviour disorder.

Table 6.2 Signalment and behaviour disorders of the dogs involved in the pilot study

Breed	Behaviour disorder	Sex	Age (years)
1. Mixed (Working breeds)	Human Aggression	Male entire	2
2. Mixed (Toy breeds)	Separation related disorder	Female neutered	6
3. Poodle	Noise sensitivity	Female neutered	5
4. Collie	Dog Aggression	Male neutered	4
5. Airedale	Over excitement	Male neutered	3

All the dogs in this pilot study complied with the selection criteria determined from previous studies: they were recruited from referral to the ABC from veterinary surgeries, over 18 months of age, lived within a 25 mile radius of Southampton University, had a behaviour disorder and were amenable to handling.

In order to compare the dogs' behaviour patterns between visits the behavioural consultations were carried out in the owner's home, as it was easier to control their home environment and prevent variability in behaviour through habituation that may occur during follow up visits to an unfamiliar clinic environment with potential interruptions. The behaviour consultation followed a standard format (Section 2.3.1) and provided the opportunity to test the feasibility of using recording methods to achieve a precise measure of the focal dog's behaviour patterns, as well as testing the appropriateness of the assessment questionnaire.

6.2.1.2 Collection of behavioural data

(i) Direct observation

The dogs were observed for the 15 - 45 minute period of the consultation using hand held video recording equipment and for the 45 - 90 minute period of the consultation tripod held recording equipment was used (Samsung VPA208).

(ii) Assessment questionnaire

The assessment questionnaire (Section 2.4.3; Figure 6.1; Appendix 5) was designed for use in this study only. The aim of this questionnaire was to determine the effect of the behaviour modification programme between visits i.e., 0-6 weeks and 6-12 weeks. The counsellor completed the questionnaire at the end of every follow up visit. The counsellor based her answers on the information provided by the owners about their dog's behaviour, on her own observations of the dog's behaviour and by comparison of the change in behaviour from the previous visit. The answers to the questionnaires were based on a 5-point Lickert scale. The ends of each scale represented behavioural improvement or no improvement; in order to encourage maintenance of attentiveness whilst completing the questionnaire the scales on some scales were reversed. For example, on question two an improvement would be indicated by a mark on the left hand side of the scale, whereas in question three an improvement would be indicated by a mark on the right hand side of the scale. This questionnaire was used to achieve a measure of overall behavioural change between visits.

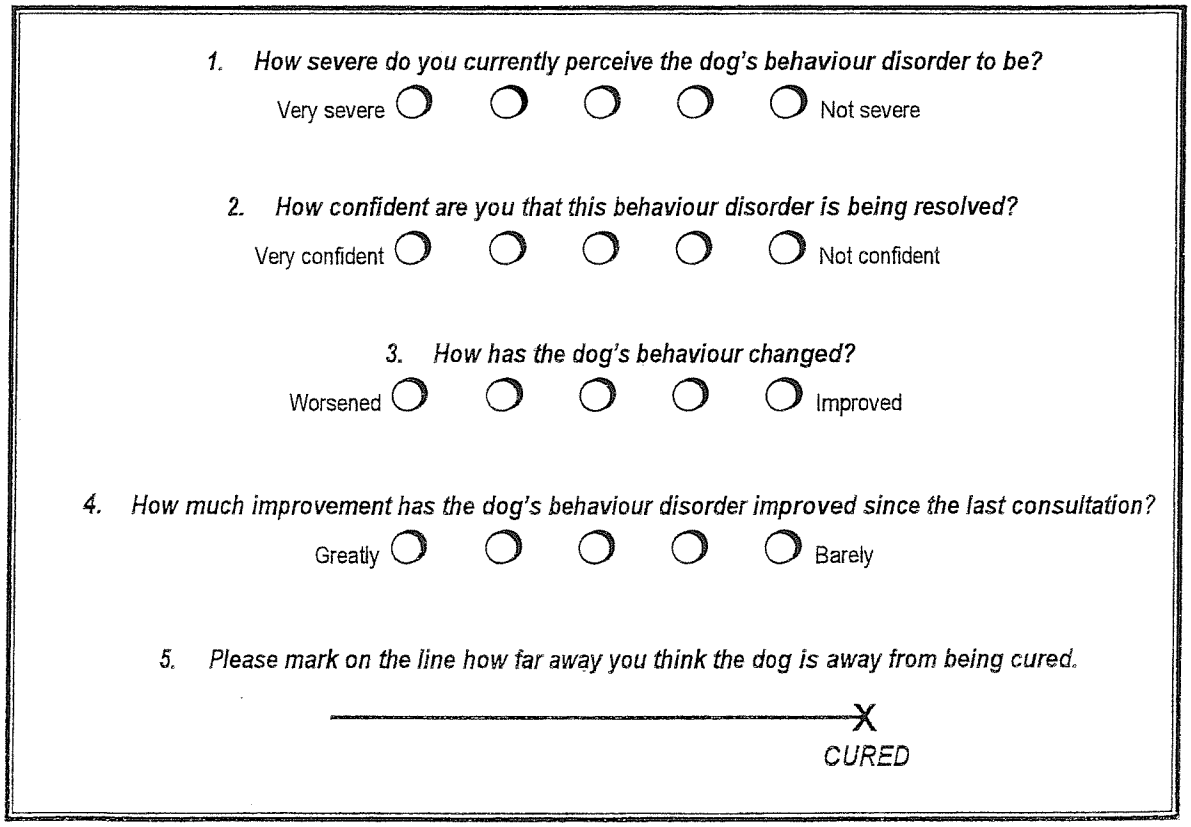

Figure 6.1 The counsellor assessment questionnaire with 5-point Likert scale.

6.2.1.3 Collection of hormonal data

The pilot study determined that blood sampling in the dog's home environment was feasible. Repeat visits were arranged for the same part of the day (morning or afternoon) in order to avoid the diurnal variations in hormone concentrations (Hedge *et al* 1987; M°Geown 1996). A measure of cortisol concentration for every dog at each visit was required. Ideally, salivary cortisol would have been the preferred method as salivary cortisol samples are less subject to fluctuation as a result of acute stressors than plasma samples. However, if the plasma sample is collected within a 3-minute period then the cortisol concentration of the sample is likely to be reflective of the stress status of the dog rather than reflective of the stress that may be caused due to the sampling procedure (Tuber *et al* 1996). A preliminary study had indicated that collection of salivary cortisol and analysis by the DELFIA method as used for human saliva, did not give consistent results when dog saliva samples were used (Appendix 13) and therefore plasma cortisol concentration was measured by RIA (Appendix 11).

6.2.1.4 Results

The pilot study indicated that when consultations were conducted in the owner's home the history-taking period of the consultation could last for up to two hours but after one hour did not provide any more relevant additional information about the dog's behaviour disorder. Therefore, prior to the consultation the owners were informed that the consultation would last for two hours. The first hour would involve their description of the history and development of the problem and in the second hour the counsellor would discuss the cause and treatment of the behaviour disorder.

(i) Direct observation

The pilot study indicated that some dogs responded to the observer when observed using a hand held camera. The owners confirmed that exhibition of such behaviour patterns was uncharacteristic of their dog. The incidence of such atypical behaviour patterns appeared to reduce when a tripod was used for videotaping, but frequently the focal dog was out of frame. It was therefore decided that this data was best collected by direct observation by the observer during the behaviour consultation.

(ii) Assessment questionnaire

The owner's assessment of changes in their dog's behaviour appeared likely to be unreliable, as they found it difficult to recall what behaviour patterns their dogs had displayed between visits. Therefore, the behaviour counsellor assessed the progression of the behaviour modification programme between visits and completed the questionnaire.

6.2.1.5 Discussion

The most suitable method for the collection and determination of cortisol was plasma and RIA respectively. The measurement of behaviour patterns using either a hand or tripod held video camera

was unsuitable for use in this study. Some consequential findings from the pilot study included the necessity for the veterinarian (who was also a qualified behaviour counsellor) to conduct routine procedures, e.g., the monitoring and administration of vaccinations. This was required as veterinary research suggests that vaccinations can contribute to the onset of autoimmune reactions that can interfere with testing for hypothyroidism and therefore any vaccines should be given at least two weeks before or immediately after testing for hypothyroidism, however this suggestion has yet to be confirmed. All procedures carried out on dogs in their home environment were only done with the permission of the dog's referring veterinary surgeon. The possibility that dogs may develop clinical hypothyroidism during the course of the behaviour modification was also identified and a development of hypothyroidism questionnaire was designed for us in the experimental study (Section 6.2.2).

6.2.2 Experimental study

The methods used in this study are described in Chapter 2 and are based on the results of the pilot study (Section 6.2.1).

6.2.2.1 Recruitment of sample

The eleven dogs used in this study were aged between 19-72 months (median 48 months), from eight different breed groups and comprising neutered males and females and entire females (Table 6.3). All dogs were recruited from veterinary referral as described previously (Section 2.3.2) and filled the selection criteria introduced in the pilot study (Section 6.2.1). All the dogs used in this study were diagnosed to have a fear or stress related behaviour on the basis that these may be more likely to be associated with an altered thyroid state (Section 1.3.4). Cases were recruited from a maximum 25-mile radius of Southampton University, in order to return blood samples to the laboratory for separation of plasma and storage before the anticoagulant expired and coagulation occurred and before significant changes in plasma cortisol levels prior to freezing.

Table 6.3 Signalment of sample

Dog Name	Age (months)	Breed	Sex
Buck	60	Collie cross	Male neutered
Daisy	72	Labrador cross	Female neutered
Dan	96	English Springer Spaniel	Male neutered
George	60	Lurcher	Male neutered
Hula	24	Labrador cross	Female neutered
Ki	48	Border Collie	Female entire
Millie	36	English Springer Spaniel	Female entire
Muddy	48	Collie cross	Male neutered
Sage	23	Bernese Mountain	Female neutered
Spanner	19	Cocker Spaniel	Male neutered
Zoe	24	Golden Retriever	Female neutered

6.2.2.2 Collection of behavioural data

All dogs were seen at their [1]homes on three occasions at 6 ± 1-week intervals. The initial [2]consultation lasted for approximately two hours in which the development of the disorder was established and a behaviour modification programme recommended. The discussion was based around a questionnaire, which was completed by the owner prior to the day of the consultation; the process was identical to consultations conducted at the ABC, (Section 2.4.1). For the two follow up visits the behaviour modification programme was reiterated, but no additional advice for any other behaviour disorder or for any other pet in the house was given. In such cases where the owner required assistance with the behaviour disorder of another pet this was addressed after completion of this study. Behavioural data was collected on each visit via: -

a) Direct observation

The dogs were observed for a 45-minute period of the consultation, as previously justified in Chapter 2 (Section 2.4.5). Instantaneous time sampling was used every 20 seconds of the 45 minute observation period to determine the behaviour patterns of the [3]focal dog. The behaviour patterns were noted on check sheets.

b) The owner interaction questionnaire

The owner interaction questionnaire (Section 2.4.2) was useful for the assessment of behavioural change directed towards familiar people between visits. In cases where the dog had more than one owner the same owner completed the questionnaire on the initial and subsequent follow up visits.

c) The assessment questionnaire

The assessment questionnaire (Section 2.4.3; Figure 6.1; Appendix 5) was designed for use in this study only and remained unchanged from its use in the pilot study (Section *6.2.1.4*).

6.2.2.3 Collection and analysis of hormonal data

At the end of every consultation a blood sample was taken (Section 2.3.1). Plasma was analysed for concentrations of T4 (Appendix 11), T3 (Appendix 11), TSH (Appendix 11), TgAA (Appendix 11), rT3 (Appendix 11) and cortisol (Appendix 11). Ideally, it would have been advantageous to measure TgAA and rT3 for the entire sample of dogs used in used in Chapters 3-6, however, this was not financially viable. As this study had a smaller sample size it was possible to measure TgAA and rT3 for each dog for which there was sufficient plasma. The role of TgAA and rT3 are summarised in

[1] The veterinarian and assistant (myself) reviewed the relevant health and safety guidelines prior to attending behaviour consultations in client's homes.
[2] All behavioural consultations were free of charge, however several owners made a donation to the University of Southampton.
[3] In multi dog households only the referred dog with the behaviour disorder was observed.

Sections 1.5.1 and 1.5.5. However, from a clinical and diagnostic perspective the measurement of TgAA allows the early diagnosis of autoimmune thyroiditis, whilst rT3 measures support a diagnosis of hypothyroidism that may be due to non-thyroidal factors, e.g., illness or fasting. Therefore, both TgAA and rT3 measures were beneficial for the examination of individual case studies in which the influence of other factors that may influence a dog's mental or physical state could be examined in depth.

6.2.3 Statistical analysis

In order to examine the effect of behaviour modification on hormonal concentration it was necessary to assess any underlying relationships between the hormones and to examine the possibility that any subsequent associations between behaviour and hormones may be influenced by the relationship between the hormones themselves. Therefore analysis of covariance (ANCOVA) was used to test hypothesis 3 and to examine the relationships between the hormones, for which the stress hormones, rT3 and cortisol, were the covariates and dog and visit were the main factors. Hypotheses 1, 2, 4 and 5 were then investigated using Spearman's Rank order correlation to investigate the relationships between behavioural and hormonal measures. The reliability of the *p*-values from these tests would have been improved if a larger sample had been studied, but insufficient time was available to study more cases.

6.2.3.1 Exploring behaviour patterns

(i) Direct observation

In order to focus the data analysis only the behaviour patterns that had been found to be weighted on meaningful factors in previous PCA were considered, i.e., Activity and Aggressivity (Section 3.2.2 *Behavioural variables* and Section 3.3.3). Each behaviour pattern was scored according to the intensity of its component behaviour patterns (Table 6.4).

Table 6.4 Behaviour patterns and the scores comprising the factors Activity and Aggressivity

Factor	*Behaviour pattern*	*Intensity of behaviour*	*Score*
Activity	Head position	Up	1
		Level	0
		Down	-1
	Whimper	Whimpering	1
	Body Position	Stand	1
		Sit	0
		Lay	-1
Aggressivity	Ear Position	Up	1
		Down	0
	Growl	Growling	1
	Proximity	Near familiar	1

As behaviour consultations were subject to interruptions there were differences in the total observation period for each dog (Section 2.2.2.2). Therefore, a rate was calculated for each behaviour pattern by dividing the total score for each behaviour pattern by the total number of observations made. Lastly, for each factor the mean was calculated by dividing the rate by the number of behaviour patterns comprising the factor.

(ii) Owner interaction

As with the direct observations of behaviour ((i) above) only the behaviour patterns found to be important in previous investigations were considered (see Section 4.3.2). These behaviour patterns were scored on the intensity of the behaviour pattern (Table 6.5), then added together and the means were calculated by dividing the total score by the number of behaviour patterns comprising the factor.

Table 6.5 Behaviour patterns and the scores comprising the factor Sedentary Posture

Factor	*Behaviour pattern*	*Scored on intensity*
Sedentary Posture	Lay	1
	Sit	0
	Roll	-1

6.2.3.2 Counsellor assessment

Data collected using the assessment questionnaire were first tested for reliability using Cronbach Alpha, which is based on the average inter-item correlation (SPSS v10). All scales were scored from -2 (negative state or change) to +2 (positive state or change). A reliability coefficient (alpha) of >0.7 indicates that the questions are not meaningfully different (Siegel and Castellan 1988) and the results are suitable for averaging. The answers to the five questions comprised in the counsellor questionnaire were averaged for both visits two and three.

6.2.3.3 Hormonal associations

Analysis of covariance (ANCOVA; General Linear Model Univariate procedure; SPSS v10) was used to examine the effect of visit and dog on T4, T3 or TSH. Additionally, the effects of the covariate interactions (cortisol and rT3) with the hormones was also examined (hypothesis 3). This was necessary in order to rule out any underlying relationships between the hormones that may influence the results of investigations into the association between behavioural and hormonal measures. The association between cortisol and rT3 was tested using Spearman's rank correlation.

Prior to carrying out the ANCOVA tests transformation of data was required as ANCOVA is a parametric test that combines linear regression and ANOVA. In order to determine the most suitable

method of transformation a larger dataset supplied by the Endocrine Unit Michigan State University (Chapter 7) of hormonal measures T4 (n=218), T3 (n=218) and TSH (n=218) was explored. The results of this were extrapolated to include rT3, TSH and cortisol as these hormones are subject to the equivalent metabolic parameters as T4, T3 and TSH. The data was square root transformed because after examining the square root and log transformation the square root transformation equalised the variation on the mean. As several variables contained zero measures the transformation used was therefore $\sqrt{(x+0.5)}$, where x is either T4, T3, TSH, rT3 or cortisol.

6.2.3.4 Hormonal and behavioural associations

In order to compare the behavioural with hormonal data the measures needed to be comparable. Therefore the difference between visits two and three was then calculated for the counsellor assessment, Activity, Aggressivity and Sedentary Postures and hormonal measures (T4, T3, TSH, rT3 and cortisol) for each dog individually. Spearman's Rank Order Correlation was used to test the correlation between the behavioural and hormonal measures. The changes in behaviour as a result of behaviour modification (as assessed by the behaviour counsellor) as well as the changes in each hormone are shown for each visit in Section 6.3).

6.2.4 Individual case studies

6.2.4.1 Why case studies?

The use of several well-structured and closely monitored case studies (in addition to empirical research) provides valuable information for research of this kind for which there is little published work. All the behavioural and hormonal data collected from this sample was analysed collectively, as described above. A considerable amount of individual data was also collected and is presented descriptively. Several factors that were inappropriate for consideration in the statistical analysis were considered from the information compiled in the case studies. These include:

i. The association between subtle changes in behaviour, which may point to possible internal motivations, with changes in hormones.
ii. The identification of a specific onset of hypothyroidism deduced from hormone measurements and the possibility that this may be linked to behavioural change. More specifically, the environmental factors that may have triggered behavioural change, e.g., the dog's reaction to changes in household composition.
iii. The onset of global incidents that may have had an indirect influence on dog behaviour patterns, e.g., the restrictions enforced by the foot and mouth outbreak in summer 2001.
iv. The occurrence of non-pathological injuries, e.g., wounds and how this relates to hormonal and behavioural change.

6.2.4.2 The development of hypothyroidism

In order to determine whether or not during the course of treatment the dogs were developing any behavioural or physiological symptoms associated with clinical hypothyroidism the owners were asked to confirm if their dog showed any of the symptoms associated with a hypothyroid condition. At each consultation a list of symptoms possibly associated with hypothyroidism was read out to the owners. These included behavioural symptoms to which the owner was asked to reply with "increase", "decrease" or "no change", e.g., "since our last visit has (dog's name) showed an increase, decrease or no change in aggressive behaviours directed towards people?" (Appendix 4). Owners were also asked about the development of physiological symptoms to which they were asked to reply with a "Yes" or "No", e.g., "Hair loss, scaly skin, constipation" (Appendix 4). In order to collect all the required information both the behaviour counsellor and myself attended each visit. This information was then compiled in each dog's case profile.

6.3 Results

The results for all eleven cases are presented first, followed by the case profiles.

6.3.1 Counsellor assessment

The Cronbach alpha indicated that the responses to the questions compiled in the counsellor assessment questionnaire were highly intercorrelated (alpha = 0.91) and therefore the answers to questions 1-5 were averaged. If stress is causing an increase in cortisol and subsequent reduction in T4, T3 and/or TSH then as the behaviour modification programme progresses and the dogs become less stressed an increase in T4, T3 and/or TSH would be expected, while both cortisol and rT3 would decrease. Six dogs had an improvement in behaviour on visits two and three, two dogs showed an improvement only on visit two, two dogs showed an improvement only on visit three and one dog showed no improvement (Table 6.6).

These assessments were considered with the changes in thyroid hormone concentration. T4 (Figure 6.2) was generally reduced between visit one and two, but showed an increase at visit three for three dogs (Sage, Mille and Ki; Section 6.3.4 see case profiles) all of whom showed an improvement in behaviour at visit three. Four dogs had an increase in T3 (Figure 6.3) at visits two and three, two of these dogs (Zoe and Daisy; Section 6.3.4 see case profiles) showed an improvement in behaviour at visit three only, whilst the two other dogs (Millie and Ki; Section 6.3.4 see case profiles) showed an improvement in behaviour at both visits two and three. Five dogs had the same measures of TSH at every visit (Figure 6.4), for three dogs the measure of TSH remained unchanged at each visit (Zoe, Daisy and Hula; Section 6.3.4 see case profiles) and two dogs (Dan and Spanner; Section 6.3.4 see case profiles) their TSH value reduced between visit one and two and remained the same at visit

three. There was no general pattern of behavioural change between visits. Of the eight dogs for which cortisol measures are available for each of the three visits, five dogs at visit two and four dogs at visit three had an improvement in behaviour but showed an increase in cortisol (Figure 6.5). Of the dogs that showed an improvement on behaviour at visit two and three only one (Muddie; Section 6.3.4 see case profile) had a decrease in rT3 on both visits (Figure 6.6).

Table 6.6 Behaviour counsellor's assessment of the behaviour disorder at visits 2 and 3. Assessments were made on comparing the dog's behaviour to the previous visit.

Dog	Visit 2	Visit 3
Buck	Improved	No improvement
Daisy	No improvement	Improved
Dan	No improvement	No improvement
George	Improved	Improved
Hula	Improved	Improved
Ki	Improved	Improved
Millie	Improved	Improved
Muddy	Improved	Improved
Sage	Improved	Improved
Spanner	Improved	No improvement
Zoe	No improvement	Improved

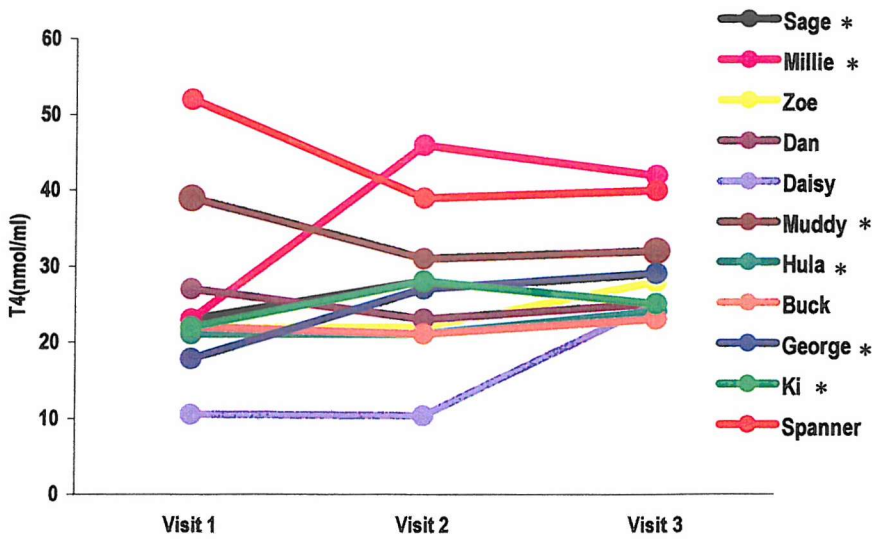

Figure 6.2 T4 changes between visits
***Dogs for which the behaviour disorder is reported by the counsellor as improved, at both visits 2 and 3. Sage and Ki have the same measures of T4 at visits 2 and 3**

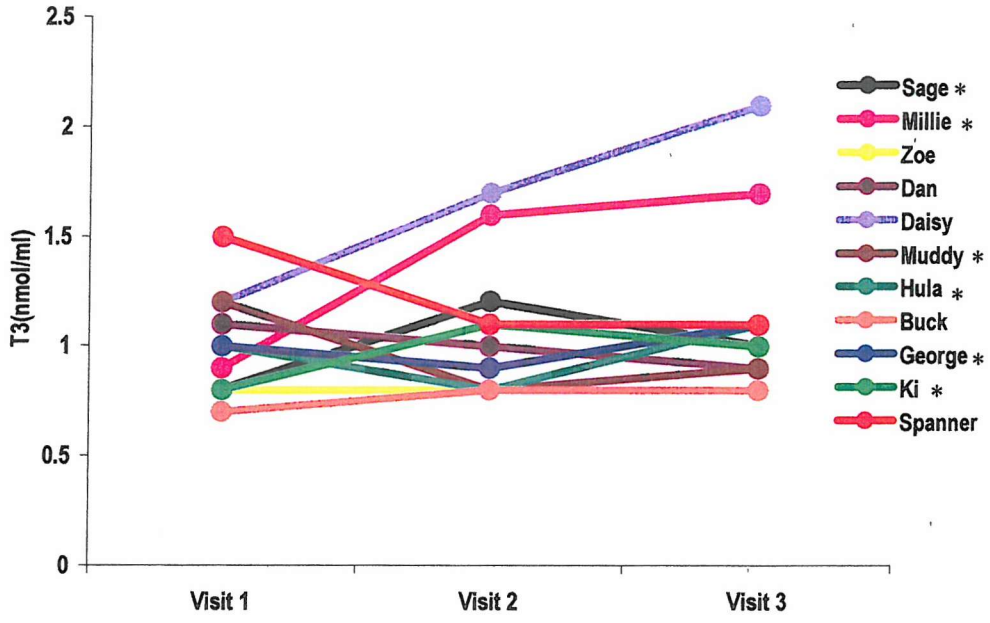

Figure 6.3 T3 changes between visits.
***Dogs for which the behaviour disorder is reported by the counsellor as improved at each visit, Zoe and Muddy have the same measures of T3 at visits 2 and 3.**

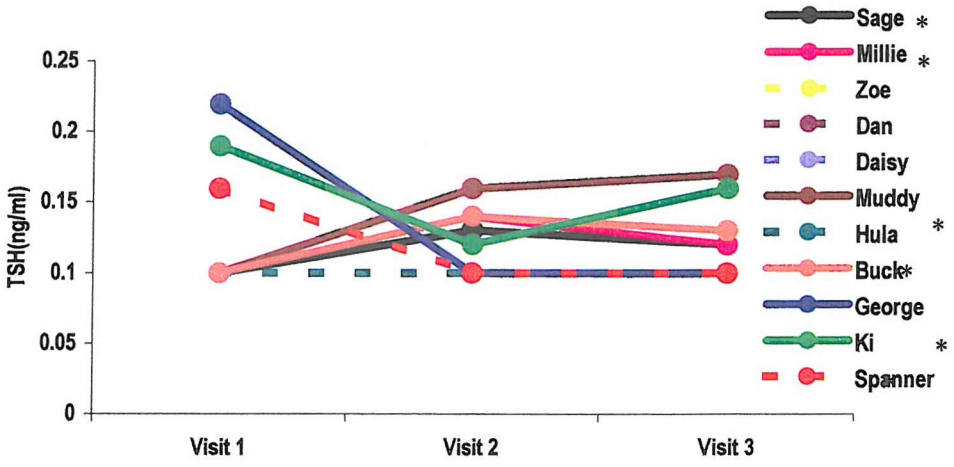

Figure 6.4 TSH changes between visits
***Dogs for which the behaviour disorder is reported by the counsellor as improved at each visit, Spanner and George have the same measures of TSH at visits 2 and 3.**

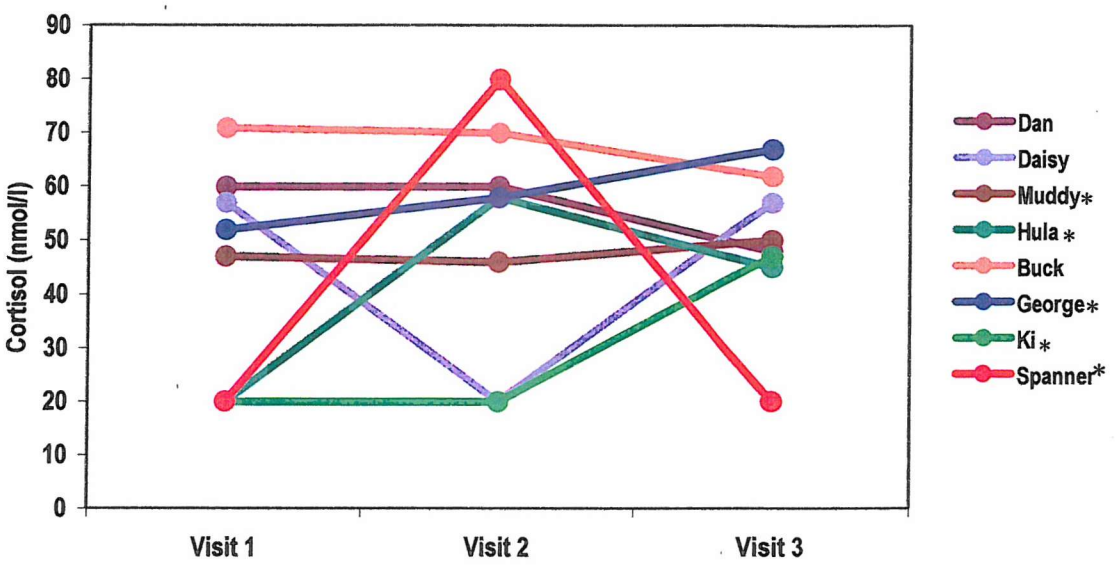

Figure 6.5 Cortisol changes between visits.
***Dogs for which the behaviour disorder is reported by the counsellor as improved at each visit.**

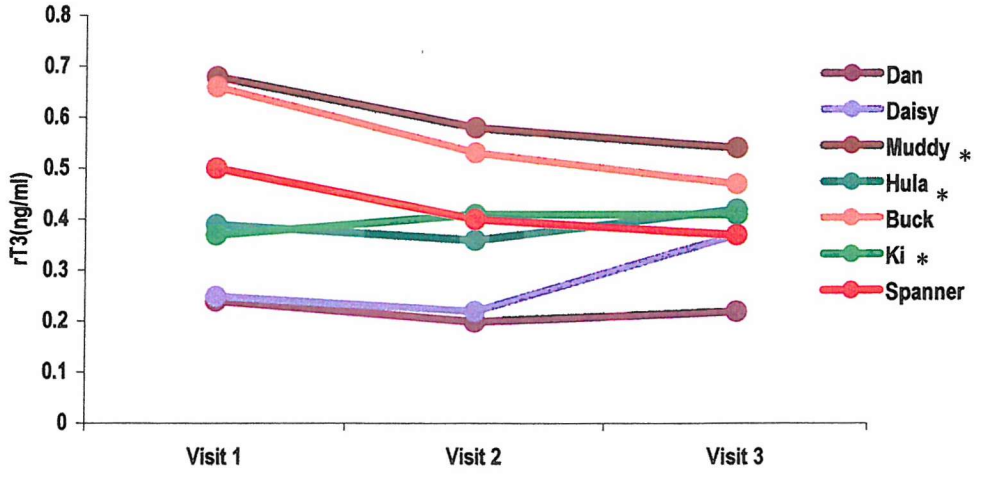

Figure 6.6 rT3 changes between visits
***Dogs for which the behaviour disorder is reported by the counsellor as improved at each visit.**

One dog (Daisy) had a [4]T4 titre below the normal range, 10.5nmol/l and 10.3nmol/l at visits 1 and visit 2 respectively, and her [5]rT3 values for visits 1 and visit 2 were abnormal measuring 0.25ng/ml and 0.22ng/ml respectively. Daisy's behaviour only improved at visit three when her T4 and rT3 titres were in the normal range. Two other dogs had an abnormal rT3 reading. Muddy's rT3 measured 0.68ng/ml at visit one, but this had decreased by visit two and further reduced at visit three when his behaviour was reported to have improved. This is what I would have predicted as when stress decreases rT3 is expected to decrease. Dan's rT3 measured 0.24ng/ml at vist one and remained lower than normal at visits two and three; he was the only dog to show no improvement in behaviour throughout the study. One dog (Ki) gave a positive result for TgAA and this was measured on visit 3, Ki's behaviour was reported to have continually improved throughout the study and he was referred to his vet for re-testing for TgAA.

6.3.2 Relationships between hormones

The relationship between T4 and T3 is well documented both in the endocrinology literature and throughout this thesis (Chapters 3-5). Whilst the association between rT3 and the thyroid hormones T4 and T3 is also well acknowledged I have investigated the relationship between these hormones for this sample. Results from the ANCOVA determined a relationship between rT3 with T4 ($F_{1, 14}$=26.68, p<0.0001; Figure 6.7) as well as rT3 and T3 ($F_{1, 14}$=20.00, p=0.001; Figure 6.8).

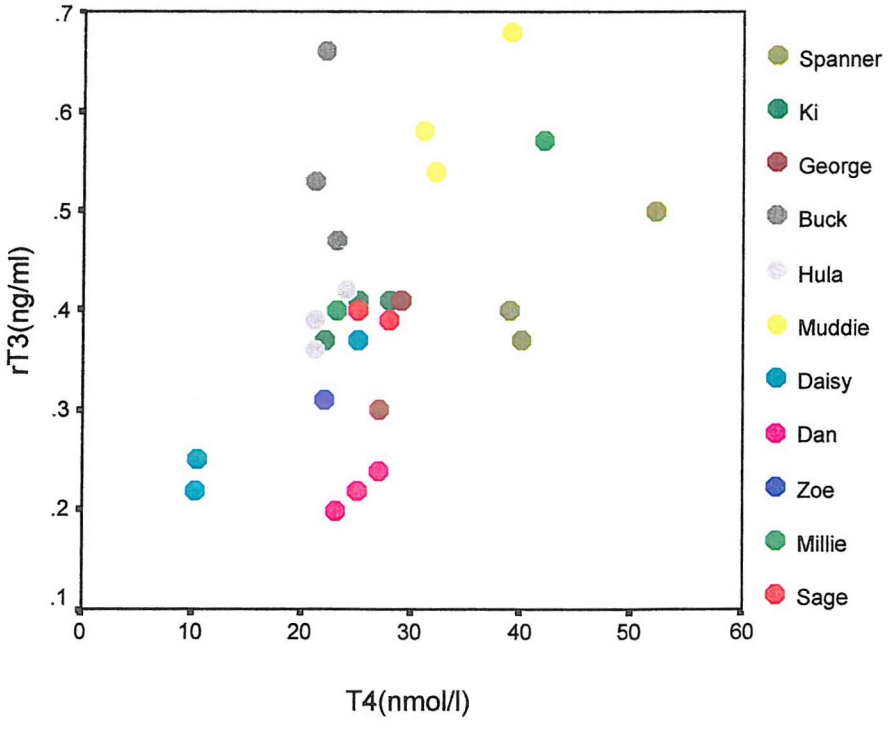

Figure 6.7 Association between T4 and rT3 with individual dogs highlighted.

[4] Normal range for T4 is 13-53nmol/l.
[5] Normal range for rT3 is 0.27-0.62ng/ml.

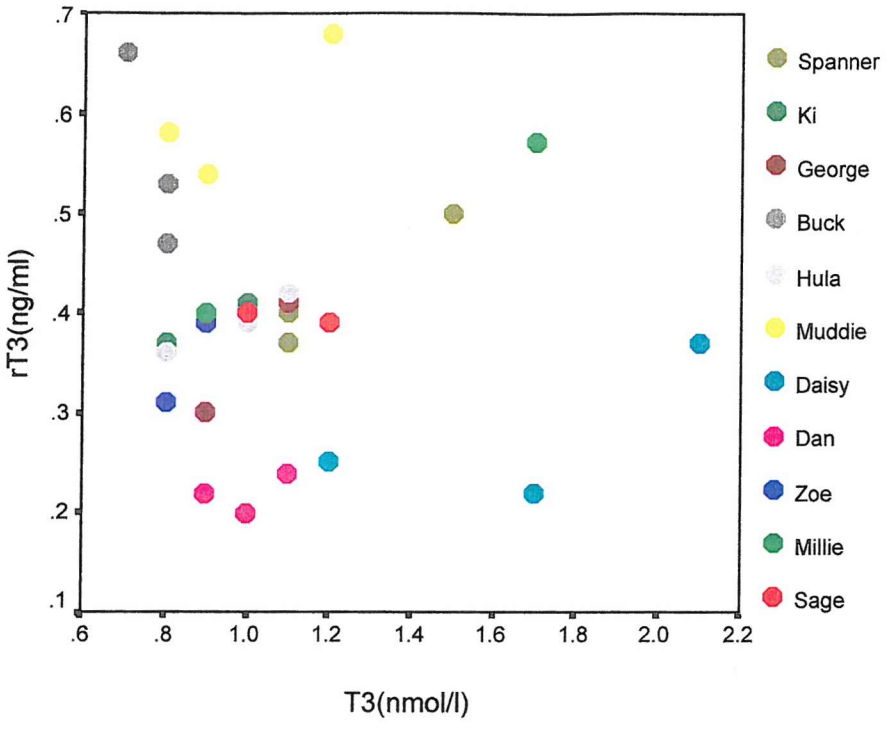

Figure 6.8 Association between T3 and rT3 with individual dogs highlighted.

A significant association between rT3 and T4/T3 is what I would have expected as T4 acts as a precursor to T3 (Section 1.3.2) and rT3, rT3 being a more common product in a cortisol rich environment. Cortisol was not significantly associated with T4 ($F_{1, 14}$=0.29, p=0.60) or with T3 ($F_{1, 14}$=1.84, p=0.20), or rT3 (r_s = 0.23, p=0.50).

6.3.3 Relationship between hormones and behaviour

A significant negative correlation was found between T3 titre and Activity (r=-0.86, p<0.01; Figure 6.9). The change in T3 titre correlated negatively with Activity in that increased T3 was associated with a decrease in Activity. Whilst the association between Activity and T3 was expected, the direction of the correlation is unusual as a reduction in T3 would usually be associated with a reduction in Activity and therefore a positive correlation coefficient would have been predicted. No significant relationships were found between Aggressivity or Sedentary posturing with the hormones.

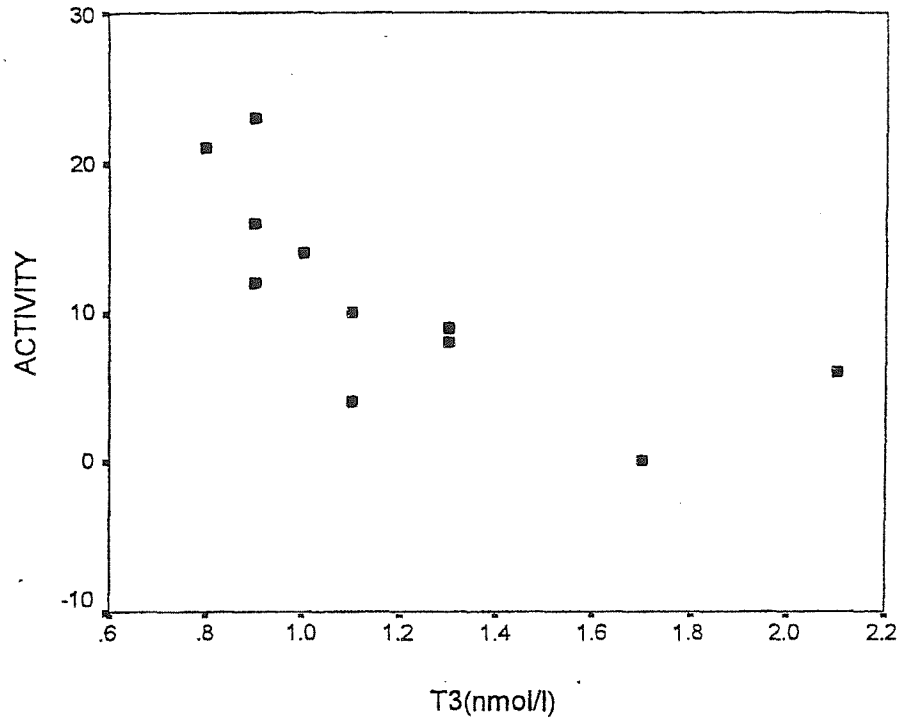

Figure 6.9 Association between Activity and T3. 11 points = one per dog as mean values were taken for visits 1-3 for both Activity and T3(nmol/l).

6.3.4 Case profiles

The data for each case was analysed collectively. The information collected on the development of hypothyroidism (6.2.5) was collected in order to rule out the possibility that some dogs were developing hypothyroidism during the course of treatment for their behaviour disorder as this could have influenced the results. I have included a case profile for each dog in order to examine the subtle information not appropriate for statistical analysis. However, the results can be summarised in that six cases required no further treatment and the behaviour modification programme was considered successful by the counsellor and the owner. In the remaining seven cases, although the behaviour of the dogs had improved substantially, additional behaviour modification was required in order to complete the treatment.

Case Profile 1: Sage

Age:	*23 months.*
Sex:	*Female, neutered.*
Breed:	*Burnese Mountain dog.*
Homed from at age:	*Breeder at six weeks.*
Household composition:	*Three people; Two females, one aged between 30-40 (Mrs A), one aged between 60-80 (Mrs A's mother) and a male aged between 40-50 (Mr A). Three Burmese Mountain dogs; two females aged 32 months and six months, and a male aged 17 months. All dogs were neutered.*
Medical history	*Successful surgery on inverted eyelids.*
Behaviour disorder	*Learnt undesirable attention seeking behaviour patterns.*
Onset of disorder:	*16 months of age.*
Origin of disorder:	*Sage was homed when she was six weeks of age and she developed a very strong attachment towards Mrs A. Sage exhibited behaviour patterns that were successful in obtaining Mrs A's attention, these included jumping up on and biting her on the hand, for which Sage was sometimes punished (pushing the dog to the floor and shouting) and sometimes given positive attention (petting and play).*
Reason for continuation of disorder:	*The inappropriate use of punishment and reinforcement by Mrs A resulted in Sage exhibiting further aggressive behaviour due to a motivational conflict between wanting to approach and fear of punishment whilst in the presence of her owner; which compounded the disorder.*
At visit 1	*A treatment programme was recommended in which the main priority was the emphasis of consistent interactions in order to return the predictability to the dog-owner relationship. Mrs A was taught how to maintain the control of the resources that the dogs perceived as valuable (food, toys and attention from people mainly herself) and how control of resources can prevent the requirement for punishment techniques. This programme was applied to all the dogs. The second part of the behaviour modification programme was focused on teaching Sage that jumping was no longer a successful behavioural strategy in order to receive attention from Mrs A. For this, Sage was ignored when she jumped up and/or mouthed Mrs A's hand, and she was rewarded with attention for exhibiting calm behaviour patterns such as sitting and lying down.*
Blood test results for visit 1	*T4 =23.0 nmol/ml* *T3 = 0.8 nmol/ml* *TSH =<0.1 ng/ml* *TgAA =Insufficient plasma* *rT3 = Insufficient plasma* *Cortisol = Insufficient plasma*
At visit 2	*Mrs A described Sage's behaviour to have changed, in that there was a decrease in inappropriate mounting, fearful /nervous behaviour and aggressive behaviour patterns. Veterinary assessment noted that Sage had gained weight.*
Blood test results for visit 2	*T4 =28.0 nmol/ml* *T3 =1.2 nmol/ml* *TSH =0.13 ng/ml* *TgAA = Negative* *rT3 =0.39 ng/ml* *Cortisol =<20.0 nmol/L*
At visit 3	*Mrs A described an increase in exploratory behaviour patterns, inappropriate mounting, a further decrease in aggressive behaviours towards people and a decrease in excitable and vocal behaviour patterns. Veterinary assessment observed Sage's weight increased attributable to approaching maturity.*
Blood test results for visit 3	*T4 =25.0 nmol/ml* *T3 =1.0 nmol/ml* *TSH =0.12 ng/ml* *TgAA = Negative* *rT3 =0.40 ng/ml* *Cortisol =<20.0 nmol/L*

Case Profile 2: Millie

Age:	*36 months.*
Sex:	*Female, entire.*
Breed:	*English Springer Spaniel.*
Homed from at age:	*Breeder at nine weeks.*
Household composition:	*Five people; three females, one aged between 30-40 (Mrs A), one aged between 60-80 (Mrs A's mother) and a girl aged two years, two males one aged between 40-50 (Mr B) and a boy 10 years of age. One female cat aged 18 years and one rabbit aged nine months.*
Medical history	*No significant medical problems. Possible pseudopregnancy at time of visit one.*
Behaviour disorder	*Learnt fearful aggressive behaviours directed towards strangers visiting the house and food raiding from the food preparatory surfaces in the kitchen.*
Onset of disorder:	*No specific date of onset given.*
Origin of disorder:	*During her socialisation period Millie was not exposed to people outside of her normal social group and hence developed a fear of unfamiliar people.*
Reason for continuation of disorder:	*Millie learnt that barking and growling when people approach results in their retreat. As Millie is fearful of unfamiliar people she quickly learnt that exhibition of aggressive behaviour patterns was an effective way of preventing people approaching her and she became more confident that the exhibition of such behaviour was successful.*
At visit 1	*Recommended a treatment programme in which the main priority was the emphasis of Mrs A as having control of situations and therefore Millie need not become aroused in situations involving visitors to the house. Additionally, a desensitisation and counter-conditioning programme was recommended to treat Millie's fear of unfamiliar people. Lastly, a remote punishment technique was used to prevent Millie raiding the bin and counter top. Extra activities i.e., toys, were also suggested for periods of respite in which Millie need not be concerned about people's actions when present in her immediate environment.*
Blood test results for visit 1	*T4 =23.0 nmol/ml* *T3 =0.9 nmol/ml* *TSH =<0.1 ng/ml* *TgAA =Negative* *rT3 =0.40 ng/ml* *Cortisol =52.0 nmol/L*
At visit 2	*Mrs A reported a decrease in vigilance, fearful and nervous behaviour patterns and an increase in exploratory behaviours (could be related to environmental changes), obedience, and inappropriate mounting. Veterinary assessment determined that Millie had lost weight.*
Blood test results for visit 2	*T4 =46.0 nmol/ml* *T3 =1.6 nmol/ml* *TSH =0.14 ng/ml* *TgAA =Insufficient plasma* *rT3 = Insufficient plasma* *Cortisol = Insufficient plasma*
At visit 3	*Mrs A reported a further decrease in excitable, aggressive, fearful and nervous behaviour and an increase in obedient, vocal and mounting behaviour patterns. Veterinary assessment determined that Millie had increased weight. The foot and mouth epidemic during summer 2001 resulted in the closure to the public of several areas of the New Forest and therefore Millie was not exercised on a daily basis, this may have accounted for the increase in weight.*
Blood test results for visit 3	*T4 =42.0 nmol/ml* *T3 =1.7 nmol/ml* *TSH =0.12 ng/ml* *TgAA = Negative* *rT3 =0.57 ng/ml* *Cortisol =97.0 nmol/L*

Case Profile 3: Zoe

Age:	*24 months.*
Sex:	*Female, neutered.*
Breed:	*Golden Retriever.*
Homed from at age:	*Breeder at eight weeks.*
Household composition:	*Two adults, Mr and Mrs A, both retired.* *Two dogs one female Golden Retriever aged 24 months and one male cross breed aged 15 years.*
Medical history:	*Repaired cruciate ligament in left rear leg in 1999.*
Behaviour disorder:	*A specific noise phobia.*
Onset of disorder:	*When Zoe was aged 22 months.*
Origin of disorder:	*An aversive experience during a visit to the countryside. One evening Zoe was left in a caravan and outside there were several loud gunshots that caused her to become fearful.*
Continuation of disorder:	*When Zoe is left alone during the evening she becomes very fearful and is sometimes destructive.*
Visit 1	*Recommended a treatment programme in which the main priority was building Zoe's confidence and independence. A desensitisation and counter-conditioning programme was used to treat her fear of noises and of particular contexts, such as the caravan, which had become associated with the aversive experience. In addition, Zoe's fearful behaviour was no longer reinforced by Mr and Mrs A's attention.*
Blood test results for visit 1	*T4 =22.0 nmol/ml* *T3 =0.8 nmol/ml* *TSH =<0.1 ng/ml* *TgAA = Insufficient plasma* *rT3 = Insufficient plasma* *Cortisol = Insufficient plasma*
Visit 2	*Mr and Mrs A reported that Zoe had shown an increase in her vigilance and restlessness, excitable, aggressive behaviour towards strange dogs and increased urination. The lack of improvement in Zoe's condition was influenced by a change in her usual walking patterns due to the restrictions enforced by the outbreak of Foot and Mouth disease.*
Blood test results for visit 2	*T4 =22.0 nmol/ml* *T3 =0.8 nmol/ml* *TSH =<0.1 ng/ml* *TgAA = Negative* *rT3 =0.31 ng/ml* *Cortisol =52.0 nmol/L*
Visit 3	*Mr and Mrs A reported that Zoe had shown a decrease in her vigilance behaviour and distress related behaviours when left alone and an increase in fearful and nervous behaviour patterns. Prior to visit 3 and contrary to the veterinary advice, Zoe was given skullcap with valerian to assist in the treatment of anxiety, excitability and nervousness (Dorwest Herbs).*
Blood test results for visit 3	*T4 =28.0 nmol/ml* *T3 =0.9 nmol/ml* *TSH =<0.1 ng/ml* *TgAA = Negative* *rT3 =0.39 ng/ml* *Cortisol =<20.0 nmol/L*

Case Profile 4: Dan

Age:	*96 months.*
Sex:	*Male neutered.*
Breed:	*Springer Spaniel.*
Homed from at age:	*Springer Spaniel rescue centre at 48 months of age*
Household composition:	*Four people; Mrs A aged between 40-50, Mr Mrs A aged between 40-50, and their two sons aged between 20-30. One female Bassett Hound aged approximately 96 months.*
Medical history:	*No significant medical problems.*
Behaviour disorder:	*Separation related behaviour disorder.*
Onset of disorder:	*When Dan was four years of age.*
Origin of disorder:	*Dan was not efficiently habituated to being left alone as a puppy.*
Continuation of disorder:	*Mrs A has contributed to Dan's anxiety by using inconsistent punishment techniques for misbehaviour.*
Visit 1	*Advised treatment programme included promoting positive social interactions between Mrs A and Dan as well as a desensitisation and counter conditioning programme to enable Dan to be left alone without becoming anxious.*
Blood test results for visit 1	*T4 =27.0 nmol/ml* *T3 = 1.1 nmol/ml* *TSH =0.16 ng/ml* *TgAA = Negative* *rT3 = 0.24 ng/ml* *Cortisol =60 nmol/L*
Visit 2	*Behaviour modification programme continued, although it was necessary to reiterate the advice given on the last visit, as Mrs A appeared to have misunderstood the use of rewards. Dan also developed a lump in his ear; this was examined by his veterinarian and diagnosed as a minor cyst.*
Blood test results for visit 2	*T4 =23.0 nmol/ml* *T3 = 1.0 nmol/ml* *TSH =<0.10 ng/ml* *TgAA = Negative* *rT3 = 0.20 ng/ml* *Cortisol =60 nmol/L*
Visit 3	*Behaviour modification programme continued, however, due to a change in the owner's circumstances Dan had to be placed in a kennel for one week, however, this did not appear to have any effect on his behaviour.*
Blood test results for visit 3	*T4 =25.0 nmol/ml* *T3 = 0.9 nmol/ml* *TSH =<0.1 ng/ml* *TgAA = Negative* *rT3 = 0.22 ng/ml* *Cortisol =48nmol/L*

Case Profile 5: Daisy

Age:	*72 months.*
Sex:	*Female, neutered.*
Breed:	*Labrador / Collie cross.*
Homed from at age:	*Private house at eight weeks.*
Household composition:	*Two adults; Mr and Mrs A both aged 50-60, two cats; a male and a female both aged around seven years and neutered.*
Medical history	*No significant medical problems.*
Behaviour disorder	*Learnt fearful aggressive behaviours directed towards children.*
Onset of disorder:	*Daisy was 36 months.*
Origin of disorder:	*A 12-year-old boy was tormenting her with food and she growled and snapped at him, she was then punished.*
Continuation of disorder:	*The misuse of punishment techniques caused Daisy to learn that when children are present she needs to behave defensively. Daisy learnt that people ignore warning signals therefore she stopped signalling prior to snapping. As snapping has worked for Daisy on previous occasions to get people to move away from her, the behaviour pattern was repeated to get the same outcome.*
At Visit 1	*Recommended a treatment programme in which the main priority was to decrease Daisy's fear of children and therefore prevent her motivation to behave aggressively. This was done through a carefully controlled desensitisation and counter-conditioning programme. For safety reasons Mr and Mrs A were also told to avoid situations were crowds of people and food was present.*
Blood test results for visit 1	*T4 =10.5 nmol/ml* *T3 =1.2 nmol/ml* *TSH =0.10 ng/ml* *TgAA = Negative* *rT3 =0.25 ng/ml* *Cortisol =57.0 nmol/L*
At Visit 2	*Owners reported a no changes in behaviour or development of symptoms.*
Blood test results for visit 2	*T4 =10.3 nmol/ml* *T3 =1.7 nmol/ml* *TSH =<0.1 ng/ml* *TgAA = Negative* *rT3 =0.22 ng/ml* *Cortisol =<20.0 nmol/L*
At Visit 3	*Daisy was placed in a kennel whilst both owners were overseas; therefore the behaviour modification had not progressed. Daisy has shown a decrease in play behaviour. Owners suggest that Daisy is less reactive to strangers.*
Blood test results for visit 3	*T4 =25.0 nmol/ml* *T3 =2.1 nmol/ml* *TSH =<0.1 ng/ml* *TgAA = Negative* *rT3 =0.37 ng/ml* *Cortisol =57.0 nmol/L*

Case Profile 6: Muddy

Age:	*48 months.*
Sex:	*Male, neutered.*
Breed:	*Collie cross.*
Homed from at age:	*Private house at seven weeks*
Household composition:	*Two adults; Owner and lodger, both females aged 20-30 and two other dogs, mixed breeds, one male aged 60 months and a female aged 156 months.*
Medical history	*No significant medical problems.*
Behaviour disorder	*Fearful of people and strange dogs.*
Onset of disorder:	*No specific date of onset given.*
Origin of disorder:	*Attended training classes and some crowded events, which Muddy appeared to consider aversive possibly due to previous lack of socialisation to such circumstances.*
Continuation of disorder:	*Fear unwittingly reinforced by owner.*
Visit 1	*Reduce Muddy's fear of people coming to the house and reduce Muddy's fear of people outside the house using a desensitisation and counter conditioning programme.*
Blood test results for visit 1	*T4 =39.0.0 nmol/ml* *T3 =1.2 nmol/ml* *TSH =0.10 ng/ml* *TgAA = Negative* *rT3 =0.68 ng/ml* *Cortisol =<20.0 nmol/L*
Visit 2	*Owner reported a decrease in vigilance and restlessness, fearful / nervous behaviour, aggressive behaviour patterns and an increase in excitable behaviour, disobedient behaviour and an increase in eating faeces.*
Blood test results for visit 2	*T4 =31.0 nmol/ml* *T3 =0.8 nmol/ml* *TSH =0.16 ng/ml* *TgAA = Negative* *rT3 =0.58 ng/ml* *Cortisol =<20.0 nmol/L*
Visit 3	*Owner reported an increase in play, excitable behaviour and aggressive behaviours to dogs outside. A decrease in fearful / nervous behaviour, excitable behaviour, disobedient and vocal behaviour.*
Blood test results for visit 3	*T4 =32.0 nmol/ml* *T3 =0.9 nmol/ml* *TSH =0.17 ng/ml* *TgAA = Negative* *rT3 =0.54 ng/ml* *Cortisol =<20.0 nmol/L*

Case Profile 7: Hula

Age:	*24 months.*
Sex:	*Female, neutered.*
Breed:	*Labrador cross.*
Homed from at age:	*Labrador Rescue at age unknown.*
Household composition:	*One adult female aged 20-30 (owner).*
Medical history	*No significant medical problems.*
Behaviour disorder	*Separation related disorder.*
Onset of disorder:	*18 months of age.*
Origin of disorder:	*Hula's howling when left alone indicates her anxiety was due to a lack of habituation to being apart from her social group. Hula is not very confident and this contributed to her developing her anxiety. However, her owner has also reinforced Hula's fearful behaviour with attention.*
Continuation of disorder:	*Fearful response reinforced by owner and inconsistent positive and negative interaction as Hula was rewarded and punished for displaying the identical behaviour patterns in similar situations.*
Visit 1	*Advised treatment programme included promoting positive social interactions between Hula and her owner as well as a desensitisation and counter conditioning programme to enable Hula to be left alone without becoming anxious.*
Blood test results for visit 1	*T4 =21.0 nmol/ml* *T3 =1.0 nmol/ml* *TSH =<0.10 ng/ml* *TgAA = Negative* *rT3 =0.39 ng/ml* *Cortisol =<20.0 nmol/L*
Visit 2	*Owner reported an increase in alertness, urination, play behaviour, excitable behaviour, disobedient behaviour and aggressive behaviour. Less anxiety when left alone.*
Blood test results for visit 2	*T4 =21.0 nmol/ml* *T3 =0.8 nmol/ml* *TSH =<0.10 ng/ml* *TgAA = Negative* *rT3 =0.36 ng/ml* *Cortisol =58.0 nmol/L*
Visit 3	*Owner reported an increase in vocalisations, exploratory behaviour, coprophagia, disobedient and nervous behaviour, and an increase in weight, also a decrease in fearful / nervous behaviour to people, aggressive behaviour, a slight decrease in destructive behaviour.*
Blood test results for visit 3	*T4 =24.0 nmol/ml* *T3 =1.1 nmol/ml* *TSH =<0.10 ng/ml* *TgAA = Negative* *rT3 =0.42 ng/ml* *Cortisol =45.0 nmol/L*

Case Profile 8: Buck

Age:	*60 months*
Sex:	*Male, neutered*
Breed:	*Collie cross*
Homed from at age:	*Rescue at 18 months*
Household composition:	*Two adults; Owner and lodger, both females aged 20-30 and two other dogs, mixed breeds, one male aged 60 months and a female aged 156 months.*
Medical history	*No significant medical problems*
Behaviour disorder	*Separation related behaviour disorder and stereotypical behaviour patterns – repetitive circling.*
Onset of disorder:	*No specific date of onset.*
Origin of disorder:	*Lack of habituation to being left alone.*
Continuation of disorder:	*Owner reinforcement of behaviour disorder.*
Visit 1	*Recommended treatment programme involved owner controlling the start and finish of interactions and desensitising Buck to being left alone.*
Blood test results for visit 1	*T4 =22.0 nmol/ml* *T3 =0.7 nmol/ml* *TSH =<0.10 ng/ml* *TgAA = Negative* *rT3 =0.66 ng/ml* *Cortisol =<71.0 nmol/L*
Visit 2	*Buck's behaviour has improved in that the circling has decreased. Although the owner had received a written report, the information needed to be reinforced verbally due to the owner's lack of basic literacy skills.*
Blood test results for visit 2	*T4 =21.0 nmol/ml* *T3 =0.8 nmol/ml* *TSH =0.14 ng/ml* *TgAA = Negative* *rT3 =0.53 ng/ml* *Cortisol =70 nmol/L*
Visit 3	*No further reduction in stereotypical circling and there was an increase in barking when Buck was left alone.*
Blood test results for visit 3	*T4 =23.0 nmol/ml* *T3 = 0.8 nmol/ml* *TSH =0.13 ng/ml* *TgAA = Negative* *rT3 =0.47 ng/ml* *Cortisol =62.0nmol/L*

Case Profile 9: George

Age:	*60 months.*
Sex:	*Male, neutered.*
Breed:	*Lurcher.*
Homed from at age:	*Rescue, at 48 months.*
Household composition:	*A retired male (owner).*
Medical history	*No significant medical problems.*
Behaviour disorder	*Fear aggression towards other dogs.*
Onset of disorder:	*12 months ago.*
Origin of disorder:	*Unknown. Possibly learnt fear from aversive event with other dogs whilst in kennel situation.*
Continuation of disorder:	*Fearful aggressive behaviour unwittingly reinforced by owner.*
Visit 1	*A desensitisation and counter-conditioning programme through gradual increase in contact to other dogs was advised.*
Blood test results for visit 1	*T4 =17.8 nmol/ml* *T3 =1.0 nmol/ml* *TSH =0.22 ng/ml* *TgAA = Negative* *rT3 = Insufficient plasma* *Cortisol =52.0 nmol/L*
Visit 2	*Owner reported a decrease in vigilance, fearful / nervous behaviour, aggressive behaviour, an increase in exploratory behaviour, play behaviour, excitable behaviour, disobedient and vocal behaviour. Although owner describes that he has not has the opportunity to implement the behaviour modification programme in full.*
Blood test results for visit 2	*T4 =27.0 nmol/ml* *T3 =0.9 nmol/ml* *TSH =<1.0 ng/ml* *TgAA = Negative* *rT3 =0.30 ng/ml* *Cortisol =58.0 nmol/L*
Visit 3	*Owner reported an increase in exploratory, vocal and play behaviours, and a decrease in fear and aggression to dogs also less nervous of people.*
Blood test results for visit 3	*T4 =29.0 nmol/ml* *T3 =1.1 nmol/ml* *TSH =<1.0 ng/ml* *TgAA = Negative* *rT3 =0.41 ng/ml* *Cortisol =67.0 nmol/L*

Case Profile 10: Ki

Age:	*48 months.*
Sex:	*Female, entire.*
Breed:	*Border Collie.*
Homed from at age:	*Breeder at nine weeks.*
Household composition:	*Two adults, male and female aged 30-40 and three adolescent girls.*
Medical history	*No significant medical problems.*
Behaviour disorder	*Aggression towards other dogs.*
Onset of disorder:	*Six months of age.*
Origin of disorder:	*Companion lab (Mac) died, who was very tolerant and Ki learnt that when he directed aggressive behaviour patterns towards Mac there was no response or consequences. However, when Ki directed the same behaviour patterns towards other unknown dogs they responded with defensively aggressive behaviour patterns. From this Ki learnt to be fearful towards unknown dogs and he behaves defensively aggressively towards them.*
Continuation of disorder:	*Fearful behaviour unwittingly reinforced by owner by tightening the lead and shouting whenever Ki is walked and an unknown dog becomes visible.*
Visit 1	*A desensitisation and counter-conditioning programme through gradual increase in contact to other dogs was advised.*
Blood test results for visit 1	*T4 =22.0 nmol/ml* *T3 =0.8 nmol/ml* *TSH =0.19 ng/ml* *TgAA = Negative* *rT3 =0.37 ng/ml* *Cortisol =<20.0 nmol/L*
Visit 2	*Owner reported an increase in play and aggressive behaviour towards people. Ki also developed very scaly skin.*
Blood test results for visit 2	*T4 =28.0 nmol/ml* *T3 =1.1 nmol/ml* *TSH =0.12 ng/ml* *TgAA = Negative* *rT3 =0.41ng/ml* *Cortisol =<20.0 nmol/L*
Visit 3	*Owner reported an increase in vigilance, fearful / nervous behaviour, excitable behaviour, vocal behaviour and aggressive behaviour toward people. Symptoms include hair loss, increased aggressiveness and lethargy.*
Blood test results for visit 3	*T4 =25.0 nmol/ml* *T3 =1.0 nmol/ml* *TSH =0.16 ng/ml* *TgAA = Positive* *rT3 =0.41ng/ml* *Cortisol =47.0 nmol/L*

Case Profile 11: Spanner

Age:	*16 months.*
Sex:	*Male, neutered.*
Breed:	*Cocker Spaniel.*
Homed from at age:	*Breeder at eight weeks.*
Trained:	*Obeys two commands.*
Household composition:	*Two male adults.*
Medical history	*No significant medical problems.*
Behaviour disorder	*Separation-related disorder.*
Onset of disorder:	*10 months of age.*
Origin of disorder:	*Spanner was not appropriately habituated to being left alone as a puppy.*
Continuation of disorder:	*Owners unwittingly reinforced separation related behaviour patterns by enthusiastic reunions on returning home after leaving Spanner and rewarding his anxious behaviour when leaving the house.*
Visit 1	*Advised treatment programme included a desensitisation and counter-conditioning programme to enable Spanner to be left alone without becoming anxious and not interacting to Spanner prior to departure or on return until he was calm.*
Blood test results for visit 1	*T4 =52.0 nmol/ml* *T3 =1.5 nmol/ml* *TSH =0.16 ng/ml* *TgAA = Negative* *rT3 =0.50 ng/ml* *Cortisol =<20.0 nmol/L*
Visit 2	*Increase in play and stereotypical behaviour pattern (circling), and Spanner has become more obedient, less aggressive and less destructive.*
Blood test results for visit 2	*T4 =39.0 nmol/ml* *T3 =1.1 nmol/ml* *TSH =<0.10 ng/ml* *TgAA = Negative* *rT3 =0.40 ng/ml* *Cortisol =80.0 nmol/L*
Visit 3	*Owners reported an increase in urination, play, repetitive behaviour, excitable behaviour and a decrease in destructive behaviour and some mounting.*
Blood test results for visit 3	*T4 =40.0 nmol/ml* *T3 =1.1 nmol/ml* *TSH =<0.10 ng/ml* *TgAA = Negative* *rT3 =0.37ng/ml* *Cortisol =<20.0 nmol/L*

6.4 Discussion

After evaluation of the results I will return to the initial hypotheses and consider the aim of this study, (to examine the effect of a behaviour modification programme on the hormonal indicators of stress and thyroid function). I will also consider the implications and applications of the research findings and suggest areas valuable for future research.

The counsellor's assessment determined that whilst most dogs' behavioural disorders were alleviated by treatment, some dogs showed little improvement. I suggest that the predominant reason for little or no change in behaviour following the implementation of a behaviour modification programme is likely to be due to a lack of consistency during the actual delivery of the programme and commitment and motivation of the owner. On starting a behaviour modification programme it is required that owners start to change their behaviour toward their dogs. Subsequently, dogs can become very frustrated, as previously solicited behaviour patterns no longer result in the expected outcome. For example, owners are often advised to ignore unwanted behaviours exhibited by their dogs in order to get attention, such as jumping up on to the owner. When the dog realises that jumping up is no longer a successful behavioural strategy to get attention it often starts to exaggerate its behaviour and jump higher, or jump higher and bark simultaneously. At this point it is crucial that owners maintain consistency and do not reward this new exaggerated behaviour pattern by acknowledging it, as the dog will then have learnt that displaying this new and more dramatic behaviour pattern is a successful way of getting their owner's attention.

Even when owners apply behaviour modification as instructed, stress hormones may increase for the short term after starting a behaviour modification programme. This is because the production of stress hormones is linked with frustration, fear and anxiety (Carlson 1998) and is therefore likely to increase when the previously learned behaviour patterns do not elicit the expected outcome. It should be noted that not all behaviour disorders are the result of anxiety; on some occasions a dog learns that behaviours that are often associated with anxiety e.g., seeking close contact with the owner, are reinforced and in some cases exacerbated by for example, their owner's attention. This scenario can be diagnosed by establishing how the behaviour pattern started. These behaviour patterns usually start as a behavioural response to an environmental stressor, but the dog learns which behaviour patterns are rewarded and subsequently displays these behaviours because they are perceived as rewarding and not therefore necessarily indicative of stress. Case profile number eight (Section 6.3.4) showed stereotypical circling behaviour. This behaviour pattern was considered to be anxiety related as there was a decrease in circling and a decrease in stress related hormones from visits 1-2, however, there was a further decrease in the stress hormones and no further decrease in circling between visits 2-3. Therefore it is possible that between visits 2-3 rather than using circling as an outlet for stress it

had become now a behaviour pattern that was successful in getting attention from people not involved in the delivery of the behaviour modification programme. Equally animals can use such behaviours as mechanisms for dealing with different sources of stress, and it is frequently the case that they result from progressively less severe forms of a stressor.

Other than irregularity in the delivery of a programme environmental factors also hindered the treatment of some dogs. The Foot and Mouth outbreak of summer 2001 resulted in the closure of the New Forest where many of the owners take their dogs for exercise. This had two main effects, firstly dogs were not exercised as frequently as they were on starting their programme, and in some cases this may have lowered the dogs threshold for frustration; secondly, there was a higher number of dogs attending public parks such as Southampton Common, which did not provide an opportunity for dogs who were fearful of other dogs and people to get exercise without reinforcing their fears.

The relationships between the hormones included the examination of TgAA, rT3 and cortisol as part of the diagnostic criteria, in order to investigate the influence of the stress hormones on the production of thyroid hormones, and to help to determine if lowered thyroid hormone levels were due to a primary hypothyroid state or were a result of non-thyroidal illness. Whilst results showed that there was no significant association between the thyroid hormones and cortisol, there was a significant association between rT3 and T4 and rT3 and T3. These results are not surprising and are predictable from the endocrinology of the thyroid gland. In instances of high cortisol the conversion of T4 to T3 is reduced and T4 is also converted to rT3 (Section 1.5.5), therefore I also expected rT3 and cortisol to be correlated significantly, as they are in other species (Mitsuma and Nogimori 1982; Laurberg and Boye 1984; Brabant *et al.* 1987). The lack of correlation found may be due to the small sample size with much individual variation between dogs, but the results do not suggest a tight link between cortisol and the thyroid hormones in dogs, particularly with regard to plasma cortisol.

The relationship between hormones and behaviour

The negative correlation between Activity and T3 is a paradox and difficult to interpret. Activity and T3 have been associated in both Chapters 3 and 4 in that low titres of T3 are associated with low levels of Activity. This is the expected finding as the principal symptom of hypothyroidism is lethargy as a result of decreased metabolic function associated with a decrease in the thyroid hormones. The sample used in this study has suggested the opposite the reverse relationship between Activity and T3 but it is possible that this is an artefact due to the small sample size.

Case Profiles

In 55% of cases the behaviour modification programme was successful in that there was an improvement in the dogs behaviour on both visits two and three. The owner's understanding and application of a behaviour modification programme is a primary factor in determining its success.

Whilst in some cases owners claimed that they were applying the behaviour modification procedures, the dog's behaviour indicated the contrary. For example, one owner told her dog (Dan) *"I am going to ignore you now"* when the dog had exhibited a behaviour pattern that should not have been reinforced. As an owner's interaction is a primary reinforcement for dogs, telling the dog it was going to be ignored was actually rewarding the behaviour and therefore increasing the possibility that the unwanted behaviour would occur again.

The behaviour treatment itself may cause dogs to be initially stressed as the dogs suddenly find that previously effective method of getting a desired outcome are no longer effective, and they have to develop alternative patterns of behaviour to get what they want. This can cause frustration reflected by an initial increase in cortisol titre (e.g. Spanner). In such cases the dog's behaviour also appears to initially get worse rather than better as a result of the behaviour modification programme. Other factors that could possibly influence the development of a behaviour modification programme include changes in household composition, as in one case (e.g. Spanner) the owners separated and one member vacated the household. This disruption in household members may influence the social hierarchy and may well affect the dog's behaviour. Confinement due to the foot and mouth restrictions (e.g. Chuck) may have caused them to become anxious and less receptive to the behaviour modification and may also have affected the physiological parameters. No dogs developed any medical problems that would have influenced the progression of a behaviour modification or thyroid hormone levels. No dogs were on any medication that would have influenced the progression of behaviour modification, or thyroid hormone levels. One case (Daisy) was given a herbal remedy for anxiety, however the effect of this treatment was not monitored.

The hypotheses

Hypothesis 1: Reject the null hypothesis in favour of the alternative hypothesis, as there is a significant relationship between Activity and T3 but the direction of this association was not as predicted. No association was found to support the anecdotal relationship between aggression and lowered thyroid hormone titre. Although this is a small sample size the lack of an association between lowered thyroid hormone and aggression is consistent with the study presented in Chapters 3-5.

Hypothesis 2: Accept the null hypothesis in that there was no detectable association between the changes in observed behaviour patterns, and changes in stress hormones. There was no association between changes in Activity and Aggressivity with cortisol and rT3. Over a longer period of time I would have expected a reduction in the stress hormones as the behaviour modification programme reduced the dogs' anxiety. However it is possible that in the short term, as the behaviour modification programme gets underway and the owner's behaviour changes toward their dog that this may cause the dog to become more anxious and more stressed.

Hypothesis 3: Accept the alternative hypothesis in that there was an association between rT3 and T4 and between rT3 and T3. The relationship between rT3 with T4 and rT3 with T3 is expected as T4, T3 and rT3 are precursors that interchange in an attempt to maintain physiological equilibrium.

Hypothesis 4: Accept the null hypothesis in that is there no association between the stress hormones and counsellor assessment of improvement in behaviours. Quantitative methods of measuring change in the behaviour disorder, such as temperament tests for each visit, rather than via the counsellor assessment questionnaire would have been favoured, as this would have generated entirely objective measures appropriate for statistical testing. However the ethical and/or health and safety implications of conducting temperament test on dogs with an anxiety related disorder made conducting such tests unfeasible (see future work).

Hypothesis 5: Accept the null hypothesis in that is there no association between the thyroid hormones and counsellor assessment of improvement in behaviours (see discussion in Hypothesis 4 above).

The implications and applications

The main finding of this study is the unexpected association between high Activity and low T3, which has previously been discussed. A more pertinent finding is the lack of association between aggressive behaviour and subclinical hypothyroidism as there have been several suggestions by behaviour counsellors and veterinary counsellors that hypothyroidism causes aggression (Section 1.3.4) which have not been confirmed by this study. As a result of the previous anecdotal evidence that thyroid replacement therapy has been used in some instances to treat dogs exhibiting inappropriate aggression (Section 1.3.1). This reasoning not only presumes that hypothyroidism causes aggressive behaviour but suggests that treating the dog with thyroid replacement therapy is the cure for unwanted aggressive behaviour. The inappropriate use of thyroxine has several side effects that include cardiac dysfunction and an increased mortality (Kaptein 1988; Section 1.8), therefore a diagnosis of hypothyroidism based solely on the occurrence of behavioural disorders is inappropriate. A range of diagnostic tests are essential in order to establish a diagnosis of hypothyroidism in humans and dogs. Additional behavioural cues as to the onset of hypothyroidism are valuable but should be in addition to and not instead of direct measures of the thyroid hormones. Additionally, the detrimental effects of a stressful lifestyle are well known in human medicine and the effects of stress on the health of the domestic dog should not be underestimated.

Areas for future work

Future work should be aimed particularly at: -

i. Establishing invasive and non-invasive indicators of stress and their association with the behaviour of the domestic dog living as a companion animal.

ii. Establish the association between rT3 and cortisol concentration for dogs.

iii. Refining the methods used in the measurement of dog behaviour patterns. Ideally, hidden cameras would provide the most objective measures of dog behaviour provided there was an extended period of habituation for both the human and non-human members of the household to adjust.

iv. A longitudinal study that examines dogs with the same behaviour disorders and following a standard behavioural treatment programme in order to reduce variability, over a longer period of time to establish if chronic changes in physiology result from behaviour modification.

6.5 Concluding comments

This study explored several relationships between stress, thyroid hormones and behaviour of the domestic dog. A paradoxical association was found with Activity and T3. Individual case studies were useful to examine specific associations between hormones and behaviour. Future research would be useful firstly to confirm the correlation between Activity levels and T3 in dogs with behaviour disorders and the physiological relationship between the thyroid and stress hormones in a larger population of dogs. Secondly, the effectiveness of behaviour modification in terms of welfare measurements in companion dogs should be established, then the relationship between behaviour disorders and stress; stress hormones and cortisol; and rT3 and the other thyroid hormones, can be fully established.

Chapter 7

Clinical hypothyroidism and dog behaviour patterns

7.1 Introduction

In Chapters 3, 4 and 5 the thyroid status of dogs presented to the ABC with behaviour disorders was examined and exhibition of Inactivity (Chapters 3 and 6), and Sedentary Postures (Chapter 4) was found to be associated with lowered thyroid state. In this chapter I describe a study based on a different approach to investigating such associations. Instead of investigating the thyroid status of dogs with behavioural disorders, I have investigated the behaviour disorders exhibited by a clinical population of dogs with altered thyroid state, which has enabled me to examine the behavioural status of a large sample of dogs at different stages and types of clinical and subclinical hypothyroidism. Using this approach, it should be possible to obtain further evidence to determine whether any link between altered thyroid state and dog behaviour is a cause and effect relationship.

This investigation is intended to shed light on the claim that the thyroid hormones have a direct effect on the expression of aggressive behaviour by dogs. This suggestion is based on the observation that, in some single cases, aggressive behaviour, with underlying hypothyroidism, has been alleviated by thyroid replacement therapy (Dodds 1992, Dodds 1996; Dodman 1995). However, this is not indicative of a causal link between reduced thyroid hormone titres and dog behaviour patterns as the primary function of the thyroid gland is to control metabolism, and changes in metabolic rate are likely to influence several bodily functions (Chandler *et al.* 1994). Therefore, thyroid replacement therapy may influence behaviour via its effects on metabolic rate and does not necessarily indicate that low thyroid hormone titre has caused behavioural change other than its general effect on activity levels. The aim of this study was to compare the incidence and type of behaviour disorders between dogs with different category of thyroid function. I utilised methods of data collection similar to those described in Chapter 3 that enables the findings to be compared with my previous studies.

This study was conducted with the assistance of The College of Veterinary Medicine, Animal Health Diagnostic Laboratory (AHDL), Michigan State University (MSU). The Endocrine Diagnostic Section of MSU, AHDL, has been existence since the mid 1970's. This unit, under the headship of Dr Raymond Nachreiner, specialises in the laboratory diagnosis of endocrine disease of animals. Over recent years they have received around 110,000 submissions per annum for the investigation of endocrine disorders. Approximately 80,000 of those are for the investigation of thyroid disorders in dogs. Most submissions to the unit come from the United States and Canada although samples are frequently submitted from locations around the world.

The sample of dogs used in this study was categorised into five groups according to their thyroid status; one of these groups was euthyroid. The sample of dogs was comprised of dogs that were referred for diagnostic testing as a result of showing symptoms of hypothyroidism as well as dogs that

were being screened for signs of a malfunctioning thyroid gland prior to use for breeding. For the purpose of this study it has been assumed that the euthyroid group is predominantly drawn from the latter, although this could not be confirmed from the information available. The behaviour of each dog in the population was investigated via a questionnaire completed by the dog's owner. This questionnaire provided information about the dog's behaviour patterns as well as whether or not the owners considered their dogs to exhibit inappropriate behaviour. This study examined the behaviour patterns and behaviour disorders in dogs at different stages of hypothyroidism and aimed to test the null hypothesis that "there is no difference in the incidence and types of behaviour disorders/patterns between subclinically and/or clinically hypothyroid dogs and euthyroid dogs".

There is at least one mechanism by which the behaviour of the dog could have a direct effect on the development of thyroid dysfunction rather than *vice versa*. Some research veterinarians suggest that there appears to be a large proportion of canine hypothyroidism, which is due to "idiopathic follicular atrophy" with no apparent inflammatory component (Beale *et al.* 1990; Haines *et al.* 1984; Lucke *et al.* 1983). It is known that "palpation thyroiditis" can occur in people following the handling of their thyroid glands during a physical examination (Carney *et al.* 1975) and this inflammation is associated with the measurable release of anti-thyroid antibodies into the circulation. When dogs pull on their leads the pressure exerted on the neck could cause mechanical trauma to the thyroid gland (Figure 7.1). This may be of particular relevance for dogs that were restrained with choke chains (Figure 7.2) as when a dog pulls on a choke chain more pressure is exerted than compared with a lead and collar (Figure 7.3). Whilst the majority of canine hypothyroidism is the end-result of an immune mediated thyroiditis, it would be useful to determine the degree to which other factors might contribute to the proportion of hypothyroidism that does not occur with inflammation. Therefore as a matter of further interest I examined the possibility that the incidence of hypothyroidism was increased in dogs that pulled on their leads and who were controlled with choke chains.

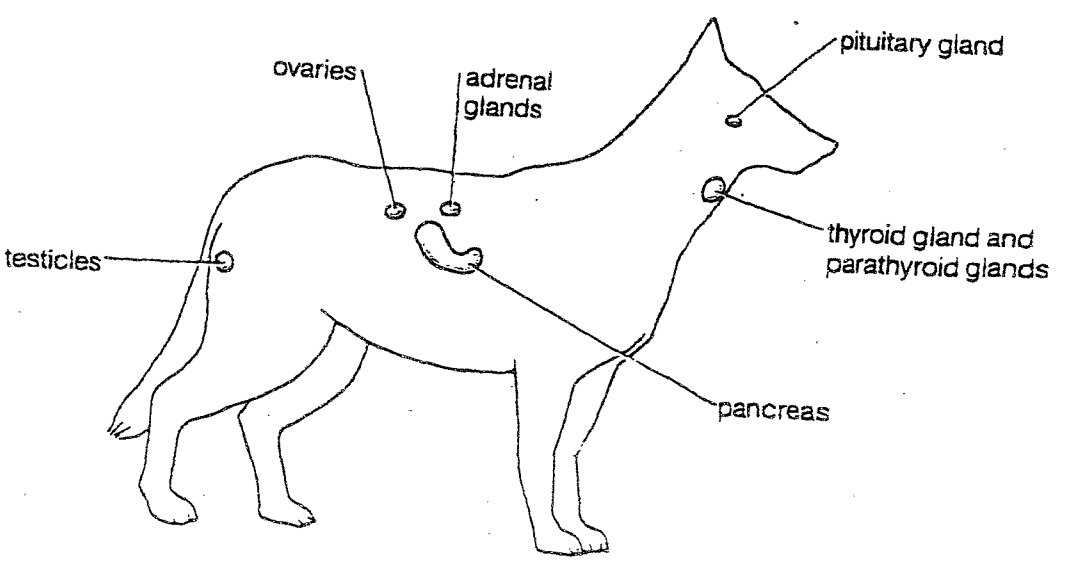

Figure 7.1 Position of the thyroid gland in the dog (Turner 1994)

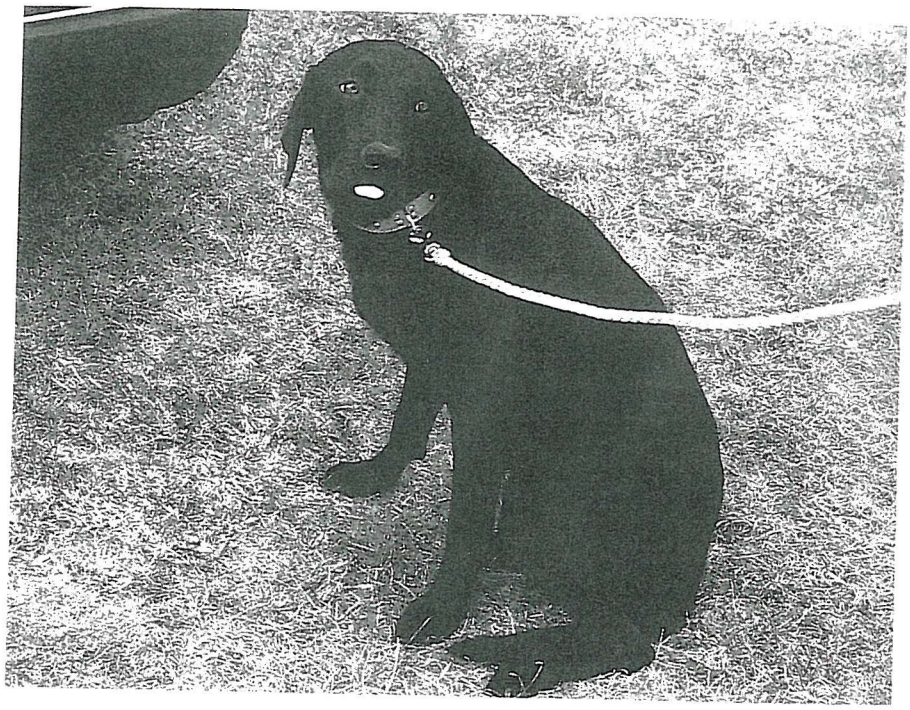

Figure 7.2 Lead and collar on a dog (Turner 1994)

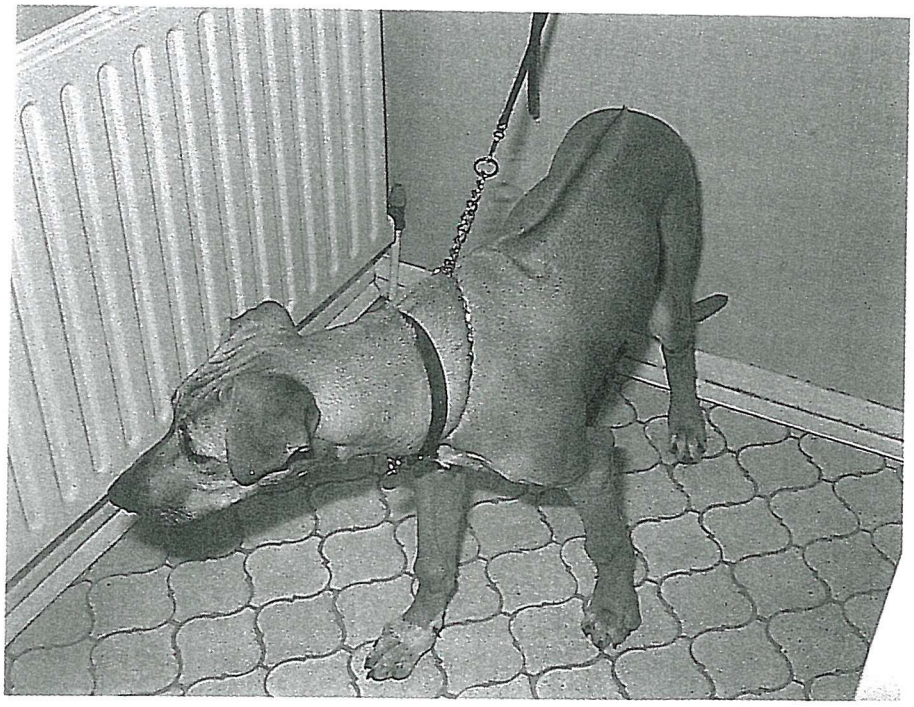

Figure 7.3 Choke chain on a dog (Turner 1994)

7.2 Methods

7.2.1 Recruitment and signalment of subjects

A behaviour questionnaire and covering letter (Appendix 7) was sent to all referring veterinary surgeries requesting their participation with this research project. Willing veterinary surgeries then forwarded the questionnaire to the owners of dogs tested for thyroid function. A business reply envelope was also enclosed in order for the clients to return the completed questionnaires to the Laboratory. Once all the questionnaires were collected they were forwarded to the Anthrozoology Institute, UK for analysis. Only dogs for which questionnaires were returned were included in the data set.

Five groups of dogs were categorised by AHDL; Euthyroid (ET); TgAA negative hypothyroid (ANH); subclinical hypothyroid (SCT); TgAA positive sub-clinical hypothyroid (SCH); TgAA positive hypothyroid (APH). Each dog was categorised (Table 7.1) on the basis of the thyroid tests: -

(i) Thyroxine (T4).

(ii) Free thyroxine by equilibrium dialysis (fT4d)

(iii) Thyroid stimulating hormone (TSH)

(iv) Thyroglobulin antibody (TgAA).

The following diagnostic cut-off values have been validated for the identification of thyroid dysfunction or pathology: tT4 <15nmol/L or fT4d <6pmol/L, TSH >0.68ng/mL (Dixon and Mooney 1999) and TgAA >200% (ELISA results expressed as a percentage of the optical density (OD) of the negative control. Normal dogs did not have an OD that was >2 times (200%) of the negative control, Nachreiner *et al.* 1998*)*. In addition to these measures dogs were routinely tested for total triiodothyronine (tT3), free triiodothyronine (fT3), T4 autoantibodies (T4AA) and T3 autoantibodies (T3AA). The values for T3, fT3, T4 and T4AA were used for the comparison of behaviour disorders and thyroid status.

Table 7.1 Criteria for the categorisation of dogs based on thyroid hormone measures

	TgAA	T4	fT4d	TSH
Euthyroid (ET; N=41)	Negative	Normal	Normal	Normal
Subclinical thyroiditis (SCT; N=44)	Positive	Normal	Normal	Normal
TgAA positive sub-clinical hypothyroid (SCH; N=36)	Positive	Normal	Normal	Elevated
TgAA positive hypothyroid (APH; N=51)	Positive	Decreased	Decreased	Elevated
TgAA negative hypothyroid (ANH; N=46)	Negative	Decreased	Decreased	Elevated

The questionnaire completed by the owner contained the signalment information about the dogs. The population was comprised of mixed sex (Figure 7.4), breed (Figure 7.5), age (Figure 7.6) and weight (Figure 7.7). All the dogs were aged over 18 months. The weight of dogs was recorded in order to determine if it could be a contributory factor influencing any association between neck trauma, pulling on the lead and hypothyroidism.

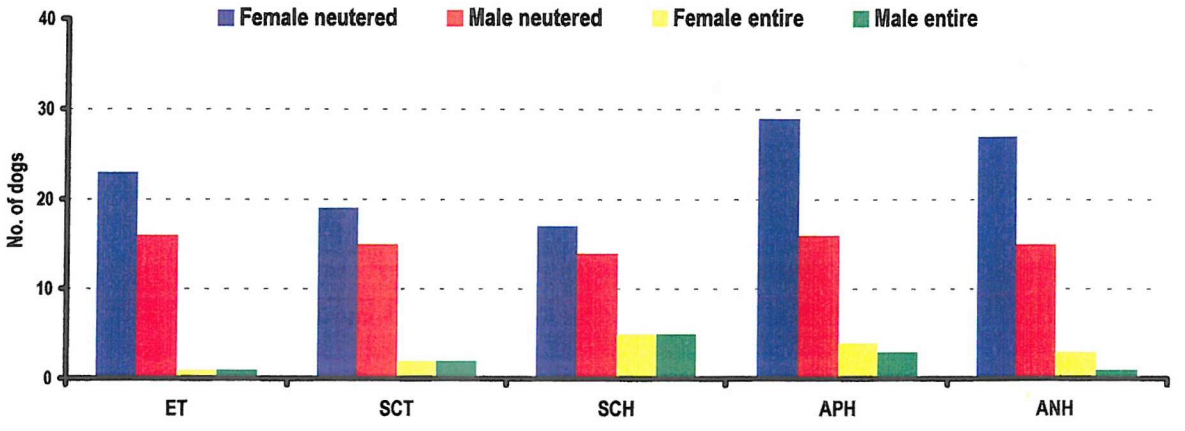

Figure 7.4 Sex distribution of dogs (N=218)

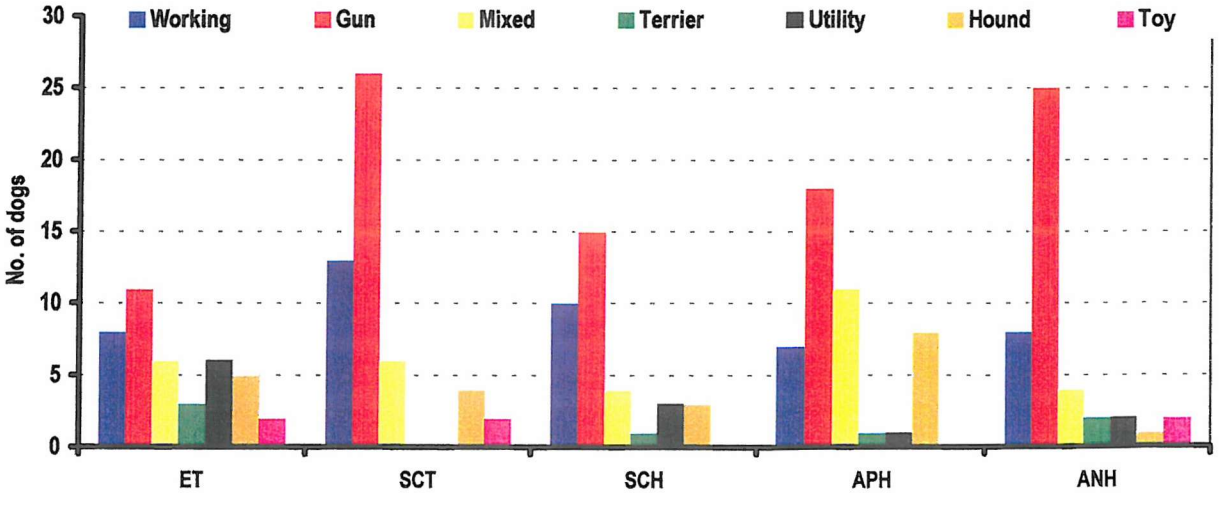

Figure 7.5 Breed distribution of dogs (N=218)

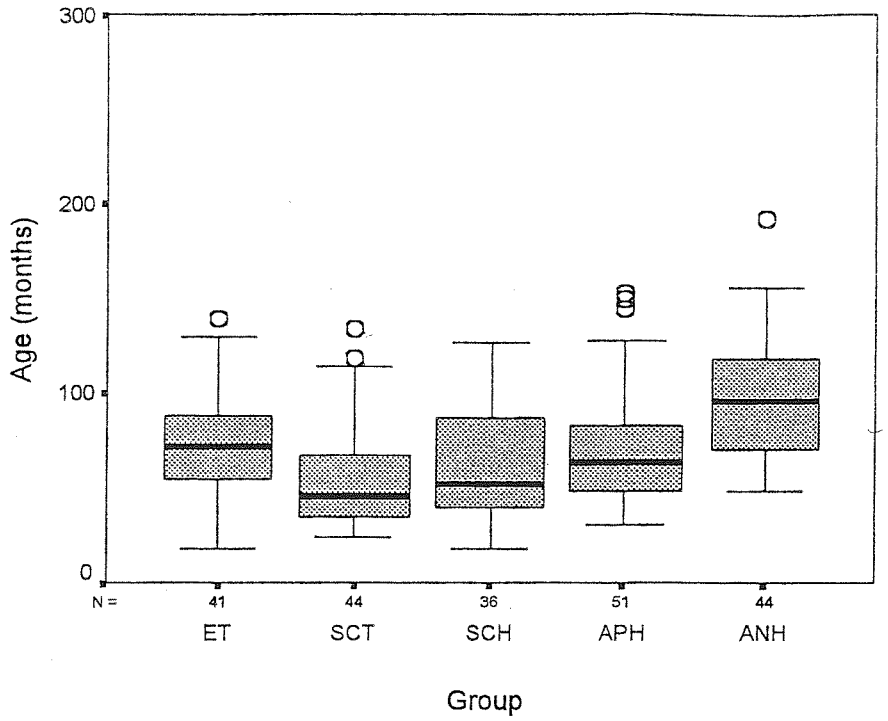

Figure 7.6 Box and whisker plot of the distribution of Age (months) in the five thyroid categories. The box represents the inter-quartile range that contains the central 50% of dogs. The whiskers are lines that extend from the box to the highest and lowest individuals, excluding outliers, which are represented by circles. The black horizontal line in the box indicates the median value.

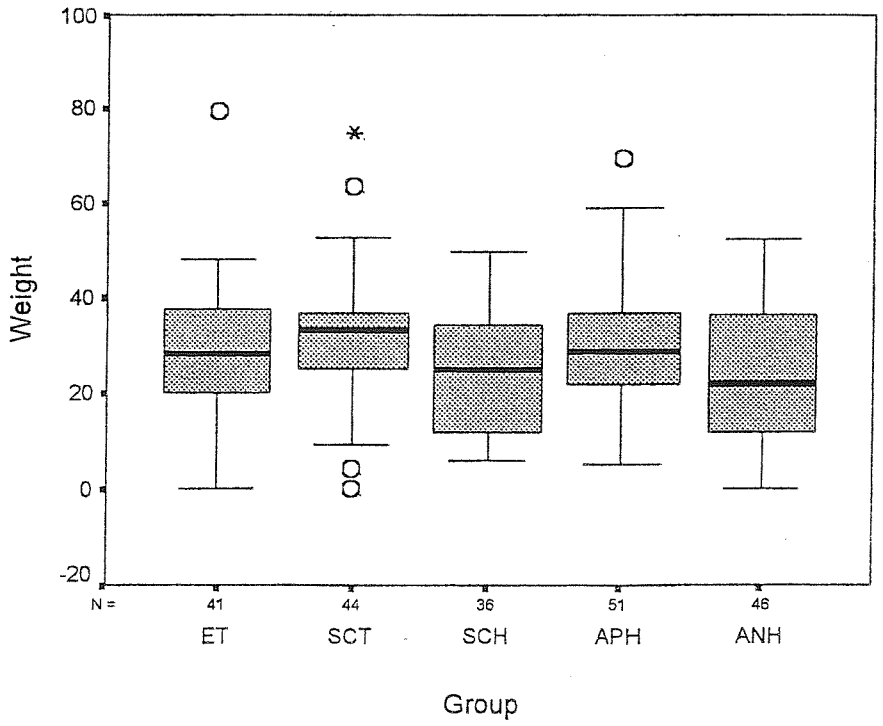

Figure 7.7 Box and whisker plot (see Figure 7.5) for the distribution of Weight (kg) of the five thyroid categories.

7.2.2 Collection of thyroidal and behavioural data

Hormonal measures: AHDL laboratories determined the concentrations of T4, fT4d, TSH and TgAA. The measures of autoantibodies by AHDL were provided as continuous measures rather than results of "positive" or "negative" as provided by Cambridge Specialist Laboratory Services. Both AHDL laboratories and Cambridge Specialist Services operate similar laboratory techniques for the determination of thyroid hormones; however, it is likely that there is some intra-assay variation and therefore results cannot be quantitatively compared to those in previous chapters. The criteria for all the groups was complied by Dr Peter Graham, Michigan State University. Antibody positive and antibody negative refers to the type of autoimmune hypothyroidism, a diagnosis of antibody positive thyroiditis being reached on detecting antibodies to the thyroid gland (Section 1.5; Table 7.1).

Behavioural measures: Out of a total of 750 questionnaires distributed (150 to each group), 229 were returned (31%). The behaviour questionnaire (Appendix 4) was comprised of a combination of the ABC questionnaire (Section 2.4.1) and an additional section requesting information about the presence of behaviour disorders. Owners were asked general questions about their dog's history, training, toys, type of housing and relationship with family members in order to accumulate additional information, which would help to assist in the categorisation of behaviour disorders. For example, in some cases owners might not have considered their dog to have a behaviour disorder *per se* and therefore might may not have completed a section entitled "Behaviour Disorders": however, asking them for information about how their dog interacts other members of the household provided an alternative opportunity for owners to describe how their dog interacts with other people. Questions pertaining to exercise, diet and medical history were also asked in order to determine any other conditions that may have contributed to changes in thyroid status. Questions were aimed at determining the presence or absence of aggression towards people, aggression towards dogs, fearful behaviour, excitable behaviour, separation related behaviour patterns, training disorders, repetitive behaviour patterns, coprophagia/pica, inappropriate mounting and a category for other behaviour disorders was also included.

7.2.3 Statistical analysis

The Kruskal Wallis test was used to test the difference between the age distribution and the weight distribution with all the categories of thyroid state, and the Chi Square test was used to test the difference between the sex distributions for all the categories of thyroid state.

The Chi square test was also used to test the difference between the types of behaviour disorders for all the categories of thyroid state. Significant findings were investigated further using 2x2 contingency tables to test for differences between each of the four categories of thyroid disease (SCT, SCH, APH, ANH) with the euthyroid group.

The Mann Whitney U test was used to examine the difference in the concentrations of thyroid hormones for those dogs that did or did not show behaviour disorders found to be significant in the preceding Chi square analysis. The purpose of the Mann Whitney analysis was to test for associations between the reported behaviour disorders and all the thyroid hormones and antibodies supplied by AHDL, i.e., T4, fT4d, TSH, TgAA, T3, fT3, T4AA and T3AA, not solely those used in the categorisation of thyroid states. For this analysis all thyroid measures were used as continuous variables as the association between T4, fT4d, TSH, TgAA (which were used to determine the categorical thyroid states) and behaviour disorders was previously identified (Section 7.2.1). Medians were calculated for the comparison of thyroid hormone values for different the behaviour disorders.

Lastly, a Chi square test was used to test for the difference in the incidence of hypothyroidism between dogs that wore a choke chain and pulled on their lead as compared to those dogs that did not wear a choke chain and did not pull on their leads.

7.3 Results

7.3.1 Preliminary data exploration

There was a significant difference between the age distributions of dogs between the groups (ET, ANH, SCT, SCH, APH (Kruskal Wallis, χ^2=46.7, d.f. =4, p<0.0001). The antibody negative hypothyroid group contained the oldest dogs and the subclinical thyroiditis group contained the youngest dogs (Figure 7.6). The median age was 65 months. There was no significant difference between groups for sex (female neutered, male neutered, female entire and male entire) for the five categories of thyroid state (Chi square, χ^2=10.3,d.f. =12, p=*0.59*), however, as this calculation was based on the basis that 10 cells contained expected counts less than five the analysis was repeated combining the sex categories male entire and female entire but no significant differences between the groups were found (Chi square, χ^2=9.6,d.f. =8, p=*0.30*). There was no significant difference between the weight distributions of dogs between the groups (Kruskall Wallis, d.f. =4, χ^2=7.9, p=0.10) (Figure 7.7). The median weight of the dogs was 28kg.

7.3.2 Hypothyroidism and behaviour disorders

A significant association was found between the categories of thyroid state (ET, ANH, SCT, SCH, APH) and the incidence of Separation related behaviour patterns (Chi squared test, χ^2=13.1, d.f. =4, p=0.01), Training disorders (Chi squared test, χ^2=16.8, d.f. =4, p<0.01) and Copraphagia/Pica (Chi squared test, χ^2=10.1, d.f. =4, p<0.05).

In order to deduce which groups were unusual, contingency tables (2 x 2) were used to test for differences between each of the thyroid state categories ANH, SCT, SCH, APH with the ET category.

This was repeated for each of the significant behaviour disorders in turn (Separation related behaviour patterns, Training disorders and Copraphagia/Pica). Significant differences were found (Table 7.2) for Training disorders, which were more frequent in the subclinical hypothyroid group than in the euthyroid group (Chi squared test, χ^2=9.7, d.f. =1, p<0.01), Separation related disorders, which were less frequent than expected in the subclinical hypothyroid group (Chi squared test, χ^2=7.2, d.f. =1, p<0.01) and TgAA positive hypothyroid (Chi squared test, χ^2=7.2, d.f. =1, p<0.01) and lastly, Copraphagia/Pica, significantly more frequent in the subclinical thyroiditis group (Chi squared test, χ^2=4.3, d.f. =1, p<0.05), and also, though not significantly, in the subclinical hypothyroid group (Chi squared test, χ^2=2.8, d.f. =1, p=0.09).

Table 7.2 Percentage of dogs in each group with a behaviour disorder.

Incidence of behaviour disorders (%) for each category of thyroid state.
Asterisked values indicate significant differences with the euthyroid group (*p<0.05, **p<0.01)

Group	Aggression towards people	Aggression towards dogs	Fearful	Excitable	Separation related disorder	Training disorder	Repetitive behaviour	Eating non-foodstuffs	Mounting	Other
ET	24	27	56	76	29	15	2	15	0	7
SCT	20	14	36	68	14	16	5	**34***	0	2
SCH	25	28	55	53	**6****	**47****	0	31	0	8
APH	25	35	57	65	**8****	22	4	16	0	8
ANH	13	26	63	63	23	15	4	13	2	4

The finding that the consumption of non-foodstuffs was particularly relevant due to the metabolic implications and associated dietary changes caused by hypothyroidism (Section 1.5.2) therefore this was investigated further. Faeces was the most commonly eaten material accounting for 61%, paper accounted for 17%, both grass and toys accounted for 9% each and the least commonly consumed material was fabric which accounted for 4% (Figure 7.8).

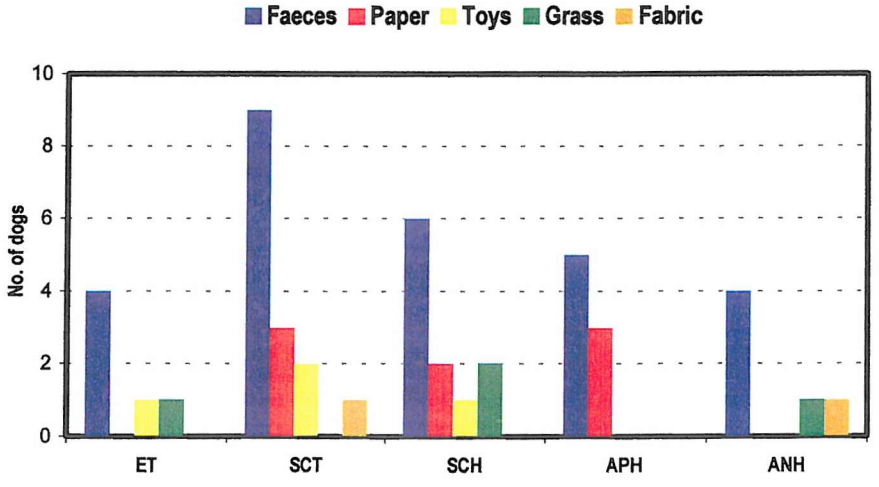

Figure 7.8 Dogs consuming non-foodstuffs (N=46; 21% of the population)

7.3.3 Thyroid hormone concentration and behaviour disorders

Mann Whitney U tests were used to test for associations between each thyroid measure taken individually, Training disorders, Copraphagia/Pica and Separation related disorders. Results from Mann Whitney U test determined no significant associations between Training disorders with any of the thyroid measures or autoantibodies. However, one significant association was found between the thyroid hormones/autoantibody titres with Separation related behaviour patterns, and for the Copraphagia/Pica some trends were found (Table 7.3).

Table 7.3 Associations between thyroidal measures and behaviour disorders (U=Mann Whitney statistic, *p*= significance level and figures in bold are significant or show trends)

Thyroidal measure	Training Disorder (N=48)	Copraphagia /Pica (N=46)	Separation related disorders (N=35)
T3	U=4053 *p*=0.94	U=3950 *p*=0.99	U=2891 *p*=0.36
T4	U=3845 *p*=0.54	U=3433 *p*=0.17	U=2951 *p*=0.36
fT3	U=4050 *p*=0.93	U=3450 *p*=0.18	U=3040 *p*=0.63
fT4	U=3407 *p*=0.08	U=3400 *p*=0.14	U=3024 *p*=0.60
T3AA	U=4025 *p*=0.89	**U=3232 *p*=0.06**	U=2697 *p*=0.14
T4AA	U=3726 *p*=0.36	U=3744 *p*=0.58	U=2917 *p*=0.40
TSH	U=3752 *p*=0.40	U=3409 *p*=0.15	U=3007 *p*=0.51
TgAA	U=3769 *p*=0.42	**U=3249 *p*=0.06**	**U=2380 *p*<0.05**

Examination of median figures (Table 7.4) highlighted that the dogs with Copraphagia/Pica had higher TgAA levels confirming that dogs with a normal profile but elevated autoantibodies, especially TgAA, were likely to display Copraphagia/Pica. Dogs with Separation related disorders had markedly lower TgAA titres than those without Separation related disorders. Overall, fewer significant results were found than when hormone profiles were tested (Table 7.2), indicating that it is the whole disease state, rather than the titres of individual hormones that is associated with abnormal behaviour.

Table 7.4 Median values for the association between thyroidal measures and behaviour disorders, (*p*= significance level)

Thyroidal measures	Copraphagia/Pica Median values		Separation related disorder Median values	
	Present (N=46)	Absent (N=172)	Present (N=35)	Absent (N=183)
T3 (nmol/L)	0.7	0.6	0.6	0.6
T4 (nmol/L)	20.0	15.0	20.0	16.0
fT3 (pmol/L)	7.3	6.4	6.3	7.0
fT4d(pmol/L)	15.0	11.0	13.0	12.0
T3AA (%)	**8.0** (*p*<0.10)	**6.0** (*p*<0.10)	4.0	6.0
T4AA (%)	13.0	12.0	12.0	12.0
TSH (ng/mL)	47.0	63.0	29.0	60.0
TgAA (%)	**727.0** (*p*<0.10)	**448.0** (*p*<0.10)	**128.0** (*p*<0.01)	**709.0** (*p*<0.01)

Due to the large difference in median figures for the presence and absence of separation related disorders the distribution of TgAA readings at each stage of hypothyroidism was examined further (Figure 7.9).

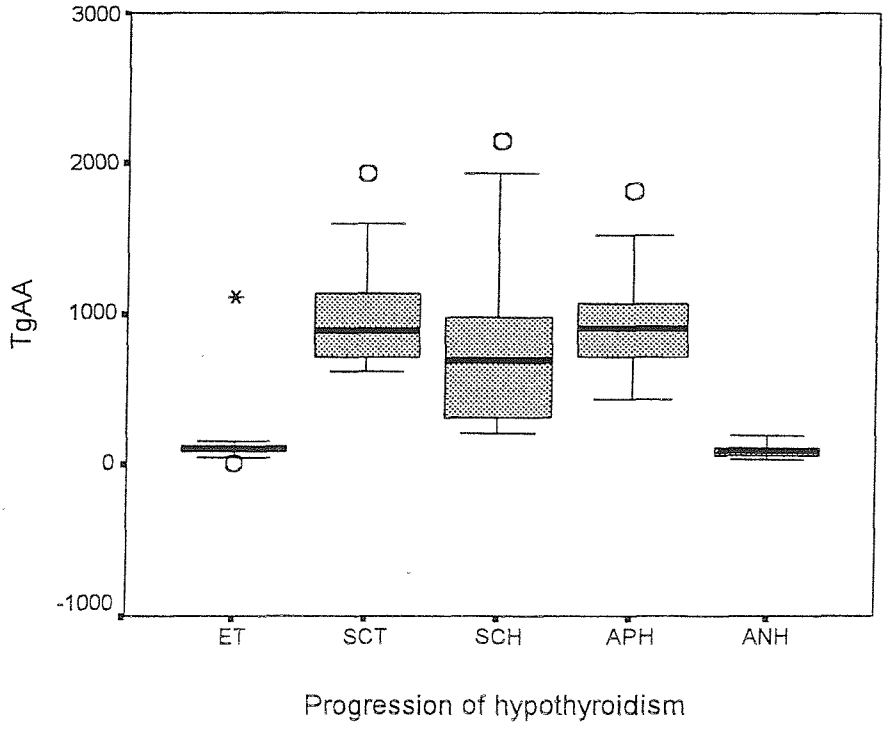

Figure 7.9 Distribution of TgAA readings for dogs with different stages of disease.

At the euthyroid (ET) stage the dogs are healthy and there is a very low TgAA presence. There is an increase in TgAA in the progression to the subclinical thyroiditis and a further increase at the subclinically hypothyroid stage. As the thyroid gland is damaged further at the antibody positive hypothyroid stage there is a decrease in the TgAA as the gland itself is destroyed and finally when the entire gland has perished TgAA return to a very low level as in the antibody negative hypothyroid group.

7.3.4 Neck trauma and hypothyroidism

Examination of the percentages of dogs in each group that wore choke chains and pulled on their leads suggested that there was a similar incidence of dogs wearing choke chains between the groups, with the exception of the TgAA negative hypothyroid group that had a lower percentage of dogs that wore choke chains and pulled on their leads (Table 7.5). Significant differences were found on comparing the number of dogs that pull and wear a choke chain against those that do not pull and do not wear a choke chain (Chi squared test, χ^2=11.7, d.f. =4, p=0.01). This relationship was examined further using 2x2 chi square tests that again identified a significantly lower proportion of dogs in the TgAA negative hypothyroid group than in the euthyroid group (Chi squared test, χ^2=7.33, d.f. =1, p<0.01; Table 7.5). It is possible than the number of dogs that pull in the TgAA negative hypothyroid group is lowered because lethargy is a key symptom of hypothyroidism and as choke chains are usually used to control boisterous dogs this may also explain why there is a lower incidence of dogs wearing choke chains in this group. Pulling between groups was considered independently and there was no significant difference between the groups.

Table 7.5 The percentage of dogs wearing choke chains and pulling on leads in each group

	Wear choke chain (%)	Pull (%)
ET (N=41)	21	21
SCT (N=44)	20	22
SCH (N=36)	29	17
APH (N=51)	20	24
ANH (N=46)	11	16

There was an imbalance in the variety of breeds to test the effect of breed groups on the incidence of pulling and/or choke chain usage and therefore it was not possible to examine this relationship statistically.

7.4 Discussion

Younger dogs were more likely to be included within the two subclinical forms of hypothyroidism. This is likely to be due to the progressive nature of the disease and hence it is more likely that the early stages of the disease is seen in younger dogs and the latter stages seen in older dogs. The higher prevalence of older dogs in the antibody negative group can be explained, as this is a more advanced stage of hypothyroidism where no antibodies are detectable as the thyroid gland is largely destroyed. The age distribution was therefore as expected.

Whilst Haines *et al.* (1984) suggests that hypothyroidism has a biased development in female dogs (and in particular spayed females) my findings are similar to those of several authors who suggest that there is no difference between sex and/or neuter status (Eckersall and Williams 1983; Jaggy *et al.* 1994; Dixon *et al.* 1999c; Dixon 2001).

The most popular breed represented in the dogs tested for hypothyroidism was the Golden Retriever and that is a breed considered to have a high risk of developing hypothyroidism (Nelson and Ihle 1987; Milne and Howard 1981; Panciera 1994). While Dixon *et al.* (1999c) and Simko (1992) suggests that small breeds aged six to nine years are most commonly affected by hypothyroidism my findings are consistent with Reinhard (1978), Dodman and Shuster (1998), Turner (1994) and Dixon (2001) which states that there is a higher prevalence for hypothyroidism in medium to large breeds as there were very few small and toy breeds represented in the sample. The sample may not have been entirely representative of hypothyroidism in different breeds as dogs tested for hypothyroidism prior to breeding and not presenting with symptoms of hypothyroidism were included in the sample.

Hypothyroidism and behaviour disorders

The finding that training disorders were more frequent in the subclinically hypothyroid group than the euthyroid group could be explained by considering the thyroid hormone changes that occur in people with Hashimoto's disease (a form of hypothyroidism). In humans during the early stages of the disease the relative concentrations of T4/T3 can fluctuate (Volupe 1996): these changes in hormones have not been confirmed for dogs. However, Hashimoto's disease is histologically identical to hypothyroidism in Beagles (Volupe 1996). It is therefore possible that if the thyroid hormones fluctuate in dogs during the onset of hypothyroidism the metabolism and bodily functions will also be affected those associated with perception. Such changes are likely to influence interspecific communication and an owner may describe their dog as disobedient or un-trainable, and as behavioural change is often the first sign of ill health then it is feasible that the behavioural changes reported by an owner as disobedience or training problems could be the result of changes in T4/T3.

The incidence of separation related disorders in the euthyroid group (25%) is broadly consistent with surveys conducted in the UK (Bradshaw *et al.* 2002) and the USA (Dalgleish 2002). In order to explain the finding that separation related disorders are less frequent in the subclinical hypothyroid group and the antibody positive hypothyroid group than in the euthyroid group and in the antibody negative hypothyroid group it is necessary to consider the common elements at the different stages and the advancement of the disease. Both the antibody positive hypothyroid group and the subclinical hypothyroid group have positive TgAA and elevated TSH, however the antibody positive hypothyroid group represents a further advancement of the disease when T4 is decreased. It is possible that as the disease progresses from the subclinical thyroiditis to subclinical hypothyroid stage to the antibody positive hypothyroid stage in which there is a progressive decrease in the reporting of separation related disorders. The progression of thyroid disease may simply be making the behavioural signs of separation disorders less obvious to owners, for example, as the dog becomes more lethargic it may bark less frequently. However, this explanation does not account for the relatively high level of reported separation related disorders in dogs with the most advanced forms of the disease, antibody negative hypothyroidism. These dogs should have been lethargic, but were evidently motivated to display obvious separation related behaviour. However, the high levels of excitability in the antibody negative group (Table 7.2) suggests that this particular population may have been atypical: possibly their veterinarians had ordered assays from the AHDL precisely because in many cases their signs of hypothyroidism had been contradictory (e.g. hair loss without reduction in activity). Investigation of other populations would be needed to investigate this further.

In considering the finding that coprophagia and pica was more frequent in the subclinical thyroiditis group it is necessary to examine the status of T4. T4 is normal in the subclinical thyroiditis group and this is an indicator that the metabolic status can be considered as functioning normally. Therefore it cannot be assumed that the increase in the consumption of non-foodstuffs is as a result of an increased appetite as the incidence of coprophagia and pica would have increased in both the antibody negative hypothyroid and the subclinical hypothyroid groups. One explanation is that a potential cause of raised TgAA (e.g., stress) might also be the cause of the coprophagia (displacement activity). Alternatively, dogs eating faeces may be punished by their owners, which cause the dogs stress, possibly leading to a raised TgAA. However, the association between eating disorders and thyroid status warrants further investigation since consumption of faeces may be an indicator of incipient clinical hypothyroidism. The finding that faeces were the most commonly eaten material is possibly because this is the most commonly available form of organic matter.

Thyroid hormone concentration and behaviour disorders

The paucity of significant associations between titres of individual thyroid hormones and behavioural disorders indicates that such associations as these are, are with the whole disease state, rather than directly linked to individual hormones. It may therefore be more profitable in the future to record

complete thyroid hormone and antibody profiles when investigating potential links with behaviour that is not simply a reflection of lethargy.

Neck trauma and hypothyroidism

The findings are not consistent with the hypothesis that pressure on the throat by a choke chain can damage the thyroid gland, as although there was a significant difference between the groups, the euthyroid group had a higher incidence of dogs that pull and a higher incidence of dogs that wore choke chains than the TgAA negative hypothyroid and the subclinical thyroiditis groups. It is possible however, that the owners of dogs in non-euthyroid groups had previously used choke chains but as the disease developed and the dogs became more lethargic the choke chain was no longer required, but their thyroid gland may have been damaged prior to the discontinued use of the choke chain. The possibility that the bigger dogs exerted more pressure on their lead and therefore neck was also considered but the association is independent of dog size and weight as there was no significant difference in weight of dogs between the groups. Similarly pulling independently of a choke chain was not significantly associated between the groups. This area warrants further investigation. The euthyroid group contained both dogs that were screened for hypothyroidism prior to breeding and therefore not showing symptoms of hypothyroidism as well as dogs that were tested for hypothyroidism as a result of exhibiting symptoms of hypothyroidism. This may have influenced the effectiveness of the euthyroid group as a comparative group, however, without further information on behaviour patterns and symptoms exhibited at the time of testing this cannot be investigated. The finding that antibody positive hypothyroid group exhibited more pulling than the antibody negative hypothyroid group is consistent with the stage of the disease as at the antibody negative stage the hypothyroidism is very advanced and the dog is likely to be extremely lethargic and therefore not pulling on the lead.

7.5 Concluding comments

The results of this investigation suggest that the null hypothesis should be partially rejected as a difference has been found in the incidence and types of behaviour disorders between hypothyroid and euthyroid dogs. These differences are generally consistent with the theory that hypothyroidism has behavioural symptoms. Separation related disorders (negatively) and training problems (positively) are associated with hypothyroidism and can be explained by considering the associated type of hypothyroidism. The findings from this study are similar to the findings of previous chapters and does not support the hypothesis that hypothyroidism is associated with behavioural change in the form of inappropriate aggression as I have found no evidence to suggest that hypothyroidism is associated with aggressive behaviour patterns and this is contrary to the suggestions in anecdotal reports (Section 1.3.4).

Chapter 8

General Discussion

The aim of this chapter is to reconsider the general hypothesis, review and discuss the principal findings, compare the experimental approaches, consider the applications, examine the limitations and recommend suggestions for future work.

8.1 A review of the general hypothesis

As anecdotal evidence suggested that aggressive behaviour could be caused by a hypothyroid condition, the general hypothesis stated that, *"there is a link between aggressive behaviour and hypothyroidism in the dog"*.

8.2 A review of the key results

The key results from the clinical population of dogs (Chapters 3, 4 and 5) suggest relationships between Activity and T3, Activity and TSH, Aggressivity and T4, Sedentary Postures and T3. The key results from the thyroid hormone population of dogs (Chapter 7) suggest relationships between subclinical thyroiditis and both training disorders and coprophagia.

A relationship between Activity and T3 was found in both Chapters 3 and 6. The results of chapter 3 suggested that a low level of Activity was associated with reduced levels of T3, however the findings of Chapter 6 found that low level of Activity was associated with increased levels of T3. I suggest that the findings of Chapter 3 are more reflective of the effects of reduced T3 as the relationship between lowered thyroid hormone and reduced behavioural activity are well established (Section 1.5.2), additionally the analysis of the behavioural and hormonal data in chapter 6 was based on a small sample size.

Reduced TSH was associated with a reduction in Activity levels (Chapter 3). This finding is consistent with the finding that a low level of Activity was associated with reduced levels of T3, and may be explained by the following mechanism: as TSH stimulates the thyroid gland to produce T4 which is then converted to T3, low T3 concentrations results from low TSH concentrations.

Reduced T4 was associated with a reduction in Aggressivity (Chapter 3), which is contradictory to the anecdotal suggestions by some veterinary clinicians that reduced T4 is associated with an increase in aggressive behaviour.

High levels of Sedentary Postures were associated with low titres of T3 (Chapter 4). This finding is entirely consistent with the relationship between Activity and T3 found in Chapter 3.

Separation related disorders were under-reported in dogs with both subclinical hypothyroidism and with TgAA positive hypothyroidism (Chapter 7). Subclinical hypothyroidism differs from TgAA positive hypothyroidism in that the TgAA positive hypothyroidism form has decreased T4 and elevated TSH whilst in the subclinical hypothyroidism both T4 and TSH are at normal levels, yet both these forms of hypothyroidism have positive TgAA readings. Owner reports of separation related disorders are often inaccurate in detail (Blackwell pers. comm.) presumably because by definition the owners are not present to observe the dog's behaviour. The most likely explanation for this finding is that, as thyroiditis progresses to the stage of TgAA antibodies being detected, symptoms of separation related disorders became less obvious to owner. There is no reason to assume that the underlying motivation for separation related disorders changes at this stage of the disease – it merely becomes less evident in behaviour.

Coprophagia/pica was associated with subclinical thyroiditis: Subclinical thyroiditis represents the relatively early stages of clinical hypothyroidism and changes in behaviour and eating habits are often the first sign of ill health, but further investigation is required to determine the mechanism by which these are related.

The results from this thesis do not support the initial hypothesis that hypothyroidism (or reduced thyroid function) is related to the exhibition of aggressive behaviour in dogs. The results do support the long established relationship between reduced behavioural activity and reduced thyroid hormone titre (Figure 8.1).

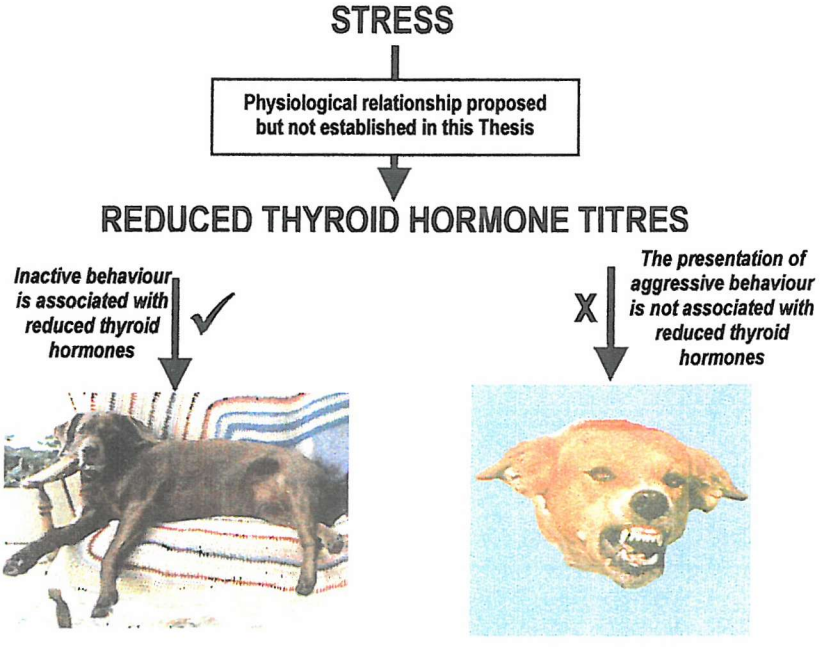

Figure 8.1 Summated thesis results

8.3 A discussion of the principal findings

8.3.1 Aggression and thyroid function

The results of this project do not support the general hypothesis that aggression is predisposed by low circulating levels of thyroid hormones, but conversely present evidence from the clinical population that aggressive behaviour patterns are positively correlated with tT4. Thus, low tT4 is generally associated with low incidences of aggression, and since hypothyroid dogs have low tT4 this refutes the idea that hypothyroidism and the occurrence of aggressive behaviour are linked. Low incidence of aggression among hypothyroid dogs is most probably a side effect of their inactivity (Section 8.3.2). The hypothesis that thyroid hormone concentrations exert a direct influence on the incidence of canine aggression is therefore improbable (Section 1.3.4). Although in human medicine it has been suggested that the brain is sensitised to the thyroid hormones that can alter behaviour and cause conditions such as ADHD (Ciaranello 1993), there is no evidence to suggest that this is the case in dogs.

8.3.2 Activity and thyroid function

Measures of activity determined from direct observation and from the behaviour patterns of dogs reported by their owners, showed that activity is correlated with tT3. Hypothyroid dogs with low tT3 are likely to be inactive. This is because the thyroid hormones are principally responsible for controlling metabolism and therefore the reduced physical activity is as a result of reduced metabolic rate in hypothyroid dogs. The association between low titres of thyroid hormone, both (T4 and T3), and lethargic behaviour in both dogs (Chandler *et al.* 1994) and humans (Werner and Ingbar 1978) is well established.

8.3.3 Behaviour and HPT axis feedback

In Chapter 3 I found that increased TSH was associated with increased activity. TSH concentrations are largely influenced by feedback from the thyroid gland to the pituitary, therefore both increased and decreased TSH could be justifiably associated with inactive behaviour patterns, depending on the stage of hypothyroidism. It is possible that in the early stages of hypothyroidism the relative concentrations of T4 / T3 can be increased or decreased depending on the type and cause of hypothyroidism or can fluctuate as in Hashimoto's disease in people (Volupe 1996) which in Beagles and chickens is histologically identical to the human disease (Volupe 1996). There is a very close relationship between Graves (hyperthyroidism) disease and Hashimoto's disease (hypothyroidism) in humans and it has also been suggested that there is an autoimmune basis for these diseases but the evidence for this is circumstantial (Volupe 1996). T4 and T3 concentrations are shown to be associated with changes in dog behaviour and are also the principal factors determining the production of TSH as a result of feedback on the pituitary gland.

8.3.4 Behaviour, subclinical and autoimmune thyroiditis

Behaviour, subclinical hypothyroidism and autoimmune thyroiditis are to be considered together as in Chapter 7 it was hypothesised that the subclinical hypothyroidism may be associated with dog behavioural disorders. This hypothesis was based on the possibility that the degeneration of the thyroid gland is a gradual process with a progressive development of symptoms such as a change in the temperament of dogs possibly first exhibited in the form of training problems. These changes in behaviour are likely to be secondary to the gradual development of pathology in the thyroid gland, which cause a change in the production of thyroid hormones. The results show that behavioural disorders (coprophagia/pica and training disorders) were associated with lowered thyroid function (subclinical thyroiditis and subclinical hypothyroidism respectively). However, separation related disorders are thought to be particularly closely associated with physiological stress (Overall 1997) and these were less common in dogs with some types of thyroid disorders, and no group could be identified in which these were more frequent than in euthyroid dogs. The interaction between the HPA and HPT axes could therefore vary in the different profiles of hypothyroidism.

The association between coprophagia/pica and training disorders with the early stage of thyroid disease could be explained by considering that behaviour disorders are often associated with the physiological stress response (Overall 1997). The stress response has an inhibitory effect of the HPT axis, which can cause both the subclinical and autoimmune forms of hypothyroidism, although Joffe and Sokolov (1994) state that in humans subclinical hypothyroidism is most commonly caused by autoimmune thyroiditis.

The effect of stress (either acute or chronic) on the immune system is not fully established, but stress can alter immunity and that can have major effects on disease (Maier *et al.* 1994). Certainly, immune parameters have been used as indicators of stress and compromised welfare (Manser 1992). There are two viewpoints on the relationship between stress and diseases mediated by the immune system, the first suggests that stress causes suppression of the immune system (Khansari *et al.* 1990; Kort 1994) and the second theory paradoxically suggests that stress enhances immunity (Maier and Watkins 1999). The latter suggestion although valid is less well verified, and in their review Maier and Watkins (1999) restrict their discussion to acute stress on the basis that the stress response evolved to deal with acute rather than chronic stressors. Maier and Watkins (1999) theorise the possibility that adverse emotional events may affect the immune system without involving endocrine pathways due to a bi-directional link between the CNS and immune system, the link being nerve endings detected in the thymus, spleen and lymph nodes. Thus the immune system may function as a diffuse sense organ informing the brain about events in the body regarding infection and injury. Whilst this view appears valid the concept that stress causes suppression of the immune system is more well documented and in order to explain the findings relating separation related disorders and autoimmune forms of

hypothyroidism I shall consider briefly, a) how stress depresses immunity and b) examples of the relationship between stress and autoimmune diseases.

McMillian (1999) suggests that the stress of an unpleasant emotional state can cause immunosuppression, which is largely an endocrinological reaction. In the event of raised physiological plasma glucocorticoids or during the administration of pharmacological doses of glucocorticoids the immune system function of mice, rats, rabbits, hamsters, monkeys and man have all been shown to become compromised. However, the immunosupression is not only the result of glucocorticoids as the opioid hormones and catecholomines have also been demonstrated as having depressing lymphocyte function (Manser 1997). Hadden (1987) suggests that other enhancing hormones such as insulin, growth hormone, somatostatin and thyroxine may also be suppressive to immune function but this is not well established.

Both infectious and autoimmune diseases are more common in people experiencing stress, e.g., people with rheumatoid arthritis (Feigenbaum *et al.* 1979; Section 1.2.3;). When rats were stressed by exposure to a cat they developed a worsening of an artificially induced autoimmune disease (Carlson 1998). In a strain of rats predisposed to developing diabetes those rats that were exposed to chronic stress had a higher incidence of developing the disease (Lehman *et al.* 1991).

One of the principal stressors that have been shown to lead to immunosuppression in laboratory mice (Rabin *et al.* 1987 cited in Manser 1992), rats (Steplewski *et al.* 1987 cited in Manser 1992) and bonnet monkeys *Macaca radiata* (Laudenslager *et al.* 1982 cited in Manser 1992) is social stress due to the inappropriate size of social group, changes in the composition of the social group and separation from the social group respectively. Therefore it appears probable that separation related disorders in dogs could also be associated with a malfunction of the immune system, but this is not in agreement with the result that high TgAA was associated with low reporting of separation disorders (Chapter 7).

While behavioural disturbance appears to be the most frequent mental state associated with clinical and subclinical hypothyroidism in humans (Extein and Gold 1987; Lesser *et al.* 1987; Nomura 1994; Dorn *et al.* 1996; Lasser and Baldessarini 1997; Leo *et al.* 1997; Stein and Avni 1988; Denicoff *et al.* 1990; Sauvage *et al.* 1998) this requires further empirical studies to determine if this is also true for dogs.

8.4 A comparison of the experimental approaches

In order to examine if dogs with behaviour disorders (in particular those with aggressive behaviour) had a reduced thyroid function I examined the thyroid status of a clinical population of dogs (clinical population) exhibiting inappropriate behaviour (Chapters 3-5). I further examined this association by investigating the behaviour of a population of dogs with different forms of hypothyroidism (hypothyroid population). Inactivity in the clinical population and training disorders and coprophagia in the hypothyroid population were highlighted as associated with reduced thyroid function. The difference in these two findings is possibly attributed to the different methods of behavioural data collection for the two populations. The behavioural data for the clinical population was largely observed directly whilst the behaviour of the dogs in the hypothyroid population was via a questionnaire. However, contrary to findings suggested by Dodds (1992), Dodds (1996), Dodman (1995) and Hamilton-Andrews (1998) there was no association found between reduced thyroid hormone and the occurrence of aggressive behaviour in either the clinical population or hypothyroid population.

8.5 Common factors relating behaviour disorders

Some research findings were notable but not directly pertinent to the relationship between behaviour and thyroid function; however; these issues are of relevance to dog behaviour and welfare and shall be considered further. In the classification of behaviour disorders in Chapter 5 it became obvious that fear and/or anxiety are likely to be a fundamental motivation in the, onset, development and/or continuation of numerous behaviour disorders. Therefore the relevance of emotional state to physical welfare is noteworthy. Lack of effective socialisation of puppies is one of the principal causes of the development of a fearful response in dogs (Appleby *et al.* 2002) and thus, the adequate socialisation of puppies in order to prevent the onset of specific or general fear behaviours should be emphasised for the prevention of behavioural disease. The effect of early experience in the development of appropriate social behaviour has been documented in other species. For example, laughing gull chicks *Larus atricilla* that do not hear crooning prior to hatching have a tendency to develop abnormal vocalisations (Impekoven 1976) and in a study in humans Brown and Harris (1978) found that women who experienced an environmentally deprived childhood were more likely to develop depression in later life.

8.6 Applications of project findings

Several research findings can be applied in order to benefit dog welfare.

8.6.1 The behavioural signs of hypothyroidism

Behavioural clinicians and veterinary surgeons should not associate the onset of an aggressive temperament with hypothyroidism unless other symptoms of the latter are present, e.g., hair loss. Although in some cases aggression may occur with hypothyroidism I have found no evidence to suggest the idea that the thyroid status has caused the behavioural change; the onset of aggression is generally multifactorial, with both environmental and physical factors contributory (Reisner 1996). Aggressive behaviour is also considered to be a non-specific sign of ill health and therefore the development of other diseases should be considered in the diagnostic process. In addition to the established behavioural signs of the onset of hypothyroidism such as lethargy, there may also be changes in the type and frequency of behaviour patterns from that which is normal for the individual dog. In Chapter 7 it was revealed that dogs with subclinical thyroiditis have an increased tendency to consume faeces. Therefore the consumption of faeces could be considered a non-specific sign of the onset of hypothyroidism, certainly more reliably than the onset of aggression.

8.6.2 The treatment of behaviour disorders

As aggressive behaviour patterns do not appear to be causally related to the onset of hypothyroidism, thyroid replacement therapy should not be used as a panacea for the treatment of aggressive behaviour patterns in the dog (unless clinical hypothyroidism has been positively diagnosed by diagnostic tests). In contrast to sex hormone supplements, which have been used (rather unsuccessfully) in the past in behaviour modification programmes, the effect of thyroid supplementation on behaviour is most likely to be a secondary effect rather than having a direct effect on behaviour. There are very serious health consequences associated with the inappropriate use of thyroid supplementation, due to its direct effects on cardio vascular and metabolic systems (Section 1.8) and hence these should also not be used unless clinical hypothyroidism has been diagnosed. With further research the stress reducing value of behaviour modification programmes and especially the use of behaviour modification to treat corticogenic hypothyroidism (Otsuki *et al.* 1973) should become clear and advantageous to dog welfare.

8.6.3 Measuring the effect of stress on thyroid function

Although measures of rT3 were not correlated with cortisol this may have been due to the small sample size in which the relationship was investigated. rT3 was however, significantly correlated with T3 and T4 and due to the established physiological relationship between glucocorticoids and thyroid hormone (Kaplan *et al.* 1977; Ferguson 1984; Drazner 1987; Kaptein 1988) I suggest that

measures of rT3 should be useful for the determination of the effect of stress on thyroid function. In addition to behavioural studies, measures of the HPT axis hormones, cortisol and rT3 are necessary in order to establish the accurate relationship between stress, behaviour and thyroid function for which additional work is required (Section 8.4.2).

8.6.4 Neck trauma and hypothyroidism

The ethical and welfare implications of dog restraint techniques have recently been hotly debated in Western Societies. However, the use of choke chains is common in both the UK and the USA and therefore the association between dogs pulling on choke chains and the incidence of hypothyroidism is a welfare consideration of great consequence. Choke chains have also been implicated in trauma to the spinal column in the neck (Dr Peter Graham pers. comm.). I would suggest therefore, that choke chains are not used to restrain dogs and particularly not in those breeds with an increased tendency to develop hypothyroidism.

8.7 Limitations of project and suggestions for future work

8.7.1 Limitations of project

i. In order to make firm conclusions about the reliability of rT3 and cortisol measures it would have been advantageous to have previous quality control data for the bioassay of rT3 and cortisol. However, the measurement of rT3 is used mainly for research purposes and is not a common diagnostic test, so quality control data is sparse.

ii. When testing the hypothesis that *"thyroid function is related to the behaviour of dogs considered as having behaviour disorders"* (Chapter 3) it would have been valuable to develop a matched control group of dogs without behaviour disorders, however, logistically this was not a possibility, and there would also have been conceptual difficulties in identifying a suitable population of owners.

iii. When measuring the behaviour of dogs in a familiar environment (Chapter 4) it would have been advantageous to measure dog behaviour directly rather than using the owner as a tool to collect behavioural data via a questionnaire. Direct observation would prevent the inconsistencies in observation associated with multiple untrained observers. However, due to the practical constraints associated with conducting companion animal behaviour research in the home environment this was not feasible.

iv. The lack of a universal terminology and an effective classification system for use with companion animal behaviour disorders (Chapter 5) makes investigating the relationship between behaviour disorders and thyroid hormone concentration problematic. Unfortunately, the published classification systems did not entirely account for the underlying motivations of behaviour, that were necessary in order to postulate associated physiological mechanisms in

sufficient detail. The production of such a classification system (8.4.2) would have required an investigation beyond the scale of this project. An effective classification of behaviour disorders would have facilitated a precise diagnosis based on motivation (and related physiological status) and it would have enabled the exploration of the relationship between behaviour disorders and thyroid status in greater detail.

v. When conducting longitudinal studies examining the thyroid and cortisol status of dogs with behaviour disorders (Chapter 6) it would have been beneficial to conduct a more rigorous assessment to monitor behavioural change. For instance, a structured temperament test carried out by the owner at regular, fixed time intervals could then have recorded changes in the dogs' behaviour patterns. Unfortunately, this process would have been too time consuming and due to the difficulty in recruiting appropriate subjects was not possible in this project.

vi. In order to compare the findings of the study associating hypothyroidism with behaviour (Chapter 7) it would have been advantageous to compare the data from Idexx laboratories with that of other laboratories in the UK, such as CSLS. However, due to the inter assay variation this was not considered reliable. Therefore, it would have been useful to send identical blood samples to both laboratories and calculate the intra assay variation; this would then have allowed comparisons to make cross sectional associations.

8.8 Future work

i. Development of a classification system for the behaviour disorders of dogs and universal terminology pertaining to the ethology of companion animals is required.

ii. Assessment of the welfare implications associated with behaviour modification and the effect on the stress physiology of dogs would be valuable.

iii. Additional endocrinological research is required in order to confirm the physiological relationship between the HPA and the HPT axis in dogs.

iv. An extensive programme that monitors the behavioural and endocrine development of companion dogs (perhaps via routine veterinary visits from puppy hood) would be a tremendous advancement in the deduction of relationship between hormone fluctuation and behaviour. In human medicinal research the association between physical and mental disease is often more straightforward because a medical history is usually available. Therefore a longitudinal programme that monitors canine development would highlight any relationship between the development of endocrinopathies and behavioural changes.

v. Identification of breeds with a greater risk of developing hypothyroidism is essential to the reduction of the genetic bias towards these diseases. DNA tests to identify the genes that predisposes individuals to hypothyroidism is not given high priority as the treatment of the disease, (although long-term) is relatively cheap and straightforward. However, controlled breeding programmes and monitoring the development of behaviour patterns would

determine the strength of lineage in the familial tendency for autoimmune thyroiditis and would assist in the determination of the relationship between stress and autoimmune diseases.

I conclude with reference to Matteri *et al.* (2000 p.62)

"Integrated, multidisciplinary approaches that fully utilize emerging technologies and methods of molecular biology, neurology and endocrinology will lead to future advances in our understanding of the biology of stress and related animal well-being"

Appendices

Categorisation of dog breeds according to the UK Kennel Club.

GUNDOG Bracco Italiano, Brittany, English Setter, German Shorthaired Pointer, German Wirehaired Pointer, Gordon Setter, Hungarian Vizsla, Hungarian Wirehaired Vizsla, Irish Red & White Setter, Irish Setter, Italian Spinone, Kooikerhondje, Large Munsterlander, Novia Scotia Duck Tolling Retriever Pointer, Retriever (Chesapeake Bay, Curly Coated, Flat Coated, Golden, Labrador), Spaniel (American Cocker, Clumber, Cocker, English Springer, Irish Water, Sussex, Welsh Springer) and Weimeraner.

HOUND Afghan, Basenji, Basset Bleu de Gascogne, Basset Fauve de Bretagne, Basset Griffon Vendeen (Grand, Petit), Basset Hound, Beagle, Bloodhound, Borzoi, Dachshund (long-haired, miniature long-haired, smooth haired, wire haired, miniature wire-haired), Deerhound, Elkhound, Finnish Spitz, Foxhound, Greyhound, Ibizan Hound, Irish Wolfhound, Norwegian Lundehund, Otterhound, Pharaoh Hound, Rhodesian Ridgeback, Saluki, Segugio Italiano, Sloughi and Whippet.

TERRIER Airedale Terrier, Australian Terrier, Bedlington Terrier, Border Terrier, Bull Terrier, Bull Terrier (miniature), Cairn Terrier, Cesky Terrier, Dandie Dinmont Terrier, Fox Terrier (smooth, wirehaired), Glen of Imaal Terrier, Irish Terrier, Kerry Blue Terrier, Lakeland Terrier, Manchester Terrier, Norfolk Terrier, Parson Jack Russell Terrier, Scottish Terrier, Sealyham Terrier, Skye Terrier, Soft Coated Wheaten Terrier, Staffordshire Bull Terrier, Welsh Terrier, West Highland White Terrier.

UTILITY Boston Terrier, Bulldog, Canaan Dog, Chow Chow, Dalmation, French Bulldog, German Spitz (Klein, Mittel), Japanese Akita, Japanese Shiba Inu, Japanese Spitz, Keeshond, Lhasa Apso, Miniature Schnauzer, Poodle, Schipperke, Schnauzer, Shar Pei, Shih Tzu, Tibetan Spaniel, Tibetan Terrier.

WORKING Alaskan Malamute, Beauceron, Bernese Mountain Dog, Bouvier des Flandres, Boxer, Bullmastiff, Dobermann, Dogue de Bordeaux, Eskimo Dog, Giant Schnauzer, Great Dane, Hovawart, Leonberger, Mastiff, Collie Neopolitan Mastiff, Newfoundland, German Pinscher, Portugese Water Dog, Rottweiler, St. Bernard, Siberian Husky, German Shepherd Dog.

MIXED

Any combination of breeds.

Case Questionnaire- Canine

Azl Behavioural Referrals
University of Southampton
Biomedical Sciences Building

Rachel Casey BVMS DipAS(CABC) MRCVS
(023) 80 597 045

Date of Consultation: *Case number:*

BACKGROUND INFORMATION

Your name:

Address:

Daytime Tel No:

Home Tel No.:

Referral Veterinary Surgeon:

Address:

Tel:

Name of dog:

Breed/Type: **Age:** **Sex:**

Is your pet neutered?

If so, when was it done?

Early history :

- How old was your dog when you obtained it?
- Can you remember where he / she come from?
- Was he / she rehomed or from a rescue centre?

Case Questionnaire- Canine

Diet :

- What do you feed him/her ?
- How many times a day is he or she fed?
- When do you feed him/her?
- Do you give any supplements, e.g. vitamin pills?
- Does he / she enjoy food or are they finicky?
- Do you give any tit-bits? If so, what?

Exercise:

- What type of exercise does your dog have?
- How many hours of exercise per day?
- Does he / she tend to be alone or with other dogs?
- Do you keep your dog on a lead, or allow them to run loose?
- Does he / she enjoy their walks?
- Is there any interaction / play with other dogs?
- What is your dog's favourite toy?
- What is your dog's favourite game with people?
- Where do you keep your dog's toys? Does your dog have free access to them?

Housing:

- Where does your pet sleep at night?
- Where does he/she stay when you go out?
- Is he/she left regularly? If so, for how long?

Case Questionnaire- Canine

- Are there any problems when you leave him/her? What happens?

- Do you leave any toys or other distractions?

- Is there access to the garden?

- When you are at home, does your dog tend to follow you around the house?

Training history:

- Have you attended training classes with your dog? How old was the dog at the time?

- How long did you attend for?

- Where there any problems with the training?

- Can you remember how you toilet trained the dog? Please describe.

- Does he / she walk to heel?
- Come when called?
- Drop objects when asked?
- What other commands does your dog know?

Family members

- How many people are there in your household? Are there any children? If so, how old are they?

- Does everybody interact with the dog?

Case Questionnaire- Canine

- Do you have any other animals? (Type, age, sex)

Medical history:

- Does your dog have any current medical problems to your knowledge?

- Do you know of any previous medical problems?

- Is he/she on any current medication?

THE PROBLEM

Describe the problems you are having with your dog in as much detail as possible (Please use a separate sheet if necessary) :

What happens immediately before your dog displays these behaviours? Try to think both what you and your dog are doing when the problem occurs.

Case Questionnaire- Canine

What happens immediately after? Again, think about what you do, and what the dog does.

When did the problem begin? Can you remember the first time it happened?

When does the problem occur? Is it in any particular circumstances?

How frequently, on average, does the problem occur? Do you think it is becoming more frequent, less frequent, or staying about the same?

Where does it occur? Is it, for example, always in the same place?

Who is usually present at the time?

When was the last incident, and can you describe this?

If your dog is an entire bitch, is the behaviour related to her season, or does it change during her season?

Do any related dogs have similar problems?

Case Questionnaire- Canine

Do any dogs in contact with it have similar problems?

Have there been previous attempts to cure this problem? (If so, please describe)

OTHER PROBLEMS

Does your dog have any other problems?

For example, is he or she good :-

- With children?
- With strangers?
- With family members?
- To groom or bath?
- When you feed them?
- With cats?
- With loud noises?
- When meeting other dogs?

Would you describe your dog as :

- A fussy feeder?
- Aggressive in any situation?
- Aggressive to other dogs?
- Nervous of anything, such as strangers or loud noises?
- Bouncy and enthusiastic?
- Sociable?
- Confident?

Does your dog enjoy being groomed? What kind of brush do you use?

Case Questionnaire- Canine

Are there any other problems with the dog?

Do you need to sedate him/her when you go to the vet, or for clipping nails?

Is this your first dog (not including childhood pets)?

If not, what breeds of dog have you owned previously?

REHABILITATION

How much time do you feel able to commit to working with your dog to solve these problems?

What would you envisage happening if the behaviour problem persists?

Thanks very much for your co-operation in filling in this questionnaire. If you have any queries, please do not hesitate to contact me on (023) 80 597045.
I look forward to meeting you and your dog.

Case Studies Questionnaire

Has (dogs name) shown any, or any changes in of the following symptoms or behaviours?

1. "Was there an increase/ decrease, or no change in his/her alertness?"
2. "Was there an increase/ decrease, or no change in urination or defecation?"
3. "Was there an increase/ decrease or no change in exploratory behaviour?"
4. "Was there an increase/ decrease, or no change in play behaviour?"
5. "Was there an increase/ decrease or no change in repetitive behaviours such as tail chasing?"
6. "Was there an increase/ decrease or no change in fearful or nervous behaviour?"
7. "Was there an increase/ decrease or no change in excitable behaviour?"
8. "Was there an increase/ decrease or no change in disobedient behaviour?"
9. "Was there an increase/ decrease or no change in vocal behaviour, i.e., whining, barking and/or growling, if so, which ones?"
10. "Was there an increase/ decrease or no change in aggressive behaviours directed towards people?"
11. "Was there an increase/ decrease or no change in aggressive behaviours directed towards dogs?"
12. "Was there an increase/ decrease or no change in destructive or distress-related behaviours such as barking when left alone?"
13. "Was there an increase/ decrease or no change in inappropriate mounting of people and / or other animals?"
14. "Was there an increase/ decrease or no change in the consumption of non-foodstuffs, such as faeces, fabric, if so which?"
15. Has (dogs name) developed any of the following symptoms?
    - "Hair loss"
    - "Scaly skin"
    - "Brittle hair"
    - "Lightening of the hair colour"
    - "Lethargy"
    - "Reluctance to exercise"
    - "Mental dullness"
    - "Intolerance to cold"
    - "Irritability"
    - "Hyperpigmentation of the skin"
    - "Thickening of the skin"
    - "Gain in body weight"
    - "Increased aggressiveness"
    - "Stiff locomotion"
    - "Heat seeking"
    - "Delayed healing of wounds"
    - "Constipation"
    - "Hypertension"
    - "Seizures"
    - "Disorientation"
    - "Circling"

Breeding Dogs

- "Infertility"
- "Lack of libido"

Bitches

- "Abortion"
- "Irregular cycling"

Counsellors guide as to how behaviour therapy is progressing

1. How severe do you currently perceive the dog's behaviour disorder to be?

VERY SEVERE ☐ ☐ ☐ ☐ ☐ NOT SEVERE

2. How confident are you that this behaviour disorder is being resolved?

VERY CONFIDENT ☐ ☐ ☐ ☐ ☐ NOT CONFIDENT

3. How has the dog's behaviour changed?

IMPROVED ☐ ☐ ☐ ☐ ☐ WORSENED

4. If the dog has made an improvement how much of an improvement has it made?

MUCH IMPROVEMENT ☐ ☐ ☐ ☐ ☐ NO IMPROVEMENT

5. On the line below, if **X** *marks the desired outcome as a result of the dogs behaviour modification programme, mark the line with another* **X** *how far you think it is from the desired outcome?*

Canine Thyroid Health Study

MICHIGAN STATE UNIVERSITY

University of Southampton

12 June, 2001

Dear Pet Owner,

Re: Behavior Questionnaire

Michigan State University's Endocrinology Laboratory and the Anthrozoology Institute at the University of Southampton in England have joined forces to try to find out more about how thyroid hormones might influence behavior in dogs.

Theresa Barlow is currently conducting a three-year study on the welfare of pet dogs. A major part of her work is to examine the relationship between dog behavior, hormonal status and other factors mentioned in the enclosed questionnaire. The enclosed questionnaire is a part of her research. The results of this research will assist in the correct interpretation of dog behavior patterns, both in the USA and UK. The fundamental aim of this study is to apply our results in a way that will improve dog welfare.

We would be extremely grateful if you would find the time to look over and fill-out the enclosed questionnaire. It is designed to be filled-out by an adult about Case 0292624, who recently had a thyroid test performed by Michigan State University. There are no right or wrong answers to the questions. Your anonymity will be respected. Please answer questions in as much detail as you feel comfortable and use the back of the pages for additional information you feel might be relevant. If behaviors have changed, we prefer that you provide your answers based on the way things were at the time the thyroid test was performed in October, 2000.

Please use the enclosed Business Reply envelope to return the questionnaire before July 15th, 2001.

We really appreciate any help you can give us.

Regards,

Dr Peter A Graham
Animal Health Diagnostic Laboratory
PO Box 30076
Lansing, MI 48909-7576
1-517-353-0621
CTHS@ahdl.msu.edu

Theresa Barlow
Anthrozoology Institute, University of Southampton
Biomedical Science Building
Bassett Crescent East
Southampton SO16 7PX UK
www.soton.ac.uk/~azi/azi.htm azi@soton.ac.uk

Behavior Questionnaire

General Information

Pet

Please check our information for errors and provide additional details where appropriate.

Dog's Name: Case 0292624

Breed: CANINE, UNDET.

Age: ____ years ____ months

Approximate weight: _____ lbs or _____ Kg

Sex:

- ☐ Male
- ☐ Female

Has your dog been neutered (castrated or spayed)?

- ☐ Yes – Approx. date: _____
- ☐ No

If your dog is an non-spayed female, when was she last in season?

____ / _____ (month/year)

Owner (s)

Please provide a method for us to contact you:
☐ if you don't mind us contacting you with further questions and/or
☐ if you would like to receive the results of this study,

Name: _____

Street Address: _____

City: _____

State: _____

Zip: _____

Phone: (____)_____

Fax: (____)_____

E-mail: _____

History

1. Age of your dog when obtained: ___months ___ years

2a. From where was your dog obtained? (eg. breeder or rescue shelter) _____

2b. Details of environment at that time?

_____

Exercise

3. How often does your dog get exercise? (Fill in and circle)

____minutes / hours *per* _____day / week / month

4. Does your dog interact with other dogs? ☐ yes ☐ no

If yes, how? (e.g. playfully or aggressively)

_____

5. Type of collar used when your dog is out on a leash?

Slip/Choke	Fixed/Buckled	Other:
☐	☐	_____

6. Does your dog "pull" against its leash? ☐ yes ☐ no

Toys

7. Does your dog like playing with people?

☐ yes ☐ no

8. Does your dog like playing with other dogs?

☐ yes ☐ no

9. What is your dog's favorite game?

_____

10. Does your dog like playing with toys?

☐ yes ☐ no

Training

11. What commands does your dog obey?

_____

12. Who trained your dog, you or someone else?

_____

The Thyroid Behavior Study is a joint project of The Canine Thyroid Health Study at Michigan State University and The Anthrozoology Institute of the University of Southampton, England email: CTHS@ahdl.msu.edu

Housing

13. Where does your dog sleep at night?

_____

14. Where does your dog stay when you go out?

_____

15. Is your dog left alone regularly? ❑ yes ❑ no

If yes, for how long (e.g. 6 hrs, 5 days a week)?

_____

16. As far as you are aware, how does your dog behave while left by itself?

_____

17. Does your dog follow any member of the family around the house? _____

Diet

18. What do you normally feed your dog?

❑ Dry	❑ Canned	❑ Homemade
(main brand)	(main brand)	(main types)

19. What time(s) of day do you feed your dog?

_____

20. Do you provide dietary supplements (e.g. vitamin pills)? _____

21. Does your dog eat all its food immediately?

❑ yes ❑ no

22. Do you give your dog food treats? ❑ yes ❑ no

If yes, what types and when?_____

Family Members

23. How many adults are in the household? _____

24. How many children are in the household? _____

25. Does every member of the household interact equally with the dog? _____

_____

26. Do you have any other animals? ❑ yes ❑ no

If yes, please give details (eg. type, age and sex):

_____

_____

_____

Medical History

27. When was your dog last vaccinated? ____(month/year)

28. Does your dog have any **current** long or short-term health problems? ❑ yes ❑ no

If yes, please explain: _____

_____

29. Has your dog had any other **previous** major medical problems? ❑ yes ❑ no

If yes, please explain: _____

_____

30. Is your dog currently on any medication? ❑ yes ❑ no

If yes, please explain: _____

_____

Behavior Problems

31. Does your dog do any of the following? (check boxes and circle choices)

- ❑ Barks at people (Strangers / Everyone / Other)
- ❑ Nips people (Strangers / Everyone / Other)
- ❑ Growls at people (Strangers / Everyone / Other)
- ❑ Barks at dogs
- ❑ Nips dogs
- ❑ Growls at dogs
- ❑ Fearfulness / Nervousness in only a few certain situations, eg. noise / strangers
- ❑ Fearfulness / Nervousness in many different situations
- ❑ Excitability to certain situations
- ❑ Excitability to many different situations
- ❑ Destructive when left alone
- ❑ Noisy when left alone
- ❑ Urinates / Defecates when left alone
- ❑ Disobedient outside the home
- ❑ Does not come back when off the lead
- ❑ Mounting people or other animals inappropriately
- ❑ Repetitive behaviors such as tail chasing
- ❑ Eating non-foodstuffs, eg. feces, fabric, please specify: _____
- ❑ Other behaviors, please specify:

_____

_____

Time	Ear Position		Tail Position			Tail Movement		Head Posture			Body Posture			Movement			Familiar		Vocalizations		
	Up	Down	Tucked	Relaxed	Flag	Wag	Still	Up	Level	Down	Stand	Sit	Lay	Walk	Run	Jump	Near	Not	Bark	Growl	Whimper

Alternative methods for the measurement of canine thyroid hormones

Many assay procedures have been used in the study of thyroid physiology. I will consider these methods and justify RIA as the best method to deduce thyroid hormone concentration in plasma.

a) *Protein Bound Iodine test*, chemically determines the amount of iodine that is co-perceptible with serum proteins, however many substances can interfere with the determination of results. Butanol Extractable Iodine test separates the inorganic iodine and iodoproteins from T4. However, the radiographic dyes can interfere with the samples and so this method is not frequently used.

b) *Radioactive Iodine Uptake* test uses a counting device to measure thyroid uptake of a tracer dose of an isotope of iodine. However, specialised equipment and strict precautions are required so this test is not possible within this institution.

c) *The Basal Metabolic Rate (BMR)* was the original test used in human medicine however this requires co-operative patients and is therefore was of limited use in veterinary medicine.

d) *The T3 Resin Sponge Uptake* test measures the unoccupied sites on binding proteins. This test does not measure T3 levels and requires additional tests for T4.

e) *ELISA* is a cheaper and safer method than RIA but it is less reliable. It is important to note that total T4 and total T3 concentrations are much lower in dog than human plasma so a sensitive assay needs to be used.

f) *RIA and Total Serum Thyroxine Column Technique*, Competitive Protein Binding are procedures for direct measurement of T4, (Siegel 1980). Of these RIA is sensitive and precise. The only interfering substances are those with similar chemical structure and cross-immunologic properties. RIA techniques are simple and economical and can measure both bound and free hormones; they are very specific, sensitive, precise and practical. The Gamma-B T4 supplied by Immunodiagnostic Systems Ltd., is a liquid phase RIA. It was used to quantify thyroxine concentrations in canine serum.

Quality control data for T4 for Febuary 2001 (top two tables) and November 2001 (bottom two tables). Data supplied by CSLS.

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative	Level 2 Month	 Cumulative	Level 3 Month	 Cumulative
T4, Total	Mean	35	34	88	92	168	178
RIA	SD	0	2	5	4	0.7	8
IDS	CV	0.0	5.2	5.6	4.7	0.4	4.7
Dedicated Reagent	# Points	2	295	2	299	2	290
nmol/L	Fixed Mean	35		92		179	
	Fixed SD	2		4		6	

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative
T4, Total	Mean	23.5	23.9
RIA	SD	0.1	2.0
IDS	CV	0.6	8.4
Dedicated Reagent	# Points	2	45
nmol/L			

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative	Level 2 Month	 Cumulative	Level 3 Month	 Cumulative
T4, Total	Mean	37	35	96	92	182	179
RIA	SD	2	2	4	4	11	8
IDS	CV	4.6	5.6	4.0	4.8	6.2	4.7
Dedicated Reagent	# Points	4	461	4	464	4	453
nmol/L	Fixed Mean	35		92		179	
	Fixed SD	2		4		6	

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative
T4, Total	Mean	24.2	23.3
RIA	SD	1.4	1.7
IDS	CV	5.9	7.3
Dedicated Reagent	# Points	8	281
nmol/L	Fixed Mean	23.4	
	Fixed SD	1.6	

Quality control data for T3 for Febuary 2001 (top two tables) and November 2001 (bottom two tables). Data supplied by CSLS.

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative	Level 2 Month	 Cumulative	Level 3 Month	 Cumulative
T3, Total	Mean	0.89	0.97	1.96	2.01	3.50	3.57
RIA	SD	0.00	0.05	0.00	0.07	0.00	0.14
Amerlex	CV	0.0	4.7	0.0	3.5	0.0	4.0
Dedicated Reagent	# Points	1	44	1	41	1	30
nmol/L	Fixed Mean	0.98		2.03		3.60	
	Fixed SD	0.04		0.07		0.20	

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative				
T3, Total	Mean	0.85	0.86				
RIA	SD	0.00	0.06				
Amerlex	CV	0.0	7.3				
Dedicated Reagent	# Points	1	10				
nmol/L							

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative	Level 2 Month	 Cumulative	Level 3 Month	 Cumulative
T3, Total	Mean	0.00	0.97	0.00	2.01	0.00	3.58
RIA	SD	0.00	0.04	0.00	0.07	0.00	0.14
Amerlex	CV	0.0	4.6	0.0	3.5	0.0	3.9
Dedicated Reagent	# Points	0	71	0	65	0	48
nmol/L	Fixed Mean	0.98		2.03		3.60	
	Fixed SD	0.04		0.07		0.20	

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative				
T3, Total	Mean	0.00	0.84				
RIA	SD	0.00	0.04				
Amerlex	CV	0.0	4.9				
Dedicated Reagent	# Points	0	39				
nmol/L	Fixed Mean	0.84					
	Fixed SD	0.04					

Quality control data for TSH for Febuary 2001 (top two tables) and November 2001 (bottom two tables). Data supplied by CSLS.

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative	Level 2 Month	 Cumulative
TSH	Mean	0.45	0.49	1.55	1.52
RIA	SD	0.00	0.04	0.00	0.11
DPC Coat-A-Count	CV	0.0	8.0	0.0	7.6
Dedicated Reagent	# Points	1	58	1	58
ng/mL					

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative
TSH	Mean	0.27	0.25
RIA	SD	0.00	0.03
DPC Coat-A-Count	CV	0.0	10.0
Dedicated Reagent	# Points	1	19
ng/mL			

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative	Level 2 Month	 Cumulative
TSH	Mean	0.48	0.48	1.52	1.51
RIA	SD	0.06	0.04	0.09	0.10
DPC Coat-A-Count	CV	11.8	7.9	6.0	6.8
Dedicated Reagent	# Points	2	124	2	124
ng/mL					

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative
TSH	Mean	0.25	0.26
RIA	SD	0.01	0.03
DPC Coat-A-Count	CV	5.7	10.2
Dedicated Reagent	# Points	2	111
ng/mL	Fixed Mean	0.25	
	Fixed SD	0.03	

Quality control data for Cortisol for November 2001. Data supplied by CSLS.

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative	Level 2 Month	 Cumulative	Level 3 Month	 Cumulative
Cortisol	Mean	74	78	514	531	818	847
RIA	SD	4	5	14	29	46	50
ICN Corti-Cote	CV	5.5	7.0	2.7	5.5	5.6	5.9
Dedicated Reagent	# Points	4	461	4	464	4	458
nmol/L	Fixed Mean	79		547		880	
	Fixed SD	5		26		48	

Analyte Method Instrument/Kit Reagent Unit Temperature		Level 1 Month	 Cumulative				
Cortisol	Mean	122	135				
RIA	SD	6	8				
ICN Corti-Cote	CV	4.7	5.8				
Dedicated Reagent	# Points	11	340				
nmol/L	Fixed Mean	134					
	Fixed SD	7					

Pilot study to determine the concentration of T4 in dog plasma

Introduction

A pilot study was conducted in order to determine the feasibility of using a commercial assay kit for the detection of T4 in dog plasma.

Method

The method for the determination of T4 using a commercial kit was followed (Appendix 11). The counter produced results for: -

1. Each total (containing 200µL of ^{125}I-T4). ^{125}I-T4 contained 0.05M barbitual buffer containing aminonaptholsuphonic acid and 0.09% sodium azide as preservative. Radioactive content <185kBq (5µCi) per bottle.
2. Each non-specific bound (containing 25µL of sample, 200µL of ^{125}I-T4 and 500µL of T4 antiserum complex). T4 antiserum complex contained pre-precipitated sheep anti-T4, 52mL per bottle and 0.04M of Borate buffer containing 0.09% sodium azide as preservative.
3. Each calibrator (containing 25µL of sample, 200µL of ^{125}I-T4 and 500µL of T4 antiserum complex). Seven calibrators were used containing T4 in 1ml of horse serum, each contained 0.09% of sodium azide as a preservative.
4. Each unknown sample (containing 25µL of sample, 200µL of ^{125}I-T4 and 500µL of T4 antiserum complex).

The counts for the known concentration samples were used to construct a standard curve from which the concentration of T4 in the unknown plasma was deduced. Two assays were performed, sample A using 25uL of sample and calibrator (Figures A11.1 and Table A11.1) and sample B using 50uL of sample and calibrator (Figures A11.1 and Table A11.2). From these two assays it was possible to determine the intra-assay variation for each assay, (coefficient of variation). This measures how much variation there is within an individual assay. An intra-assay variation of <5% is considered acceptable. To determine the effect of the other plasma constituents on the assay the concentration of the plasma was doubled, hence, sample B contained at 50uL of plasma. Lastly, the percentage binding curves were constructed for each assay in order to compare inter assay reliability. It is important to note that this is a pilot study and that the only difference between samples A and B was the difference in volume of plasma so it is not expected that these two samples will differ significantly. All tests were performed in duplicate. Both samples were plasma taken from the same dog at the same time.

Results

i. Coefficient of Variation

The within assay variation was calculated, i.e., the coefficient of variation, (CV): -

Sample A
CV (%) = (Standard Deviation / Mean) x 100
= (2.030/ 25.694) x 100
= 8%

Sample B
CV (%) = (Standard Deviation / Mean) x 100
= (0.5288/ 26.944) x 100
= 2%

ii. Percentage Binding

The percentage bound (B/BO%) of each calibrator, control and unknown was calculated as follows: -

$$B/BO\% = \frac{\text{(mean counts for each calibrator – means NSB counts)}}{\text{(mean counts for '0' calibrator – mean NSB counts)}} \times 100$$

iii. Standard Curves

Sample A: T4 in 25uL of canine plasma

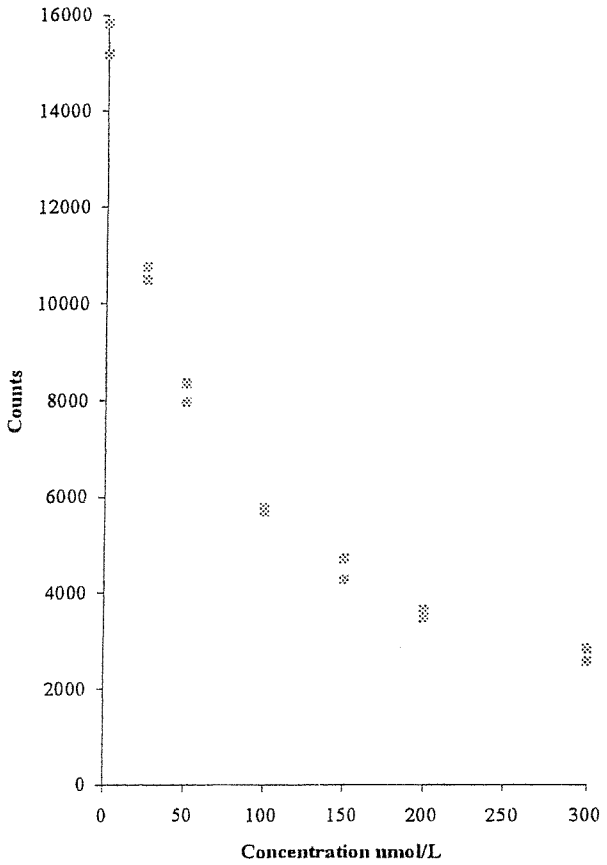

Figure A11.1 Standard Curve for Assay A (T4 at 25uL sample)

Table A11.1 Concentration of T4 from Standard Curve

[T4] nmol/L	Count
29.00	10133
27.25	10318
26.75	10373
25.80	10487
25.75	10490
24.75	10619
23.50	10761
22.75	10898

Sample B: T4 in 50uL of canine plasma

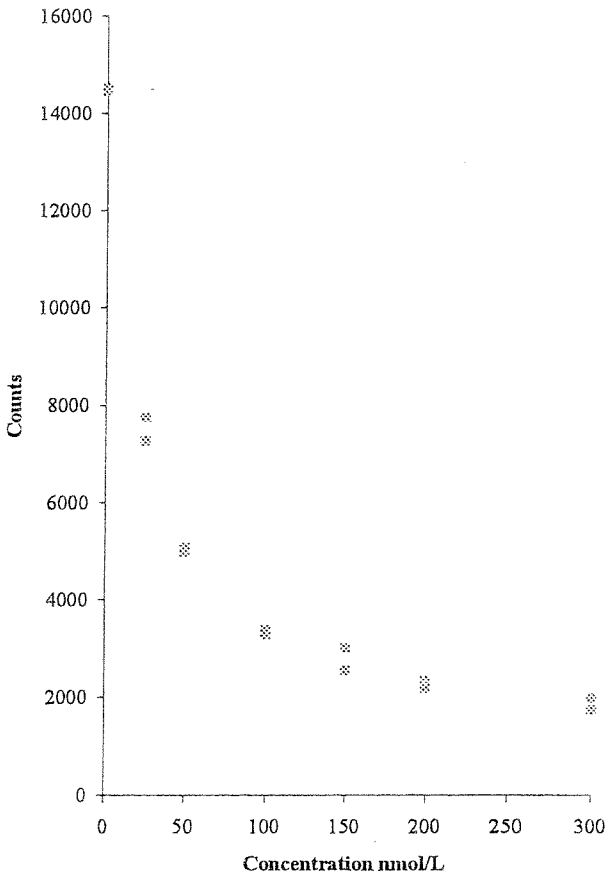

Figure A11.2 Standard Curve for Assay B (T4 at 50uL sample)

Table A11.2. Concentration of T4 from Standard Curve

[T4] nmol/L	Average Count
27.60	7132
27.50	7145
27.40	7160
27.00	7182
26.75	7256
26.70	7265
26.50	7307
26.10	7362

There was no significant difference between sample A and B, (Unpaired t-test with Welch correction, $t = 1.685$, $p>0.05$). However, on comparing the curves for both samples, sample B actually had lower values than sample A, this difference was not expected and is possibly due to errors in the assay procedure rather than the sample or commercial kit.

Discussion

There is no significant difference between the samples A and B (t test $p>0.05$). This was expected as the both samples of plasma were taken from the same animal at the same time. The coefficient of variation in assay A (8%) is more than would be ideal (5%), (Dr. A. Thomas, per. comm.). However, the variation in assay B is acceptable (2%). This shows that within assay A there was some degree of inaccuracy, this could have been at the blotting stage when all the tubes are inverted and put to drain. It is possible that each tube did not drain equally and therefore some had higher levels of radioactivity than other tubes. The percentage binding curves can be compared; in a replicated assay the curves would be exactly the same. A comparison of assays A and B shows that the curves are not identical but they are very similar and it is possible that the non-significant result is due to the small number of samples. However, the efficacy of the assay is acceptable for the detection of T4 in canine plasma samples.

Thyroxine

The gamma-B kit (supplied by Immuno Diagnostic Systems, Tyne and Wear, UK) provides a means of estimating the concentration of T4 in plasma. The kit requires sequential addition of sample, ^{125}I-thyroxine and pre-precipitated antibody complex. Following a 20-minute incubation at 37°C, the tubes are centrifuged, decanted and counted. Bound radioactivity is inversely proportional to the concentration of T4. Table A11.1 describes the procedure in full.

Table A11.1 Radioimmunoassy procedure for the deduction of T4 titre in dog plasma (Procedure provided by Immuno Diagnostic Systems, Tyne and Wear, UK)

REQUIRED MATERIALS (Not provided with the kit)	*REAGENTS* (Stored at 2-8°C)	*ASSAY PROCEDURE* (Reagents at room temperature and mixed gently prior to assay use)
Disposable 12x75mm polystyrene tubes and racks Plastic film to cover tubes Precision pipetting devices to deliver 25uL, 200uL and 500uL Vortex mixer Water bath capable of maintaining 37°C Centrifuge capable of at 1500 g for 22minutes Absorbent tissue for decanting supernatants Gamma scintillation counter	Calibrators: 7 bottles labelled A-G each containing T4 in 1ml horse serum (the exact value of each calibrator is known). Calibrators contain 0.09% (water/volume) sodium azide as a preservative ^{125}I-T4: ^{125}I-T4 is a yellow liquid there is 21 ml per bottle. 0.1% BSA-0.05M barbitual buffer containing aminonaptholsulphonic acid and 0.09% (water/volume) sodium azide as a preservative was used Radioactive content <185kBq (5uCi) per bottle T4 Antiserum Complex: Pre-precipitated sheep anti-T4, a blue liquid, 52mL per bottle). 04M-borate buffer is containing 0.09% (water/volume) sodium azide as preservative Assay Buffer: 0.4% BSA – 0.1M borate buffer, 5Ml, per bottle	1. Labelled tubes were prepared in duplicate. 25uL of each calibrator, control or unknown were added to the appropriately labelled tubes. 25uL of zero calibrator was added to non-specific binding (NSB tubes) 2. 200uL of ^{125}I-T4 were added to all tubes including two additional tubes that were set aside as total counts 3. 500uL of T4 antiserum complex was added to all tubes except TC tubes and NSB tubes 4. The tubes were vortex gently *without foaming and incubated for* between 20 and 22mins at 37 ± 2°C 5. The tubes were then centrifuged at 1500g for 20minutes on a Beckman E766, Model J-6B floor standing refrigeration centrifuge 6. The supernatants were decanted and the inverted tubes were allowed to drain on a pad of absorbent tissue. The rims were blotted to remove the remaining drops of liquid 7. The radioactivity in all tubes were counted on a LKB Wallac 1272 Clingigamma Automatic Gamma Counter for 5 minutes. In addition to the assay tubes 3 empty tubes were counted in order to deduce the radioactivity in standard tubes

Triiodothyronine

The Amerlex-M T3 radioimminoassay kit (supplied by Ortho-Clinical Diagnostics, Amersham, UK) provides a means of estimating the concentration of T3 in plasma. The kit utilizes a competitive immunoassy technique dependent on competition between T3 in the sample and ^{125}I labelled T3 for a limited number of binding sites on antibodies. The antibody bound fraction is separated and decanted. The amount of tracer bound is inversely proportional to the concentration of T3 present. Table A11.2 describes the procedure in full.

Table A11.2 Radioimmunoassy procedure for the deduction of T3 titre in dog plasma (Procedure provided Ortho-Clinical Diagnostics, Amersham, UK)

REQUIRED MATERIALS (Not provided with the kit)	*REAGENTS* (Stored at 2-8°C)	*ASSAY PROCEDURE* (Reagents at room temperature and mixed gently prior to assay use)
Disposable 12x75mm polystyrene tubes and racks Plastic film to cover tubes Precision pipetting devices to deliver 25uL, 200uL and 500uL Vortex mixer Water bath capable of maintaining 37°C Centrifuge capable of at 1500 g for 22minutes Absorbent tissue for decanting supernatants Control sera Gamma scintillation counter	4 bottles of tracer (125I-labelled T3, <370kBq) in barbitone buffer with Antimicrobal Agent (55ml) 4 bottles Amerlex-M antibody suspension (sheep anti-T3, binds≥35% of 8ng T3) in buffer with Antimicrobal Agent (55ml). 2 sets of T3 standards (0nmol/l, 0.5nmol/l, 1nmol/l, 3nmol/l, 5nmol/l, 6nmol/l, 12nmol/l) with Antimicrobal agent (1ml) 2 bottles of Amerlex-M NSB reagent in buffer with antimicrobal agent (5ml)	1. Labelled tubes were prepared in duplicate. 2. 50ul of zero standard was pipetted into NSB tubes 3. 50ul of standard, control and sample was pipetted into appropriate tubes 4. 500ul of tracer was pipetted into all tubes 5. 500ul of Amerlex-M antibody suspension was dispensed into all except NSB tubes; 500ul of NSB reagent was dispensed into NSB tubes 6. All tubes were vortexed, covered and incubated at 37°C for 60minutes 7. The rack was attached to the separatior base, left for 15minutes and decanted, drained for 15minutes and blotted 8. The radioactivity in all tubes were counted on a LKB Wallac 1272 Clingigamma Automatic Gamma Counter for 5 minutes. In addition to the assay tubes 3 empty tubes were counted in order to deduce the radioactivity in standard tubes

Thyroid stimulating hormone

The Coat-a-count TSH IRMA kit (supplied by Diagnostic Products Corporation Los Angeles, CA) provides a means of estimating the concentration of TSH in plasma. Canine TSH is captured between monoclonal anti-TSH antibodies on the inner surface of the polystyrene tubes and the polyclonal anti-TSH tracer. Unbound ^{125}I-labelled anti-TSH antibody is removed by decanting the reaction mixture and washing the tube. The canine TSH is directly proportional to the radioactivity present in the tube after the washing. The concentration of TSH in the canine sample is obtained by comparing the sample counts per minute with those obtained from the set of calibrators provided with the kit. The assay procedure has a minimum sensitivity of 0.1ng/ml. Table A11.3 describes the procedure in full.

Table A11.3 Radioimmunoassy procedure for the deduction of TSH titre in dog plasma (Procedure provided Diagnostic Products Corporation Los Angeles, CA)

REQUIRED MATERIALS (Not provided with the kit)	*MATERIALS and REAGENTS* (Stored at 2-8°C)	*ASSAY PROCEDURE* (Reagents at room temperature and mixed gently prior to assay use)
Disposable 12x75mm polystyrene tubes and racks Volumetric pipettes: 2ml and 1ml Distilled water Controls Gamma scintillation counter Absorbent tissue Plastic film to cover tubes Rack shaker Graduated cylinder for dispensing 400ml Micropipettes for 100ul Dispenser for 2.0ml Decanting rack	100 polystyrene tubes coated with murine monoclonal antibodies to canine TSH and packaged in zip lock bags Two 5ml vials of iodinated anti TSH rabbit polyclonal antibody with preservative One set of seven labelled vials, of lympholysed canine TSH calibrators in a TSH free canine serum matrix Calibrators; 0ng/ml, 0.15ng/ml, 0.3ng/ml, 0.6ng/ml, 1.5ng/ml, 4.0ng/ml, 12ng/ml One vial containing 40ml of a concentrated buffered saline solution, with surfactants and preservative	1. 14 tubes with canine TSH were labelled in duplicate 2. 100ul of each calibrator, control and canine serum was pipetted in to the prepared tubes 3. 100ul of ^{125}I TSH was added to every tube 4. All tubes were shaken for 3hours at room temperature 5. The tubes were decanted and 2ml of buffer wash added to each tube, after 1-2minutes a further 2ml of buffer solution was added and after 1-2minutes the tubes were decanted 6. All tubes were then counted for 1minute on the gamma counter

Thyroglobulin autoantibody

The VT-10 canine thyroglobulin autoantibody immunoassay kit (supplied by Oxford Laboratories Inc., Michigan, USA), uses ELISA for the determination of TgAA in canine serum. Purified canine Tg is coated on the wells of a polystyrene plate. The canine test sera and positive and negative canine control sera are added and bind to the plate, followed by enzyme labelled anti-immunoglobulin and autoantibodies that bind during incubation. The more concentrated TgAA in the sample, the higher amount of binding occurs resulting in a brighter colour. All reactions were ceased with the addition of stop solution. Table A11.4 describes the procedure in full.

Table A11.4 Elisa procedure for the deduction of TgAA titre in dog plasma (Procedure provided by Oxford Laboratories Inc., Michigan, USA)

REQUIRED MATERIALS (Not provided with the kit)	*REAGENTS* (Stored at 2-8°C)	*ASSAY PROCEDURE* (Reagents at room temperature and mixed gently prior to assay use)
Precision micropipettes with disposable tips: one 50-200 µL adjustable multiwell pipettor or 50 µL and 100 µL multiwell pipettors; 20-200 µL and 200-1000 µL adjustable pipettor. Beakers, flasks, cylinders necessary for preparation of reagents 5 mL 1N H2SO4 (stop reagent) 96-well plate washing/aspiration device 96-well plate reader for measurement of absorbance at 450 nm Deionized water	TgAA positive reference serum, 1 vial 50 µL TgAA negative control serum, 1 vial 50 µL Rabbit anti-canine IgG (HRP conjugated), 1 vial 100 µL EIA Phosphate Buffer (Blue), 125 mL TMB substrate, 20 mL Wash Buffer, 10x (Yellow), 25 mL 96 well microtiter plate precoated with canine Tg Two disposable reagent troughs	All reagents were mixed before use The number of strips required for the test was determined and all remaining strips were removed from the holder and stored at 4°C in the foil pouch provided The assay was performed in duplicate 100 µL of diluted samples was pipetted and transferred in to the thyroglobulin pre-coated wells (a 1:100 dilution was recommended) The plate was incubated for 2 hours at room temperature Post incubation the plates were washed with buffer, this process was repeated 3 times 100 µL of diluted peroxidase conjugated anti-canine IgG was added to each well and incubated for 1 hour at room temperature The plates were washed with buffer, this process was repeated 3 times 200 µL of enzyme-substrate conjugate was added to each well and allow to react for 10 minutes 50 µL of stop solution was added to stop the reaction The plate was read at an absorbance of 450 nm

Reverse triiodothyronine (rT3)

The rT3 radioimminoassay kit (supplied by BioChem ImmunoSystems, Rome, Italy) provides a means of estimating the concentration of rT3 in plasma without any initial treatment of the plasma. Antigen competes with the radioactive tracer ^{125}I-rT3 for the binding sites of the antibody. After the incubation period, the amount of tracer bound to antibody is inversely proportional to the amount of antigen present in standards or samples. In outline, the assay requires incubation of the reaction mixture for three hours at room temperature, precipitation of the immunocomplex with polyethyleneglycol, centrifugation and decantation followed by counting of the radioactivity. Table A11.5 describes the procedure in full.

Table A11.5 Radioimmunoassy procedure for the deduction of rT3 titre in dog plasma (Procedure provided by BioChem ImmunoSystems, Rome, Italy)

REQUIRED MATERIALS (Not provided with the kit)	*REAGENTS* (Stored at 2-8°C)	*ASSAY PROCEDURE* (Reagents at room temperature and mixed gently prior to assay use)
Disposable 12x75mm polystyrene tubes and racks Plastic film to cover tubes Precision pipetting devices to deliver 25uL, 200uL and 500uL Vortex mixer Water bath capable of maintaining 37°C Centrifuge capable of at 1500 g for 22minutes Absorbent tissue for decanting supernatants Control sera Gamma scintillation counter	1 vial of rT3 antiserum containing lyophilised anti-rT3 raised in rabbit, in Tris buffer with bovine serum albumin and sodium azide (<0.1%) 8 vials of rT3 standards containing lyophilised rT3 (0ng/ml, 0.025ng/ml, 0.5ng/ml, 0.1ng/ml, 0.25ng/ml, 0.5ng/ml, 1.0ng/ml, 2.0ng/ml) in phosphate buffer with bovine serum albumin and sodium azide (<0.05%) One vial of ^{125}I-rT3 in Tris buffer with sodium azide (<0.1%) One bottle of 20% TWEEN PEG solution containing polyethylenglycol (PEG) solution in phosphate buffer with Tween and sodium azide (<0.1%) One vial of Serotest containing lyophilised human serum	1. Tubes were prepared in duplicate for total radioactivity, NSB, bound antigen, standards and sample 2. 0.1ml of sample, standards and serotest was pipetted into the respective tubes 3. 0ml, 0.1ml and 0.2ml of the bound, NSB and standard were pipetted into respective tubes 4. 0.1ml of ^{125}I-rT3 was added to each tube 5. 0.1ml of rT3 antiserum was added to all the tubes except the NSB and total radioactivity tubes 6. All tubes were mixed and incubated for 3 hours at room temperature 7. 1.0ml of 20% PEG was added to all tubes except the total radioactivity tubes 8. All tubes were mixed and centrifuged for 15 minutes at 3500xg 9. The supernatant was decanted and each tube was counted on the gamma counter for 1 minute

Plasma cortisol

The Corti-Cote radioimminoassay kit (as supplied by ICN Basingstoke, Hampshire, UK) provides a means of estimating the concentration of cortisol in plasma. In the ICN pharmaceuticals Cortisol Solid Phase Component System tubes are coated with an antibody to cortisol for the separation of the bound and free forms. Post incubation the bound and free fractions are separated. The level of radioactivity bound is inversely related to the amount in the sample. Table A11.6 describes the procedure in full.

Table A11.6 Radioimmunoassy procedure for the deduction of cortisol titre in dog plasma (Procedure provided by ICN, Basingstoke, Hampshire, UK)

REQUIRED MATERIALS (Not provided with the kit)	*REAGENTS* (Stored at 2-8°C)	*ASSAY PROCEDURE* (Reagents at room temperature and mixed gently prior to assay use)
Evacuation glass tubes containing anticoagulant Water bath Aspirator Precision pipette (25ul) Semi-automatic pipette (0.5ml) Vortex mixer Gamma scintillation counter Absorbent paper	Cortisol antibody coated tubes 50ml of cortisol tracer solution (less than 5uCi (185kBq) cortisol 125I in phosphate buffer and 0.1% sodium azide as a preservative Cortisol serum standards (0ug/dL, 1.0ug/dL, 2.5ug/dL, 6.0ug/dL, 15.0ug/dL, 30.0ug/dL, 60.0ug/dL 0ug/dL) with 0.1% sodium azide as a preservative	14 antibody coated tubes were labelled 25uL of standards, controls and samples were pipetted out into the appropriate tube 500uL of cortisol tracer was added to each tube. The tubes were mixed in a vortex for 2-3 seconds Samples were incubated in a water bath at 37OC for 45 minutes The liquid was aspirated from each tube Tubes were drained for at least 3minutes on absorbent paper Each tube was counted for 1 minute on a gamma counter

Classification of behaviour disorders based on Borchelt and Voith (1982)

Classification	Description	Circumstances
Predatory aggression	Chase or bite directed to animals or humans	Preceded by stalking or moving object
Fear aggression	Bark, growl, bite directed to animals or humans, facial and body postures indicative of fear	When the dog is approached for, threatened or punished
Fear related dog-dog aggression	Bark, growl, bite directed to dogs, facial and body postures indicative of fear	When the dog is approached or threatened by a dog
Confidence related dog-dog aggression	Bark, growl, bite directed to dogs, facial and body postures indicative of confidence	When the dog is approached or threatened by a dog
Pain elicited aggression	Growl or bite directed to humans	When person attempts to groom, medicate or manipulate the painful area
Punishment elicited aggression	Bark, growl or bite directed to humans	When the dog is exposed to an aversive stimulus or a conditioned aversive stimulus.
Maternal aggression	Bark, growl or bite directed to animals or humans	When individuals approach, puppies, puppy surrogates or nesting area (may occur during pseudocyesis)
Re-directed aggression	Growl or bite redirected to person or object	When there is interference when dog is threatened or fighting
Resource related aggression to humans (confident aggression – humans)	Bark, growl, bite directed to humans, facial and body postures indicative of confidence	When the dog is approached or threatened by a human
Housebreaking	Urination and or defecation in home	Usually unrelated to presence / absence of owner. Large pools of urine.
Marking	Urination in the home	Usually unrelated to presence or absence of owner. Small numerous spots of urine.
Submissive urination	Urination	Accompanied by submissive postures when owner or stranger approaches
Excitement urination	Urination	Accompanied by excited greeting, jumping, playing etc.
Fear induced elimination	Urination / defecation	In presence of fearful stimulus.
Phobias	Whine, pant, shake, hide, run, increased heart rate.	Common eliciting stimuli include, loud noises, thunder, firecrackers, sight of strangers, traffic.
Separation related	Whine, bark, howls, elimination, destruction, depression, and psychosomatic response.	Long or short separation, may occur in the presence of people or animals, can occur at night if separated from owner, dog often stays close to owner when in the home.
Chewing, mouthing and nipping	Teething or development of play	Directed towards people or furniture.
Play related problems	Unwanted behaviours	During play
Other	Other	Other

An evaluation of salivary cortisol measures as an indicator of dog distress

Introduction

The aim of this investigation was to determine if canine salivary cortisol could be measured with the use of [1]salivettes and quantified by an in house immunoassay method developed for the evaluation of human salivary cortisol concentrations at Southampton University (Dr Peter Wood, Southampton General Hospital). When measured precisely cortisol is a reliable hormone for the assessment of acute and chronic stress, as it is a primary facilitator of the HPA axis. An accurate, simple and non-invasive and readily available method for the measurement of canine cortisol would be advantageous for dogs attending the ABC (Section 2.3), as cortisol measures would provide information on the state of their physiological stress response and may also be used to indicate the effectiveness of behavioural modification programmes (Section 1.2.7). This is valuable, as the adaptation to stress can substantially alter behaviour and prolonged stress is detrimental to welfare (Beerda *et al.* 2000a). However, for the purpose of this research project, cortisol measures used in conjunction with thyroid hormone titres may also assist in the deduction of the relationship between behaviour, stress and thyroid hormone concentrations (Section 1.7).

Whilst the collection and measurement of cortisol in dogs is a routine procedure, the method of assessment differs for pet and laboratory dogs. This is because their reactions to sampling techniques and their perception of stressors differ. Dogs kept in laboratory conditions become familiar with sampling procedures and should not react adversely to the use of either invasive or non-invasive methods required for the determination of stress. Pet dogs however, are very responsive and react readily to small environmental changes and differences in intraspecific and/or interspecific interactions, and thus obtaining a measurement of stress can in itself be stressful. This situation is further complicated when a measurement of stress is required for pet dogs with behavioural disorders. Frequently these dogs are presented at behaviour clinics; these are novel environments, in which the dogs are likely to be stressed which may result in enhanced cortisol titres.

There are several available techniques for the measurement of physiological stress. Some of the non-invasive techniques such as heart rate monitors are used for the assessment of stress in laboratory dogs. These cause minimal disturbance to dogs that are familiar with the apparatus as familiarity prevents the anticipatory responses that may induce a stress reaction (Beerda *et al.* 2000a). However, these methods are not suitable for pet dogs, as the equipment can be both cumbersome and also novel and therefore requires a lengthy period of habituation, (which is variable for each dog) in advance of stress measures. However, the habituation procedures can be stressful and may not be ethically

[1] An apparatus which assists in the collection and storage of saliva samples for the determination of cortisol concentrations.

suitable for dogs with existing behaviour disorders. In order to investigate the relationship between thyroid hormone titres, behaviour and stress an accurate method for the assessment of chronic stress in pet dogs with behaviour disorders was required. This requires a bodily fluid sample such as plasma, urine or saliva.

There are differences in the cortisol measures obtained from [2]plasma, [3]saliva or [4]urine. The measurement of salivary cortisol represents a measure of short-term stress as cortisol in its free form is readily transported to the plasma, whilst urinary cortisol represents long-term stress via pooled cortisol levels. Urinary cortisol represents the unbound biologically active form of cortisol as opposed to plasma cortisol, which is bound to plasma proteins. The venipuncture process has been shown to increase the plasma cortisol concentration in sheep (Falconer 1976), but there is evidence to suggest that if plasma samples are collected within three minutes cortisol measures are reflective of the stress status of the individual (Tuber *et al.* 1996). In humans, salivary cortisol has been validated as a non-invasive alternative to plasma cortisol and in dogs the collection of saliva and urine are established as valid, non-invasive methods to establish stress induced cortisol responses (Beerda *et al.* 2000a). Therefore, blood sampling may be replaced by saliva collection as a less invasive alternative for the measurement of cortisol levels (Beerda *et al.* 2000b), which may help to identify poor welfare in dogs that live in private homes (Beerda *et al.* 2000c) or other familiar environments.

Methods

The participants: Two dogs were used in this study; A, a neutered, Labrador Retriever / Collie cross bred, eight years of age; and B, a female, neutered, Jack Russell Terrier / Fox terrier cross bred, three years of age. The dogs were in good physical health, without behaviour problems and were not receiving any medication. Both the dogs were fed once a day at 20:00.

Collection of saliva: As cortisol levels follow a diurnal variation with the peak in cortisol secretion occurring toward the end of the dark period in diurnal animals (Manser 1992) it was necessary to take more than one sample in a day (Reimers 1990). All samples were collected when the dogs were in a familiar environment. Samples were collected within three minutes of the start of the procedure as in rodents it is known that three minutes is rapid enough to ensure that glucocorticoid levels in the samples obtained are not affected by the sampling procedure (Tuber *et al.* 1996). Saliva samples were collected by holding a pad of cotton wool (absorbent cotton wool manufactured by Robinson's) with plastic forceps and rubbing this around the mouth of each dog. The dogs were encouraged to salivate holding a piece of very strong smelling cheese in front of them, which they were given to eat after the three minutes sampling time had passed. The moist pad was then placed into a salivette

[2] Collection of plasma is invasive as veinpuncture is necessary.
[3] This procedure is less invasive than the collection of plasma but is potentially stressful.
[4] A non-invasive technique if collected post urination rather than via catheterisation.

(Figure *A13.1*) and immediately frozen at -20°C for 24 hours. This process was repeated at 11:00, 13:30, 16:30 and 19:30 on two consecutive weekdays. At each interval two samples of saliva were collected on separate pads (Sample 1 and Sample 2).

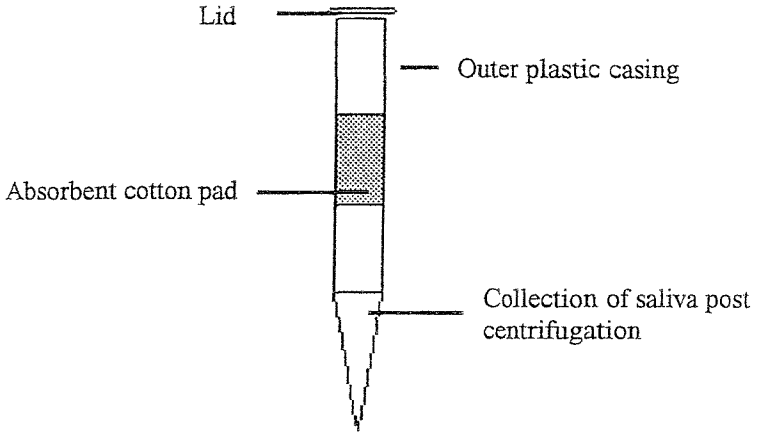

Figure *A13.1* A single salivette used for the collection and storage of saliva samples

Measurement of cortisol: The concentrations of cortisol in the saliva samples were deduced by immunoassay using the DELFIA system. This is a method developed and carried out by the Regional Endocrine Unit, Duthie Building, Southampton General Hospital, Southampton, Hants SO16 6YD. The method involved the initial coating of plates with goat-anti-rabbit-anti serum. The saliva samples were then incubated for 95 minutes with anti-cortisol antiserum and biotinylated steroid. The wells were then washed and enhancer added. The assay has been validated and recovery is in the region of 93-100% and the coefficient of variation is <12%. Freezing and thawing of saliva was essential prior to assay to ensure good recoveries of added steroid.

Results

The results for dog B are not presented, as in each sample taken there was an insufficient amount of saliva to permit assay. The results for dog A ranged from 1.9-3.3nmol/l on day one to 1.5-2.8nmol/l on day two (Figure *A13.2*). On both days the readings were higher in the morning than in the evening, but on day one the response decreased and then increased in the evening and on day two the concentration of cortisol generally decreased. On day one cortisol levels initially decreased and increased in the evening. On day two, cortisol levels showed a very slight increase (0.1nmol/l) and then decreased in the evening.

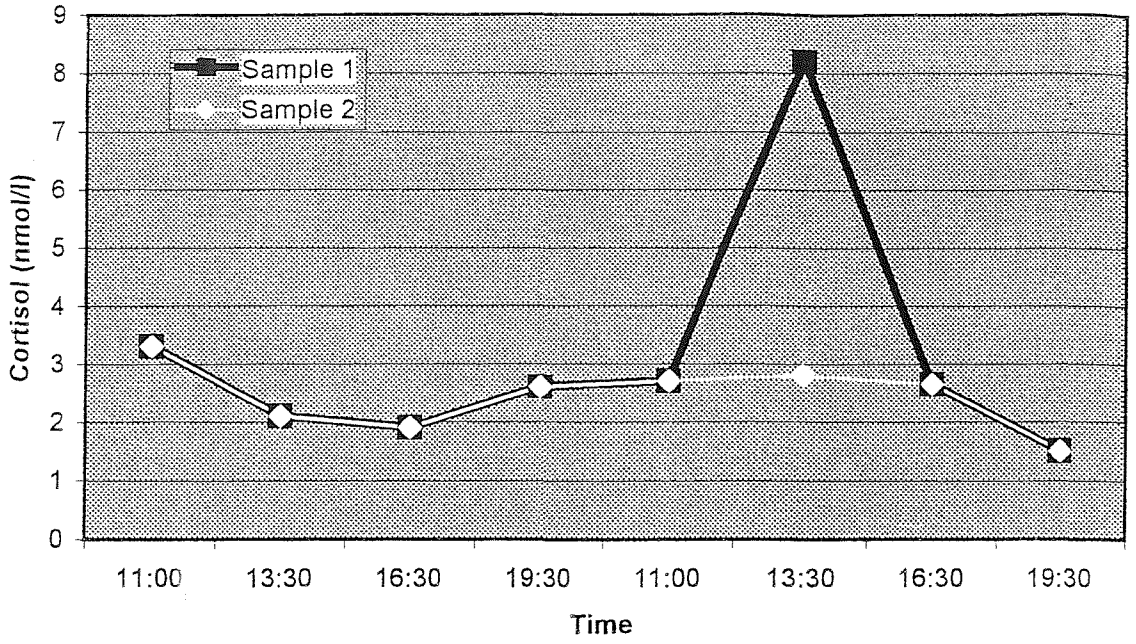

Figure *A13.2* Cortisol concentrations for Spud; samples collected in duplicate over two days

Discussion

Salivary cortisol for a non-stressed laboratory Beagle is 2.1±0.4nmol/l (Beerda 2000), which is within the same range of dog A's salivary cortisol. This suggests that the specificity of the DELFIA immunoassay method for the detection of canine cortisol is acceptable. However, the tests were done in duplicate and the results for Spud on day two at 13:30h produced results of 2.8nmol/l and 8.2nmol/l. As the samples were analysed simultaneously and in duplicate, identical results for each sample would be expected. Therefore these results suggest that the assay may not always be repeatable. Moreover, it appears that this is not suitable for dogs that do not salivate heavily.

Higher cortisol concentrations in the morning are suggestive of a diurnal variation but as this is a very small sample size this may not be the case. The general decrease in cortisol concentrations may be due to the dog habituating to the sampling procedure, however, there is an increase in cortisol at 13:30h on day one which is not consistent with day two which is difficult to interpret as both samples were taken within three minutes.

It is generally assumed that chronic stress is associated with increased cortisol levels and chronic stress is likely to indicate poor welfare (Beerda *et al.* 2000a). Salivary cortisol will change in response to acute stress but the changes will not be as sudden as for plasma cortisol. However, if measures are taken in duplicate over a long period of time then it is suggested that salivary cortisol can be indicative of chronic stress.

The use of collection tubes or pipettes to obtain saliva samples from dog cheek pouches can improve the method for the collection of saliva samples but this would require willing participants and may not be suitable for dogs with behaviour disorders such as aggression related disorders. Lemon drops have been used to stimulated saliva production in laboratory dogs (Beerda *et al.* 2000a), however, as this study required the use of volunteers with pet dogs and the use of a strong smelling cheese was considered to be more pleasant. It has also been questioned whether saliva flow rate affects cortisol titres, but saliva flow rate does not affect cortisol concentration in human subjects (Dr Peter Wood pers. comm.).

Conclusion

The results from this study indicate that determination of canine salivary cortisol is inconsistent using the DELFIA immunoassay for the detection of cortisol in humans saliva. As this assay measured cortisol in the same sample and produced different readings and ideally, in order to test the repeatability this pilot study should be repeated on a larger scale with more samples and additional dogs. However the use of salivettes for the collection and storage of canine salivary cortisol is not effective for dogs that do not readily salivate.

Autoimmune disease is a malfunction of the immune system in which the immune system inappropriately attacks the cells, tissues, and organs of an individual's own body.

Goiter is enlargement of the thyroid gland, more visible in humans and puppies than in adult dogs.

Graves' Disease is hyperthyroidism (also known as thyrotoxicosis) in humans. It is caused by an autoimmune reaction associated with exophthalmos (bulging eyes) and goitre and dermal changes. The second set of problems is caused by the excess thyroid hormone. The increased levels of thyroid hormone (see thyroid hormones) cause a disturbance in metabolism.

Hashimoto's Thyroiditis is hypothyroidism in humans, it also known as autoimmune or chronic lymphocytic thyroiditis and is the most common type of thyroiditis in humans. The thyroid gland is always enlarged, the cells of the thyroid becomes inefficient in converting iodine into thyroid hormone and "compensates" by enlarging, however, iodine uptake remains high, TSH increases in order to induce the thyroid gland to produce more T4, but T4 falls as the thyroid gland is unproductive. The individual becomes hypothyroid. The disease is confirmed by the identification of antibodies to the thyroid gland.

Hyperthyroidism is an excess of thyroxine in the blood.

Hypothyroidism is an insufficiency of thyroxine in the blood.

Reverse Triiodothyronine (rT3) an inactive form of T3 that is produced as a result of illness and/or the presence of glucocorticoids.

Thyroglobulin is a protein in the thyroid gland, a small amount of which gets into the blood.

Thyroid Binding Globulin (TBG) is a protein in the blood that binds with thyroxine (T4).

Thyroid hormones are the two principal hormones produced by the thyroid gland. These are thyroxine (T4) and triiodothyronine (T3) that travel to other body tissues via the blood primarily to regulate growth and metabolism.

Thyroid Stimulating Hormone (TSH) is a hormone produced by the pituitary that stimulates the thyroid gland to produce the thyroid hormones.

Thyroiditis is inflammation of the thyroid gland.

Thyrotoxicosis is a condition caused by excessive quantities of endogenous or exogenous thyroid hormone (see Graves disease and hyperthyroidism).

Thyroxine (T4) is the primary hormone produced by the thyroid gland. Forms include free thyroxine (fT4) and total thyroxine (tT4). Unless otherwise stated in this thesis the abbreviation T4 refers to total T4.

Triiodothyronine (T3) is the second hormone produced by the thyroid gland. Forms include free triiodothyronine (fT3) and total triiodothyronine (tT3). Unless otherwise stated in this thesis the abbreviation T3 refers to total T3. T3 is more potent than thyroxine (T4).

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