THE GERIATRIC CAT: DISEASES AND THYROID DYSFUNCTION

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ABSTRACT

This study describes the previous and current prevalence of medical diseases affecting geriatric cats in a referral population. Abnormalities of circulating thyroxine (T4) concentrations are described in sick cats and a simplified approach to treatment of hyperthyroidism with radioactive iodine (^{131}I) evaluated.

There was an increase in the number of cats presented at Glasgow University Veterinary School from, 417 between 1970 and 1972, to 835 between 1990 and 1992. Of these, 57 (13.7 %) cats of five years of age or over were referred as medical cases in the former time period, whereas 162 (19.4 %) were referred in the latter period. The most common disorders recognised between 1970 and 1972 were systemic neoplasia (21.1 %), gastrointestinal disease (19.3 %) and renal disease (8.8 %). The most common disorders diagnosed between 1990 and 1992 were hyperthyroidism (51.2 %), systemic neoplasia (10.5 %), viral infections (8.0 %) and renal disease (3.7 %). There was no conclusive clinical evidence of hyperthyroidism in cats between 1970 and 1972. Geriatric cats examined between 1970 and 1972 were significantly ($P < 0.001$) younger (mean $\pm$sd, 8.4 $\pm$4.0 years) than those seen between 1990 and 1992 (11.7 $\pm$3.3 years). Cats may now be living longer; the emergence of hyperthyroidism may be related in part to this increased longevity.

A pre-precipitated total T4 antibody radioimmunoassay kit and a free T4 equilibrium dialysis kit were validated for use with cat serum. The mean $\pm$sd (reference range) serum total T4 concentration in healthy cats ($n = 50$) was 26.00 $\pm$7.62 (10.75 - 41.25) nmol/l. The serum free T4 concentration ($n = 38$) was 24.79 $\pm$8.33 (8.14 - 41.45) pmol/l. The calculated free T4 fraction was 0.10 $\pm$0.06 (range, 0.04 - 0.37) %.

In cats with non-thyroidal illnesses ($n = 107$), serum total T4 concentrations (mean $\pm$sd, 17.35 $\pm$8.49; range, 2.00 - 45.33 nmol/l) were significantly ($P < 0.001$) lower than in healthy cats. There was a significant ($P < 0.001$) inverse correlation between mortality and serum total T4 concentrations. Serum free T4 concentrations (mean $\pm$sd, 27.70 $\pm$13.53; range 1.52 - 75.54 pmol/l) were not significantly ($P > 0.05$) different compared to healthy cats. Three cats (3.1 %) had serum free T4 concentrations below the reference range. Corresponding serum total T4 concentrations were also depressed. Twelve cats (12.2 %) had serum free T4 concentrations above the reference range. Corresponding serum total T4 concentrations tended to remain within the reference range. The calculated free T4 fraction in the sick cats (mean $\pm$sd, 0.24 $\pm$0.30; range, 0.06 - 2.10 %) was significantly ($P < 0.001$) elevated compared to healthy cats.

Serum total T4 concentrations (mean $\pm$sd, 164.02 $\pm$102.10; range, 39.69 - 575.57 nmol/l) were significantly ($P < 0.001$) higher in hyperthyroid ($n = 95$) compared to healthy cats. The serum total T4 concentration was not diagnostically elevated in three (3.2 %) hyperthyroid cats. The specificity, sensitivity and efficiency of serum total T4 estimations for diagnosing hyperthyroidism in this population were 100, 96.9 and 98.5 %, respectively. Serum free T4 concentrations (mean $\pm$sd, 233.32 $\pm$ 177.08; range, 46.88 - 687.61 pmol/l) were significantly ($P < 0.001$) higher in hyperthyroid ($n = 26$) compared to healthy cats. The serum free T4 concentration was not diagnostically elevated in one (3.8 %) hyperthyroid cat. The specificity, sensitivity and efficiency of this test were 93.9, 96.2 and 94.4 %, respectively. There was a highly significant correlation ($r = 0.92$, $P < 0.001$) between serum total and free T4 concentrations in the healthy and hyperthyroid cats. There was no significant ($P > 0.05$) difference in free T4 fractions between hyperthyroid (mean $\pm$sd, 0.11 $\pm$0.04; range, 0.07 - 0.25 %) and healthy cats.

A simple method of ^{131}I dose estimation was devised based on clinical severity of the thyrotoxicosis, serum total T4 concentration and size of goitre. The mean $\pm$sd dose of ^{131}I administered to 50 cats was 143 $\pm$24 MBq. The treatment was effective in 47 (94 %) cats. There was no significant ($P > 0.05$) difference in the outcome between cats in which ^{131}I was injected intravenously ($n = 27$) or subcutaneously ($n = 23$).

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AUTHOR'S DECLARATION

The work presented in this thesis was performed solely by the author, except where the assistance of others is acknowledged.

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Publications

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DEDICATION

To my family, both old and new.

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

Interest in the field of small animal geriatrics or gerontology has recently increased (Goldston, 1989). Despite this, there are few reports detailing the categories of disease and their prevalence in the older cat. In 1968, Griffiths surveyed diseases affecting cats over eight years of age presented to 10 veterinary practices in a six month period. Given the major advances in feline medicine, the numerical increase in the cat population and the probable increase in the proportion of aged cats since 1968, this report is outdated. Notably, feline hyperthyroidism (thyrotoxicosis) was first definitively diagnosed in 1979 by Cotter and by Peterson, Johnson & Andrews but was not known to exist prior to this date. The reasons for the emergence of hyperthyroidism remain obscure but may be related to a previous lack of awareness of the disease or to a longer average life span for cats (Peterson, Randolph & Mooney, 1994).

Since the emergence of feline hyperthyroidism as a common disease, there has been increasing interest in thyroid hormone concentrations and associated abnormalities in the cat. To date, the concentrations of circulating total (protein-bound and free) 3,5,3',5'-tetraiodothyronine (thyroxine (T4)) and total 3,5,3'-triiodothyronine (T3) in euthyroid and hyperthyroid cats have been clearly defined (Peterson, Kintzer, Cavanagh, Fox, Ferguson, Johnson & Becker, 1983b; Thoday, Seth & Elton, 1984; Mooney, 1990; Thoday & Mooney, 1992). However, a paucity of information exists regarding circulating free T4 concentrations in euthyroid and hyperthyroid cats and in cats with non-thyroidal illnesses.

In humans, it is recommended that serum free T4 concentrations are measured by the techniques of equilibrium dialysis or ultrafiltration, but interpreted with caution if other methods are used (Ekins, 1985). Absolute serum free T4 concentrations are assessed either directly, or indirectly by measurement of the free T4 fraction and subsequent calculation from serum total T4 concentrations. Refetoff, Robin & Fang (1970) suggested that the serum free T4 concentration in warm blooded vertebrates is relatively constant despite variations in serum total T4 concentrations between species. This theory has generally been extrapolated for the cat but confirmed in only a small number of cases. Hays, Turrel & Broome (1988) estimated the free T4 fraction by equilibrium dialysis in five healthy cats but the absolute concentrations were not reported. Ferguson, Peterson & Nachreiner (1989) measured the absolute serum free T4 concentration by equilibrium dialysis in 28 healthy cats, but the free T4 fraction was not calculated.

In man, the presence of severe non-thyroidal illness has a profound effect on circulating thyroid hormone concentrations (Wartofsky & Burman, 1982). Serum total T4 concentrations are markedly depressed, but serum free T4 concentrations generally

remain within the reference range, are occasionally elevated but rarely depressed. Serum total T4 concentrations are also depressed in the presence of non-thyroidal illness in the dog (Ferguson, 1988). At the time of commencement of this study, there were no reports on the effect of non-thyroidal illness on serum total T4 concentrations in the cat. During the course of the study, Peterson & Gamble (1990) reported that serum total T4 concentrations were depressed in cats with non-thyroidal diseases. However, there are no conclusive reports on the effect of illness on corresponding serum free T4 concentrations.

A diagnosis of feline hyperthyroidism is confirmed by the demonstration of an elevated serum total T4 concentration. Occasionally hyperthyroid cats have serum total T4 concentrations below the diagnostic range (Peterson, Graves & Cavanagh, 1987). In humans, serum free T4 concentrations are more consistently elevated and provide a better indication of the degree of hyperthyroidism (Woeber, 1986). Ferguson, Peterson & Nachreiner (1989) measured serum free T4 concentration in 25 hyperthyroid cats and suggested that its measurement provided no additional information over measurement of serum total T4 concentrations alone. Hays, Turrel & Broome (1988) reported that the free T4 fraction is similar in healthy and hyperthyroid cats, although only six hyperthyroid cases were examined. There are no reports comparing serum free T4 concentrations in hyperthyroid cats and cats presenting with non-thyroidal illness.

Hyperthyroidism is effectively treated by surgical thyroidectomy or the use of antithyroid drugs. However, administration of radioactive iodine is considered to be the safest, simplest and most effective method of controlling hyperthyroidism (Peterson & Turrel, 1986). The calculation of a therapeutic dose of radioactive iodine has previously relied on tracer kinetic studies and the use of sophisticated computerised nuclear medicine equipment. The administration of standard fixed doses, although applicable, have resulted in under or over treatment of a number of animals. Where the success of radioactive iodine therapy has been reported, it was recognised that the efficacy was often related to the size of the thyroid gland, circulating serum total T4 concentrations and clinical severity of the thyrotoxicosis. During the course of this study, Jones, Cayzer, Dillon & Smidt (1991) reported that the required dose of radioactive iodine for each cat could be effectively calculated from these parameters. However, although a grading system was provided for the size of the goitre and the clinical severity of the disease, no grading regimen was provided for serum total T4 concentrations, so that their work is difficult to reproduce. In addition, radioactive iodine has usually been administered intravenously, there are no studies reporting its efficacy when administered subcutaneously.

1.2 PROJECT AIMS

The present study was carried out against this background; the aims were as follows

To assess the changing trends in diseases affecting geriatric cats, by establishment of a medical referral clinic dedicated to older cats, and by reviewing case records from a similar time period 20 years ago.
To determine if any animals exhibited clinical features consistent with a diagnosis of hyperthyroidism by a retrospective study of the feline case records.
To assess the possibility that the emergence of hyperthyroidism is age related by an analysis of the ages at which cats were presented.

To fully validate a recently developed equilibrium dialysis kit method for use with cat serum and to assess absolute serum free T4 concentrations in healthy cats.
To calculate the free T4 fraction by measurement of corresponding serum total T4 concentrations, and thereby provide a complete standard base for subsequent comparison purposes.

To assess the alterations in serum total and free T4 concentrations and the calculated free T4 fraction in sick cats with a variety of non-thyroidal diseases.

To compare the efficiency of serum total and free T4 estimations for the diagnosis of hyperthyroidism, particularly in comparison to cats with non-thyroidal diseases.

To devise a simple and reproducible method of dose estimation for radioactive iodine therapy based on the clinical severity of the thyrotoxicosis, circulating total T4 concentration and size of goitre estimated by palpation.
To assess the efficacy of therapy comparing intravenous and subcutaneous routes of isotope administration.

CHAPTER 2
GENERAL MATERIALS AND METHODS

2.1 CLINICAL MATERIAL

The case material comprised a series of cats with a variety of medical illnesses referred by veterinary practitioners to the author at the Small Animal Clinic, University of Glasgow Veterinary School. Using the Jarrogate data-base system (University of Glasgow), each cat was assigned a case number with the inclusion of breed, sex, age, name and owner details. Relevant historical details were recorded and a full physical examination carried out. In almost all cases, blood was obtained by jugular venepuncture from conscious animals. The blood was collected into plain tubes for thyroid hormone assay, potassium ethylenediamine tetraacetic acid (EDTA) tubes for haematological examinations, lithium heparin tubes for routine biochemical and viral examinations and flouride oxalate tubes for glucose estimation. Haematological, biochemical and viral examinations were carried out within 24 hours of sampling. After clot retraction, serum was harvested by centrifugation and stored at -20^{o}C for future thyroid hormone assay. Further diagnostic tests, including radiography, electrocardiography, ultrasonography and biopsy were carried out as necessary. A diagnosis was subsequently made based on these findings, and in relevant cases was supplemented by post-mortem examination.

2.2 HAEMATOLOGICAL PARAMETERS

A full blood count was obtained using an ABX Minos ST Vet (Roche) haematology analyser. Buffered saline was used as a diluent and potassium cyanide as a lysing agent as described by the manufacturer. The parameters evaluated included red and white cell and platelet counts, haemoglobin concentration and haematocrit. For the differential white cell count, blood films were fixed and stained with a standard May-Grunwald-Giemsa stain, and 200 cells counted under oil immersion microscopy.

2.3 BIOCHEMICAL PARAMETERS

Plasma concentrations of urea, creatinine, phosphate, cholesterol, bilirubin, alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and glucose were measured using commercial reagent kits on a Cobas MIRA (Roche) clinical chemistry analyser. The commercial reagent kits, manufacturers and their methods are listed in Table 1.

Plasma concentrations of sodium and potassium were measured by atomic emission using a Flame Photometer 543 (Instrumentation Laboratories). Plasma concentrations of calcium and magnesium were measured by atomic absorption using an Atomic Absorption 257 Spectrophotometer (Instrumentation Laboratories). Plasma chloride

concentrations were measured coulormetrically using a Chloride Meter 925 (Corning Limited).

Plasma total protein concentrations were measured by a modified biuret reaction with blank correction and albumin by a bromocresol green automated method on a Technicon Auto Analyser AAII (Technicon Instruments Corporation). Plasma globulin concentrations were estimated by subtraction of albumin from total protein concentrations.

Quality assurance was guaranteed using commercially available control sera (Precipath U and Precipath EL, Boehringer Mannheim GmbH). Quality control was monitored by the Scottish Quality Assessment Scheme and UKEQAS for general clinical chemistry.

Serum total and free T4 concentrations were assayed as described in Chapter 4.

2.4 VIRAL EXAMINATIONS

Feline leukaemia virus (FeLV) status was assessed by the demonstration of p27 antigen using a commercial enzyme-linked immunosorbent assay (ELISA) (Petcheck FeLV antigen detection kit, IDEXX Laboratories). If positive, virus isolation in cell culture was carried out using an in-house technique. Feline immunodeficiency virus (FIV) infection was based on demonstrating the presence of FIV-specific antibodies using a commercial ELISA (Petcheck FIV detection kit, IDEXX Laboratories). Samples were screened for feline coronovirus (FCoV) antibodies by an in-house indirect immunofluorescence assay.

2.5 STATISTICAL ANALYSES

All statistical analyses, unless stated otherwise, were computed using the Statistix Version 4.0 (Analytical Software) package. This package was also used for the box and whisker plots. The level of significance was taken as $P < 0.05$.

Parameter	Reagent kit	Method
Urea	Unimate 5 UREA, Roche	Enzymatic UV test with urease and glutamate-dehydrogenase
Creatinine	T01-1927-02, Technicon	Enzymatic UV test with picric acid
Phosphate	Unimate 7 PHOS, Roche	UV test with phosphomolybdate
Cholesterol	Unimate 7 CHOL, Roche	Enzymatic colorimetric test with cholesterol esterase, cholesterol oxidase and a peroxidase catalyzed indicator reaction
Bilirubin	Bilirubin Test, Roche	4-sulfobenzenediazonium chloride (Jendrassik-Grof) reaction
AP	Unimate 3 ALP DGKC, Roche	Kinetic colorimetric test with 4-nitrophenyl phosphate
ALT	Unimate 5 ALT, Roche	Kinetic UV test with L-alanine and NADH
AST	Unimate 5 AST, Roche	Kinetic UV test with L-aspartate and NADH
LDH	MA-KIT 10 LDH SFBC, Roche	Kinetic UV test with pyruvate and NADH
Glucose	Unimate 7 GLUC PAP, Roche	Enzymatic colorimetric test with glucose oxidase and a peroxidase catalyzed indicator reaction

UV, ultraviolet
NADH, nicotinamide adenine dinucleotide

Table 1. Commercial reagent kits, manufacturers and methods used for analysing biochemical parameters on the Cobas MIRA (Roche) clinical chemistry analyser.

CHAPTER 3
AN INVESTIGATION INTO DISEASES OF GERIATRIC CATS: CURRENT AND PREVIOUS TRENDS

3.1 LITERATURE REVIEW

The aged cat

Ageing in itself is not a disease, but is a normal and complex biological process resulting in the progressive reduction of an individuals ability to maintain homeostasis under internal physiological and external environmental stresses. This decreases the subject's viability, increases its vulnerability to disease and eventually causes its death (Goldston, 1990).

In humans, the theory of ageing and attempts to retard its progress are important areas of research. There is a general consensus that ageing does not have a single cause, but is the result of a multitude of parallel and interacting processes that combine to ensure eventual decrepitude (Rusting, 1992). There are two main theories of ageing, both propose that these processes are genetically controlled (Partridge & Barton, 1993). The optimality explanation suggests that survival and fertility late in life are sacrificed for the sake of early reproduction and survival. The mutational explanation suggests that senescence has evolved because of a greater mutation load on the later, and less strongly selected, part of the life history (Partridge & Barton, 1993).

The internally controlled ageing process would eventually lead to death even in the absence of accidents, violence and infections. If ageing were retarded, this might delay much of the cancer, heart disease and other disabling conditions to which adults become increasingly vulnerable as they grow older (Rusting, 1992). The average person born in an industrialised nation today can expect to live approximately 75 years, whereas for most of history, life expectancy was closer to 30 or 40 years (Rusting, 1992). This increase is not related to modification of the ageing process but to changes in the environment including improved nutrition and the ability to control major infectious diseases by sanitation, vaccines and therapeutic agents.

Similar factors have produced longevity changes in the domestic cat population. Improved veterinary care and more advanced preventive measures, balanced nutrition and responsible ownership means that pets on average live longer (Kitchell, 1989). There are few vital statistics for cats currently available. Flower (1931) reported average longevity in the cat to be 14 to 15 years and Mellen (1940) reported it as approximately 14 years. Comfort (1956) investigating maximum ages reported 29 cats between 19 and 35 years old and suggested the cat to be the longest lived of the small domestic animals living in exceptional cases for 25 to 30 years. Morris (1994) reported that one cat was known to live to be 36 years of age. Griffiths (1968) studied 608 cats of eight years of age or over and found a marked reduction in the number of survivors after nine years of age in males and 10 to 11 years in the female. The author concluded that as in most

other species, the male cat is shorter-lived than the female. Morris (1994) reported a recent estimate indicating approximately 37 % of cats in the USA are over six years old. The cat population in the UK for 1993 was estimated at 7.05 million (Petfood Manufacturers Association Profile), thus about 2.6 million could be in excess of six years.

A recent survey undertaken by Diplomates of the American Colleges of Veterinary Internal Medicine, Veterinary Surgery, Veterinary Pathologists and the American Board of Veterinary Practitioners attempted to define the age at which cats were considered geriatric or most likely to suffer diseases associated with ageing (Goldston, 1990). The mean $\pm$ standard deviation (sd) age was reported as 11.88 $\pm$1.94 years. In the same survey the age at which dogs were considered geriatric varied depending on size but no such differentiation was noted for cats. However, Siamese cats were considered to have longer, and Persian cats shorter life spans. The survey also mentioned several other factors affecting longevity, although these factors were not differentiated by species and possible reasons were not included. Obese pets had a decreased longevity, and animals maintained strictly on balanced commercial diets lived longer than animals fed random table scraps. Feeding high fat and/or low fibre diets decreased life expectancy. Outdoor animals had shorter life expectancies than animals living indoors and neutered animals lived longer than entire animals. However, it is more practical to consider any animal over five years old at risk from age-associated changes or diseases with a definite age predisposition. Indeed Johnson & Mitzner (1989) in discussing the establishment of a geriatric programme in practice suggested that all pets over five or six years of age be selected as the nucleus of the programme.

The effects of ageing in the cat

There is limited information available regarding the normal physiological changes that occur in the aged dog and even less in the aged cat. Information is generally extrapolated from other species, primarily rats, mice and humans. The following description is largely taken from publications reviewing specific ageing changes of these species pertinent to, but rarely proven for the dog or cat (Mosier, 1981, 1989; Ross, 1989; Goldston, 1990; Mooney, 1991, 1993). Where information is available on the cat or dog, it is added.

In humans, there is a curvilinear decline in the resting metabolic rate with advancing age (Poehlman, 1992). Both total daily energy expenditure and energy intake decline with age. However, they do not decline proportionally, giving rise to a distortion in energy balance characterised by obesity or excessive leanness (Poehlman, 1992). This has not been addressed in the cat but Morris (1994) suggested a similar pattern in this species. Thus, as the metabolic rate decreases, and activity is reduced, the caloric need is decreased by up to 40 % in the last one third of normal life span.

The proportion of body fat to lean body mass increases and muscle, bone and cartilage mass decreases. The capacity for thermoregulation decreases due in part to

decreased heat production and to slow or less pronounced peripheral vasomotor reactions. Sensitivity to thirst decreases due a decrease in the number of osmoreceptors in the lateral superior hypothalamus and the development of arterio-capillary fibrosis. Sleep becomes more intermittent and the animal more restless. There is a loss of fastidiousness in excretory habits and a change in mental alertness. Generally there is a reduced reaction to stimuli and partial loss of the sensations (vision, hearing, taste and olfaction).

Few consistent age-related changes in the gastrointestinal system have been reported. Hepatic cell numbers decrease, together with a mild increase in fibrous tissue and fat accumulation. Hepatic function decreases and biliary and intestinal secretions decrease. There is a reduced rate of intestinal epithelial cell renewal. While these factors may suggest that the digestive system of older animals is less efficient, Morris (1994) argued that because of considerable reserve there may be no apparent loss of function unless seriously compromised.

The high incidence of atherosclerosis and hypertension contribute significantly to data on age-associated cardiovascular changes in humans. Since these are relatively uncommon in cats, Ross (1989) questioned the extrapolation of such data to the cat. Valtonen & Oksanen (1972) found that histopathological evidence of arteriosclerosis in 45 (77.6 %) of 58 dogs correlated with age and with endocardiosis, but the latter is an infrequent problem in cats. Baroreflex activity, blood volume, blood pressure, cardiac output and circulation time are decreased in the aged dog (Dodman, Seeler & Court, 1984). However, in the resting state, it is likely that heart function is not significantly different in the aged patient. If stressed, diminshed cardiovascular responsiveness to beta-adrenergic stimulation may become apparent (Maher & Rush, 1990).

Pulmonary function deteriorates progressively with increasing age in the dog (Dodman, Seeler & Court, 1984). This is attributable to physical changes that occur in both the lungs and chest wall. There is a decrease in lung elasticity, diffusion capacity, and pulmonary capillary blood volume (Robinson & Gillespie, 1973, 1975). Evaluation of thoracic radiographs of old dogs often reveals fibrosis, and bronchial and chondral cartilage calcification which presumably contribute to reduced ventilatory function (Dodman, Seeler & Court, 1984). There is an increase in the viscosity of lung secretions and a possible decrease in protective airway reflexes.

Renal changes associated with ageing are manifested by significant structural and functional alterations (Allen & Roudebush, 1990). The functional changes include decreased renal blood flow and glomerular filtration rate, a reduced ability to concentrate urine and to maintain sodium, water and acid-base homeostasis. There is an alteration in the distribution of vascular flow from the cortex towards the medulla. Structural changes include a decrease in the weight and volume of the kidney. The number of glomeruli decrease with an increasing percentage of sclerotic and abnormal glomeruli. The renal tubules decrease in number and proximal tubule volume and length decrease. Nephrosclerosis of varying severity, has been reported as a frequent morphologic

abnormality of geriatric dogs without biochemical evidence of renal insufficiency (Cowgill & Spangler, 1981).

Immunocompetence declines with increasing age leading to a decreased ability to ward off infections and an increased incidence of immune-mediated diseases. The leucocyte response is reduced with decreased chemotaxis and phagocytosis. In addition, the immune system may be less able to perform surveillance and eradication of neoplastic clones of cells in the early stages of the disease, thus allowing tumours to become established (Kitchell, 1989). As a rule the incidence of cancer increases with age and in general this is true for the cat (Kitchell, 1989). However, the age distribution of lymphoreticular malignancies is bimodal because of a peak associated with the early onset of cancer in cats infected with FeLV (Schneider, 1983). In addition, a notable finding in cats is that approximately 80 % of tumours are malignant (Carpenter, Andrews, Holzworth, Averill, Harbison & Moore, 1987).

Age-related reproductive changes are unique in the cat. In comparison to other species, queens do not generally lose their ability to reproduce with age. However, after six years of age, reproductive efficiency of queens generally declines as measured by inter-oestrus interval, litter size, incidence of congenital defects and frequency of problems during parturition (Harman & Talbert, 1985). Tom cats appear to have a reproductive life at least as long as queens (Ross, 1989).

In general terms, ageing results in or occurs simultaneously with the progressive and irreversible loss of organ reserve, regenerative powers of organ function, and adaptability (Mosier, 1989). Morris (1994) summarised the ageing process as a loss of functional units: neurones in the nervous system, nephron numbers in the kidney, ciliated epithelial cells in the respiratory tract; bone mass decreases and the functional ability of individual units is decreased. In themselves these changes will ultimately lead to clinical disease. Moreover, in the aged patient overt organ failure can also be precipitated when the patient is in a poor state of nutrition, when the environment is changed or where stress, of any form, is applied (Mosier, 1989). Mosier (1981) stated that recovery from disease takes 24 additional hours for each five years of age.

Diseases of the aged cat

Several recent articles have focused specifically on diseases of the aged cat including renal failure (Rubin, 1989), hyperthyroidism (Meric, 1989), neoplasia (Kitchell, 1989; Gorman, 1990), chronic respiratory diseases (Ford, 1990), diabetes mellitus (Wolf, 1989), and skin diseases (Halliwell, 1990). A contemporary edition of the Veterinary Clinics of North America focused entirely on geriatrics and gerontology (Goldston, 1989). Any current textbook on diseases of the cat provides age predispositions if applicable. However, there is only one report surveying disease conditions affecting the older cat (Griffiths, 1968).

Griffiths' (1968) survey represented an analysis of 608 cats over eight years of age seen during a six month period in 10 practices in the UK. Of these cats, 339 were male

(314 castrated and 25 entire) and 263 were female (238 ovariohysterectomised and 25 entire). Of the remaining six cases, the sex was not recorded. The preponderance of males was suggested to be due to the preference of so many people for male kittens. In all of the cases presented, a diagnosis was made on the basis of clinical examination or subsequently by post-mortem examination. The average age of the males which died or were euthanased in a terminal condition was 12 years and that of the females was 13 years.

Neoplasia occurred in 72 cats. The incidence of all other conditions was reported for the remaining 536 cases excluding neoplasia. The commonest conditions encountered, other than neoplasia were those of the respiratory system (14 % of clinical cases), teeth and gums (12 %), skin (12 %) and ears (9 %). The actual numbers of cats in each of these categories were not recorded. These conditions had a respective mortality of 9, 2, 0 and 11 %. Trauma from various causes accounted for 50 (9 %) of the cases presented. The most common were wounds or abscesses caused by bites, and these were twice as frequent in the male (8 % of clinical cases) as in the female (4 %). All of these cases survived. In addition, injuries due to accidents were seen in 17 (3 %) cases. Of these nine were probably caused by road accidents and the associated mortality rate was 24 %.

Disorders of the digestive tract were not considered to be a serious problem in the elderly cat. Only 21 (4 %) cases were presented, of which 14 were of constipation or colonic impaction. Diarrhoea was only noted in four cases and hairball and gastritis was diagnosed in three cases. All of these cats survived. Liver disease was noted in 12 (2 %) cats and was detected either by the presence of jaundice or on post-mortem examination. Nine (75 %) of these cases died or were euthanased. Nephritis was diagnosed in live animals in 23 cases and was certified as the primary cause of death in 23 further cases giving a total incidence of 9 %. A diagnosis of diabetes mellitus was made in only three cases (1 %). In one case, insulin therapy was instituted. One case was controlled by oral medication and the third case was presented in a terminal condition.

Twenty-five (5 %) cats were presented with nervous signs of which 10 were affected by "stroke", all of them over 10 years of age. Nine had paralytic conditions or paraplegia and six were affected with convulsions. Accurate diagnoses were not made and the associated mortality for this group was 63 %.

Neoplasia was the commonest cause of death in the survey. Investigation into the cause of death was possible in 138 cases, and neoplasia was implicated in 56 (41 %) cases. This represented more than twice the number of deaths due to nephritis (17 %), which was the next most common condition. In total, 72 neoplastic conditions were diagnosed giving an associated mortality for neoplasia of 77 %. The most common neoplastic condition was lymphosarcoma, accounting for 25 cases. None of these cases had been confirmed in the live animal. The remaining neoplasms were associated with the mouth and oropharynx (number of observations (n) = 12), liver (n = 8), ear (n = 6), digestive tract (n = 4), urogenital system (n = 2), mammary tissue (n = 6), labial

granuloma (n = 2), bone (n = 1) and others (n = 6). No further details on these tumours were reported. The incidence of neoplastic conditions by age were 10 % in cats of eight years, 10 % in cats of nine to 11 years, 29 % in cats of 12 to 14 years and 29 % in cats of 15 years or over.

Advances in feline medicine

Since the survey reported by Griffiths in 1968, several advances have occurred in feline medicine. Ancillary diagnostic aids are now more widely and routinely available including haematology, blood biochemistry and radiography. The existence of FeLV and its relationship to disease was discovered prior to 1968 (Jarrett, Martin, Crighton, Dalton & Stewart, 1964a; Jarrett, Crawford, Martin & Davie, 1964b), but testing for its presence, now routinely made, was only rarely performed. Holzworth (1963) described a peculiar chronic fibrinous peritonitis with a definite predilection for cats. The condition now known as feline infectious peritionitis (FIP) was described as seen most often but not invariably in kittens and young cats, often affecting several cats in a household or cattery. In 1970, Ward described its relationship to FCoV infection. Although still considered primarily a disease of younger cats, any age of cat may be affected.

Two recent discoveries pertinent to the aged cat require special mention. The isolation of FIV was first reported by Pedersen, Ho, Brown & Yamamoto in 1987, in cats from a multiple-cat household that exhibited a syndrome similar to acquired immunodeficiency syndrome. FIV is not a new virus, since antibodies to FIV have been found in stored feline sera collected as early 1975 in the UK (Gruffydd-Jones, Hopper, Harbour & Lutz, 1988). The median age of clinically healthy FIV infected cats is three years but the median age of sick cats is 10 years (Shelton, Waltier, Connor & Grant 1989). This reflects the relatively long latency period of FIV infection, which is thought to be between two and five years. Thus, FIV is considered to cause disease particularly in the older cat. In addition, male cats are more likely to be infected and infection in pedigree cats is less common than in cats of mixed breed (Hosie, Robertson & Jarrett, 1989).

Hyperthyroidism was first diagnosed as a distinct clinical entity in 1979 by Cotter and by Peterson, Johnson & Andrews. A small number of anecdotal reports analysed retrospectively suggest that hyperthyroid cats may have existed prior to this date (Mooney, 1990). However, the frequency of diagnosis has increased dramatically since 1979 (Scarlett, Moise & Rayl, 1988) and it is now accepted as the commonest endocrine disorder of domestic cats and one of the most frequently diagnosed disorders in small animal practice. In 1984 Peterson reported that one of every 300 cats presented to the Animal Medical Center, New York, irrespective of age or health status was hyperthyroid. Hyperthyroidism is a disease of aged cats and is seen almost exclusively in cats exceeding six years of age. The mean age at onset in the two largest series reported was 12.8 (Peterson *et al.*, 1983b) and 13.0 years (Thoday & Mooney, 1992). Peterson,

Randolph & Mooney (1994) reported that less than 5 % of cats are younger than 10 years of age at the time of diagnosis.

The increased frequency with which hyperthyroidism is now diagnosed may reflect an actual increase in incidence, an increased awareness of the condition by veterinary practitioners, an increased average life span for cats or a combination of these factors. However, 7, 000 cats that had necropsies performed at the Animal Medical Center, New York during the 14 year period from 1970 to 1984 have been reviewed (Peterson & Ferguson, 1989a). An average of only 1.9 cats per year were found to have gross evidence of thyroid enlargement (caused by adenomatous hyperplasia, adenoma or carcinoma) in the period before 1977. Benign adenomatous hyperplasia affecting one or both thyroid lobes is now recognised as the most common pathological abnormality associated with the condition (Mooney, 1990). The aetiology of hyperthyroidism in cats remains obscure.

In humans, the two most important forms of hyperthyroidism are Graves' disease and toxic multinodular goitre. Graves' diseases is an immune-mediated disorder in which circulating antibodies mimic thyrotropin (thyroid stimulating hormone (TSH)), thereby promoting thyroid hormone production and secretion. Despite the pathological changes in hyperthyroid cats which resemble toxic nodular goitre (Peterson & Becker, 1983), attempts have been made to identify stimulating immunoglobulins. Thyroid stimulating immunoglobulins, as found in Graves' disease have not been found in hyperthyroid cats (Peterson, Livingstone & Brown, 1987; Kennedy, Thoday & Mooney, 1989). Adenomatous thyroid tissue removed from hyperthyroid cats and transplanted into thyroxine-treated nude mice retains its histopathological appearance and continues to grow and function. Similar results are found in human toxic nodular goitre (Peter, Gerber, Studer, Becker & Peterson, 1987). Thyroid cells from hyperthyroid cats cultured in TSH-free media also continue to grow and function (Peter, Gerber, Studer, Peterson, Becker & Groscurth, 1991). Thus neither hyperfunction nor growth of toxic goitres depend on extrathyroidal stimulators. These reports suggest that intrinsic alterations within the thyroid gland lead to autonomy of follicular growth and function and subsequently to the development of hyperplastic nodules, causing thyrotoxicosis (Peter *et al.*, 1987, 1991). Increased titres of thyroid growth stimulating immunoglobulins have been identified in the sera from some hyperthyroid cats (Brown, Keating, Livingstone & Bullock, 1992). These autoantibodies stimulate thyroid growth but do not stimulate function and their precise role in hyperthyroidism is unclear. They may play a role in goitre formation. Thyroid function as indicated by *in vivo* serum total T4 concentrations did not differ in cats with increased titres of growth stimulating immunoglobulins compared to cats with no detectable titres (Brown *et al.*, 1992).

Scarlett Moise & Rayl (1988) attempted to identify possible risk factors for feline hyperthyroidism. A three to four-fold increased risk of developing hyperthyroidism was associated with regular treatment with flea sprays or powders, living strictly indoors and having reported exposure to lawn herbicides, fertilisers and pesticides. The greater the

proportion of commercial canned foods in the diet, the greater the associated risk. Cats whose diets were half or more canned food had 3.4 times the risk of developing hyperthyroidism compared to those fed no canned food. Cats fed some, but less than half canned food had 1.6 times the risk of those fed no canned food. Non-Siamese cats were approximately 10 times more likely to develop hyperthyroidism than Siamese cats. This breed predisposition has not been reported elsewhere (Peterson *et al.,* 1983b; Thoday & Mooney, 1992).

In humans, iodide administration is known to induce transient hyperthyroidism, the so-called Jod Basedow effect (Braverman, 1986). Iodide-induced hyperthyroidism frequently follows iodide repletion in endemic iodine deficient areas and occurs most frequently in patients with pre-existing thyroid autonomous nodules. Potassium iodate was added to bread throughout Tasmania (an iodine deficient area) in 1966 as a prophylactic measure against endemic goitre. After this the incidence of thyrotoxicosis at the two thyroid clinics on the island more than doubled (Connolly, Vidor & Stewart, 1970). Due to its transient nature, the Jod Basedow effect cannot explain the autonomous and progressive nature of feline hyperthyroidism. Despite this, several studies have examined the iodine content of cat foods in an attempt to implicate iodine in the cause or progression of hyperthyroidism. The iodine content of some commercial cat foods is up to 10 times recommended levels (Mumma, Rashid, Shane, Scarlett-Kranz, Hotchkiss, Eckerlin, Maylin, Lee, Rutzke, Gutenmann, Bache & Lisk, 1986). In another study, the iodine content of cat foods was found to vary more than 100-fold (Johnson, Ford, Tarttelin & Feek, 1992). Thus, certain foods are iodine deficient and some are excessive. Johnson *et al.* (1992) therefore suggested that wide fluctuations in iodine intake may eventually lead to thyroid dysfunction, particularly in individuals genetically or environmentally prone to thyroid disorders. The possible mechanisms involved were not addressed.

There is no doubt that iodine content plays a role in thyroid function in cats since serum free T4 concentrations, as measured by an analogue kit method, were found to be inversely correlated with urinary iodine excretion (Tarttelin, Johnson, Cooke, Ford & Feek, 1992). This study only examined the effect of feeding a fixed amount of iodine for two weeks and did not address if the results seen were permanent or temporary. Although not pertinent to the origin of hyperthyroidism, a change to a low iodine content diet had no effect on thyroid function tests in thyrotoxic cats (Peterson & Ferguson, 1989a). Thus the role of iodine in the pathogenesis of hyperthyroidism remains unclear. Cats may be exposed to other goitrogenic compounds (e.g. phthalates) either through their diets or in the environment that could contribute to the development of adenomatous lesions in the thyroid gland. These may be of particular importance because such hydrocarbons are metabolised by glucuronidation, a metabolic pathway particularly slow in the cat (Ferguson & Peterson, 1990).

More recent research is centred on the possible role of proto-oncogenes in the pathogenesis of hyperthyroidism (Ferguson & Peterson, 1990). Results have not yet been published.

3.2 INTRODUCTION

Griffiths (1968) surveyed disease conditions affecting cats over eight years of age. Despite the probable increase in the number of older cats presenting to veterinary surgeons today (Kitchell, 1989), there have been no recent reports on the prevalence of diseases in this population. This study was designed to assess the disease conditions affecting older cats in a referral population. A comparison with cats seen in a similar time period beginning in 1970 was made in order more clearly to assess changing trends in prevalence and their significance.

3.3 MATERIALS AND METHODS

Clinical material (1990-1992)

Using the geriatric age criteria of Johnson & Mitzner (1989) local veterinary surgeons were invited to refer sick cats of over five years of age to the medical section of the Small Animal Clinic. The study was carried out between January 1990 and June 1992. Each case underwent a full physical examination and ancillary diagnostic aids as described in Chapter 2. A diagnosis was subsequently made based on these findings, and in relevant cases was confirmed or refuted by post-mortem examination.

Historical records of clinical material (1970-1972)

The case numbers for cats seen between January 1970 and June 1972 were obtained from the Clinic day books. Microfiches of these records were then examined.

Statistical analyses

The Mann Whitney U test was used for all statistical analyses. Survival time (the time from initial presentation to death or last-follow) was determined in the non-hyperthyroid cats. A life table was calculated to determine the probability of survival and the data used to plot a survival curve as described by Colton (1974). Such a method accounted for the cats entering the study at different times, for the different lengths of time observed and the unknown endpoints for some cats at the time of study termination.

3.3 RESULTS

Clinical material (1990-1992)

The relevant case numbers are listed in Appendix 2.

Between January 1990 and June 1992, 162 cats of five years of age or over were examined. The number of cats seen represented 19.4 % of the total number (853) of cats seen at the Small Animal Clinic over this time period.

This group consisted of 81 females (74 ovariohysterectomised, seven entire) and 81 males (78 castrated, three entire). The majority of the cats were either domestic short

(n = 131) or long (n = 10) haired; the remaining 21 (13.0 %) cats represented five other breeds: Siamese (n = 13), Persian (n = 4), British Blue (n = 2), Burmese (n = 1) and Birman (n = 1).

The ages for 160 of the cats are summarised in Table 2. Two cats were of unknown age but were known to be in excess of 10 years because of the length of time in the owners possession. There was no significant (P > 0.05) difference in the ages of the female compared with the male cats. The oldest cat, at 20 years of age, was a female.

	Male	Female	All cats
n	80	80	160
Mean	11.4	11.6	11.5
sd	3.3	3.3	3.3
Minimum	5.0	5.0	5.0
1st quartile	8.5	9.0	9.0
Median	12.0	12.0	12.0
3rd quartile	14.0	14.0	14.0
Maximum	18.0	20.0	20.0

Table 2. Summary statistics of the ages (years) in the male, female and all geriatric cats grouped together, presented to the Small Animal Clinic between January 1990 and June 1992.

Diseases affecting the geriatric cats (1990-1992)

In 83 (51.2 %) cats, the main diagnosis was hyperthyroidism. The mean ±sd age at time of diagnosis in 82 of these cats where ages were known was 13.0 ±2.2 years. Only five (6.0 %) cats were less than 10 years old. The number of hyperthyroid cats of different ages compared to the total population of cats are illustrated in Figure 1.

At the time of initial presentation, 16 (19.3 %) of these hyperthyroid cats had concurrent illnesses of significance. Seven (8.4 %) had clinical and radiographic evidence of congestive cardiac failure. Four (4.8 %) had clinical and biochemical evidence of renal failure. Three of these cats were euthanased and in one renal lymphosarcoma was found on post-mortem examination. Three (3.6 %) cats were positive for FeLV antigen (n = 2) and FIV antibodies (n = 1). One cat was euthanased because of constipation. A rectal stricture and a hepatic and pulmonary adenoma were found on post-mortem examination. The remaining cat was blind as a result of damage to the retinas caused by hypertension and was euthanased at the owners request.

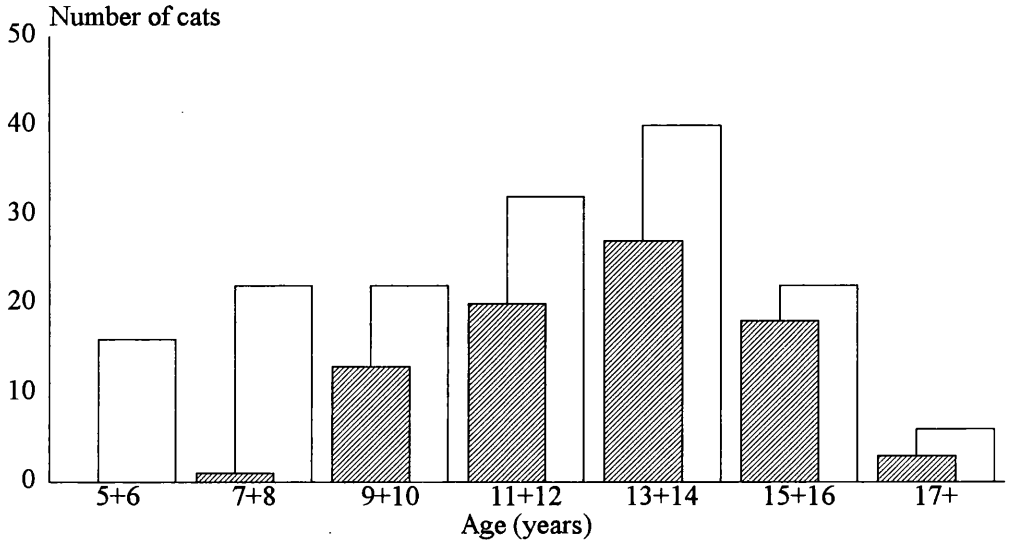


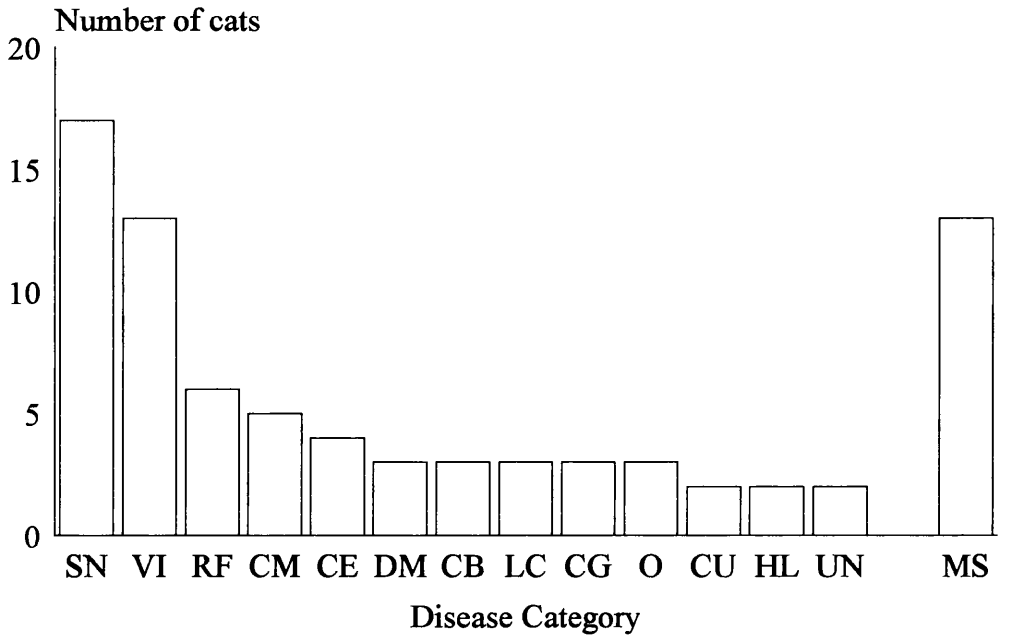
Figure 1. The number of cats diagnosed as hyperthyroid (hatched box) for different age categories compared to the total number of cats seen between January 1990 and June 1992.

The diagnoses for the 79 non-hyperthyroid cats are illustrated in Figure 2.

The most common disease category other than hyperthyroidism was neoplasia seen in 17 (10.5 %) of all cats. Lymphosarcoma was suspected clinically in six (35.3 %) cats of this group and was subsequently confirmed at post-mortem examination in four of these animals. Thyroid carcinoma was diagnosed by biopsy or post-mortem examination in three (17.6 %) cats. One case each of pancreatic adenocarcinoma, nasal carcinoma, pyloric carcinoma and pulmonary adenocarcinoma were diagnosed. In the remaining four cases pulmonary, mediastinal, abdominal or hepatic neoplasia was suspected clinically and radiographically but further investigations were refused.

FeLV, FIV and FCoV related diseases were diagnosed in 13 (8.0 %) of the cats. One cat was positive for both FeLV and FIV, while five were positive for FIV only and three for FeLV only. The remaining four cats were suffering diseases associated with FCoV. One other cat presented with a thyroid carcinoma was found to be positive for FIV antibodies. This was not considered to be clinically significant at that time.

Chronic renal failure was diagnosed as the primary problem in six (3.7 %) cats. As a secondary illness it was diagnosed in six further cases. The prevalence of the remaining diseases seen was 3.1% (five cases) for cardiomyopathy (four hypertrophic and one dilated) and 2.5 % (four cases) for chronic enteropathy. Three (1.9 %) cases each of diabetes mellitus, chronic bronchopneumonia/feline asthma, chronic gingivitis, lymphocytic cholangiohepatitis and obesity were diagnosed. Two (1.2 %) cases each of chronic upper respiratory tract infections and hepatic lipidosis were diagnosed.



SN, Systemic neoplasia
VI, Viral disease (FeLV, FIV, FCoV)
RF, Renal failure,
CM, Cardiomyopathy
CE, Chronic enteropathy
DM, Diabetes mellitus
CB, Chronic bronchopneumonia/feline asthma
LC, Lymphocytic cholangitis
CG, Chronic gingivitis
O, Obesity
CU, Chronic upper respiratory tract infections
HL, Hepatic lipidosis
UN, Undiagnosed
MS, Miscellaneous (one case each of meningoencephalitis, primary Haemobartonella felis, primary hypokalaemia, ovarian follicular cysts, feline endocrine alopecia, hyperadrenocorticism (adrenal tumour), polyarthritis, pyothorax, behavioural change, cystitis, pyloric stenosis, oesophageal reflux and intestinal parasitism)

Figure 2. Diseases of non-hyperthyroid geriatric cats seen between January 1990 and June 1992.

The survival curve for the non-hyperthyroid cats is illustrated in Figure 3. Thirty-one (39.2 %) cats died or were euthanased at the time, or within one month of presentation.

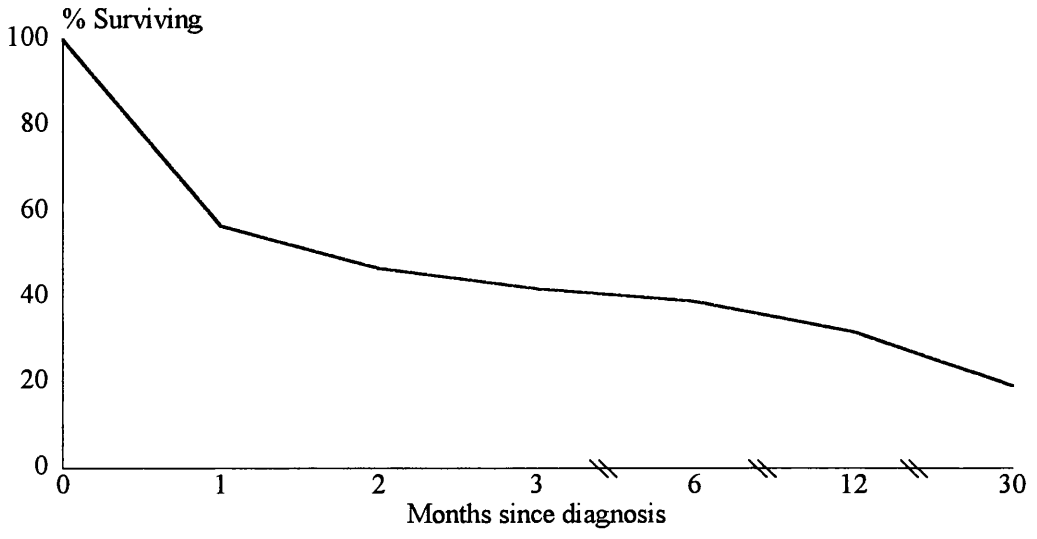


Figure 3. Survival curve for the prognosis of the non-hyperthyroid geriatric cats presented during January 1990 and June 1992.

Clinical material (1970-1972)

The relevant case numbers are listed in Appendix 3.

Between January 1970 and June 1972, 480 entries for cats appeared in the day book. Sixty-three of these entries represented external samples or post-mortem material. Of the remaining 417 entries studied, 34 (8.2 %) records were missing, 62 (14.9 %) were of unknown age, 223 (53.5 %) cats were less than five years of age and 98 (23.5 %) were five years of age or older. Of the older cats, 57 (13.7 % of all cases) were referred as medical cases.

This group consisted of 21 females (nine ovariohysterectomised, 12 entire) and 34 males (24 castrated, 10 entire). The cats were either domestic short (n = 39) or long (n = 1) haired; the remaining 17 (29.8 %) cats represented five other breeds: Siamese (n = 12), Persian (n = 2), British Short Hair (n = 1), Burmese (n = 1) and Chinchilla (n = 1).

The ages for 55 of the cats were reported, but simply described as aged in the remaining two. The mean $\pm$sd age at time of presentation was 8.35 $\pm$2.99 years. Thirty-eight (69.1 %) cats were less than 10 years of age. The oldest cat (a female) was 16.5 years. There was no significant ($P > 0.05$) difference in the ages of the female compared with the male cats. As a group, the cats were significantly ($P < 0.001$) younger than those seen between 1990 and 1992.

Diseases affecting the geriatric cats (1970-1972)

The diagnoses for these cats are illustrated in Figure 4.

Neoplasia was the most common diagnosis made, accounting for 12 (21.1 %) cases. In nine cases these were confirmed on post-mortem examination as myeloid leukaemia (n = 3), lymphosarcoma (n = 2) and one case each of salivary gland carcinoma, squamous cell carcinoma of the oesophagus, bronchial carcinoma, and pancreatic/ovarian adenocarcinoma. Alimentary lymphosarcoma, mediastinal lymphosarcoma and oesaphageal carcinoma were suspected clinically in the remaining three cats.

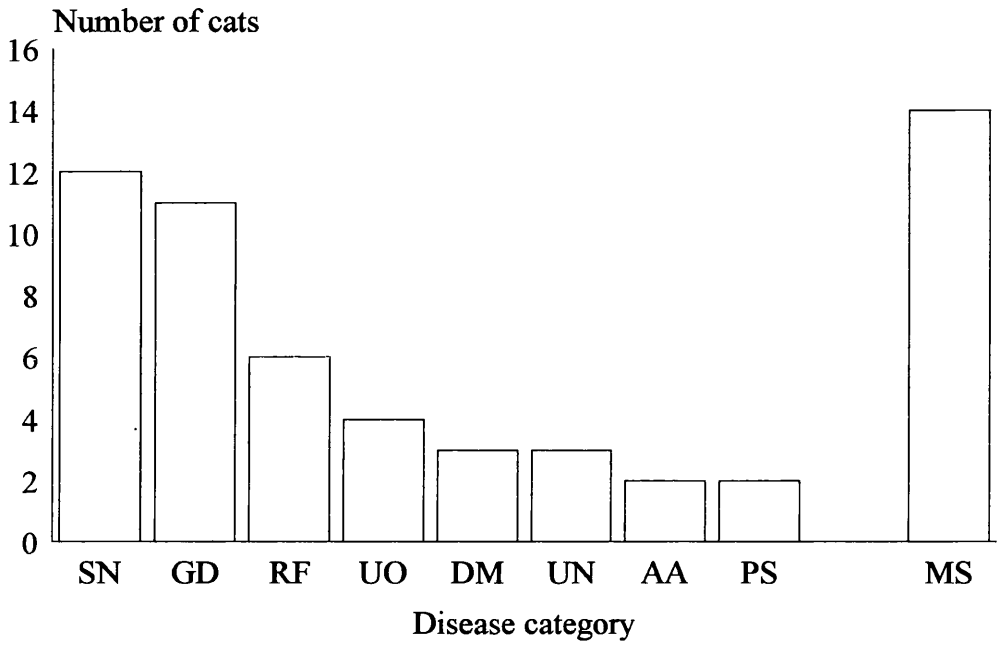
Eleven (19.3 %) cats presented with signs of gastrointestinal disease (vomiting, diarrhoea, colitis or constipation) but a diagnosis was not made in any case. The next most common diseases were renal failure (n = 6, 8.8 %), urolithiasis (n = 4, 7.0 %) diabetes mellitus (n = 3, 5.3 %) aplastic anaemia (n = 2) and pharyngitis (n = 2, 3.5 %).

It was impossible to assess the reasons for presentation or diagnoses made from three case records. The remaining 14 cases were suffering a variety of illnesses. One cat, a seven year old ovariohysterectomised Siamese, presented with polyphagia, weight loss, sudden onset blindness, polydipsia, steatorrhoea, jaundice and palpable hepatomegaly. On post-mortem examination the animal was found to have chronic pancreatitis, hepatic fibrosis and pulmonary oedema.

3.5 DISCUSSION

In comparing the geriatric medical cases seen between the periods 1990 to 1992 and 1970 to 1972 several interesting facts emerged. Approximately twice as many cats were presented to the Small Animal Clinic during the more recent period (1990 - 1992). A disproportionate increase was found in the number of geriatric medical cases presented, amounting to almost a three-fold increase. The cats presented 20 years ago were also significantly younger. These findings support suggestions that cats are now living longer (Kitchell, 1989).

In the survey reported by Griffiths in 1968, more male cats were presented than females. Whilst this was true in the cats seen between 1970 and 1972, equal numbers of male and female cats were presented between 1990 and 1992. Since Griffiths (1968) suggested that this may be due to the preference of cat owners for male kittens, this appears to be no longer true. By contrast to Griffiths (1968) report, where an increased longevity was noted for female cats, this was not found in either group of cats studied. However, the oldest cats seen in the 1970 and 1990 groups, were both females. Approximately 30 % of the 1970 cats were pedigree animals, whilst only 13 % of the 1990 cats were pedigree. This is unlikely to reflect a decline in pedigree numbers in the cat population. Rather, it may reflect a greater tendency of pedigree cat owners to present their cats for referral which may no longer be the case.



SN, Systemic neoplasia
GD, Gastrointestinal disease
RF, Renal failure
UO, Urolithiasis
DM, Diabetes mellitus
UN, Undiagnosed
AA, Aplastic anaemia
PS, Pharyngitis
MS, Miscellaneous (one case each of multiple pulmonary thrombi, old diaphragmatic hernia with liver torsion, pneumonia with cystitis, periodontal disease, pyrexia of unknown origin, chronic pancreatitis with hepatic fibrosis and pulmonary oedema, septicaemia, primary Haemobartonella felis, fibrinous peritonitis, hepatic cirrhosis, pneumonia, hydrothorax, chronic bronchopneumonia with nephrosclerosis and glomerulonephritis, FeLV)

Figure 4. Diseases of the geriatric cats seen between January 1970 and June 1972.

Hyperthyroidism was the most common disease seen in the current geriatric cats. The mean age at the time of diagnosis was similar to previous reports (Peterson *et al.*, 1983b; Thoday & Mooney, 1992). By contrast, hyperthyroidism was not diagnosed in any cat seen between 1970 and 1972. Since hyperthyroidism was not definitively diagnosed until 1979 (Cotter, 1979; Peterson, Johnson & Andrews, 1979), this could have been related to a lack of awareness of the disease. However, in retrospectively analysing the case records, only one cat was found to have historical and clinical features consistent with the presence of hyperthyroidism (Peterson *et al.*, 1983b; Thoday & Mooney, 1992). This cat exhibited weight loss despite an increased appetite, polydisia, steatorrheoa and had suddenly become blind. However, on post-mortem examination most of these signs were explained by the finding of chronic pancreatitis. A thyroid abnormality was not reported and it is doubtful that the cat was hyperthyroid. Retrospective analyses of clinical case records for the presence of hyperthyroidism has not been reported previously. The findings of the present study compliment a study of post-mortem records which showed that thyroid abnormalities were rare prior to 1977 (Peterson & Ferguson, 1989a). Thus, it would seem that hyperthyroidism has shown a true increase in incidence.

The study of the ages at which cats presented in both time periods may provide possible clues on the emergence of hyperthyroidism as a disease of significance. Between 1990 and 1992, hyperthyroidism was rare in cats less than 10 years old as has been reported previously (Peterson, Randolph & Mooney, 1994). Between 1970 and 1972, over two thirds (n = 38) of the cats were less than 10 years of age. Thus hyperthyroidism may not be a new disease. The increased frequency with which it is currently diagnosed may be related to an increased longevity of cats. Indeed, Peter *et al.* (1987) suggested that the emergence of hyperplastic follicles in thyroid tissue may be an age related phenomenon. Whether extra-thyroidal stimuli enhance this progression remains unclear.

There was an increased prevalence with which viral infections were diagnosed between 1990 and 1992 compared to the earlier period. It is likely that between 1970 and 1972 some of the diseases seen were viral-related. FeLV was discovered in 1964 (Jarrett *et al.* 1964a, 1964b). FIV is known to have existed prior to its discovery in 1987 (Pedersen *et al.*, 1987; Gruffydd-Jones *et al.*, 1988). A chronic fibrinous peritionitis was described by Holzworth in 1963, but its relationship to FCoV was not known until described by Ward in 1970. Thus, between 1970 and 1972, there was limited awareness of viral diseases and diagnostic tests were not yet, or rarely, available. There was only one case record from 1972 where FeLV status was recorded. By 1990 such testing had become routine. Because of the wide variety of clinical signs associated with FeLV and FIV related diseases (Hopper, Sparkes, Gruffydd-Jones, Crispin, Muir, Harbour & Stokes, 1989; Rojko & Hardy, 1994), it was impossible retrospectively to analyse which cats seen between 1970 and 1972 were likely candidates. However, one cat was found to have a fibrinous peritionitis which was presumably related to FCoV.

Neoplastic diseases and renal failure remained common in both groups of cats studied. They were also the two most common causes of death in the survey of Griffiths (1968). In both groups of cats, lymphosarcoma was the most common neoplastic disease diagnosed and this finding resembles Griffiths survey in 1968. Diseases of a chronic nature were common in both the cats surveyed from 1990 and 1970.

Irrespective of disease, an accurate diagnosis in life was made more frequently in the period between 1990 and 1992. This again presumably reflects the increased number of ancillary diagnostic tests available and carried out. In addition, with the aid of these tests secondary problems unrelated to the primary diagnosis were often found. This is not surprising in the older animal because of the additional stress on organ systems, which as a result of the normal ageing process have a limited reserve capacity (Mosier, 1989). The presence of a concurrent problem ultimately affected both prognosis and treatment options. This study confirms suggestions that a standard data-base for the assessment of diseases in geriatric animals should at least include haematological and full blood biochemical profiles (Mooney 1991; Mooney, 1993).

The survival prognosis for the non-hyperthyroid cats seen between 1990 and 1992 was poor. There was a sharp decline in survival within the first month of presentation, followed by a slower decline and a further decrease by 30 months. This is not surprising in a referred population of cats. Referral is often based on specialisms in a particular institute. However, chronically and severely sick animals are often also referred because of better diagnostic and treatment facilities available at the referral institute. In addition, in this study, many owners opted for referral as a final attempt to do as much as possible for their animal.

The large number of hyperthyroid cats referred was related both to the expertise available and the establishment of a radioactive iodine unit for its treatment. Providing these cats had no concurrent illnesses, the prognosis was excellent. In accord with this, many of the cats were lost to adequate follow up after successful management by surgical thyroidectomy, antithyroid drug treatment or radioactive iodine administration. Survival data were therefore difficult to calculate.

By its very nature, a study using a referred population of animals cannot provide true disease prevalences. This study did not attempt to do so, but simply highlighted some changes that presumably reflect, at least in part, similar trends in disease in the wider feline geriatric population. In carrying out a similar survey on geriatric dogs, MacDougall & Barker (1984) suggested that although information from referral centres is biased towards particular research interests and expertise, such data reflects diseases of importance worthy of further study. More importantly, in the study presented here, the paucity of information on the geriatric cat became evident. Specifically, the current proportion of older cats, average longevity and disease prevalence in the wider feline population are unknown and deserve to be the subject of further study.

CHAPTER 4
ASSESSMENT OF SERUM TOTAL AND FREE THYROXINE CONCENTRATIONS IN FELINE SERUM

4.1 LITERATURE REVIEW

An introduction to the thyroid hormones

Irrespective of species, the role of the thyroid gland is to produce iodinated derivatives of tyrosine, the thyroid hormones (Gorbman, 1986). Once released into the circulation the thyroid hormones are responsible for a variety of actions. In the adult vertebrate these actions differ from tissue to tissue as exemplified by enhancement of lipolytic activity in adipose tissue, modulation of gonadotropin secretion by the pituitary, maintenance of hair growth by the skin and stimulation of the sodium pump and glycolytic pathways leading to calorigenesis and oxidative phosphorylation in the liver, kidney and muscle. In addition, thyroid hormones alter the concentrations of certain neurotransmitters and their receptors in the brain and affect tissue metabolism of some drugs. They modulate the actions of other hormones through receptor interactions or alteration of transcriptional or translational events initiated by other hormones, and can regulate concentrations of their own receptors and, through transamination, their own metabolism (Shambaugh, 1986).

In man, a variety of iodothyronines, iodotyrosines, iodoproteins such as thyroglobulin and iodoalbumin and a small amount of inorganic iodide are released by the thyroid gland into the blood (Chopra, 1991). Only T4 and T3 are considered to have significant metabolic activity. T4 is the main secretory product of the thyroid gland. Despite this T3 is three to five times more potent than T4, and T4, although possessing inherent thyromimetic activity, is often considered to be a "pro-hormone". In health, only 10 to 15 % of circulating T3 is secreted by the thyroid gland, the remainder being produced peripherally by (outer ring) 5'-deiodination of T4 (Braverman, Ingbar & Sterling, 1970; Chopra, 1991). Two isotypes of 5'-deiodinase (type I and type II) have been identified by differences in their substrate preference, degree of susceptibility to inhibition by propylthiouracil and response to physiological perturbations. The highest type I activity is found in the thyroid gland, liver and kidney. The type II enzyme appears to generate T3 for local use in organs such as the brain and brown adipose tissue (Kohrle, DieterHesch & Leonard, 1991).

In the dog, T4 is also the main secretory product of the thyroid gland, although smaller amounts of T3, 3,3',5'-L-triiodothyronine (reverse T3 (rT3)) and various deiodinated metabolites have been identified in venous effluent from the thyroid glands (Laurberg, 1978, 1980, 1981). In the cat, T4, rather than T3, is the main compound in thyroid venous plasma (Taurog, Porter & Thio, 1964). This is supported by a recent study of six healthy cats where thyroid secretion rates of 5.6 $\pm$1.2 ug/kg/day and 0.4 $\pm$0.1 ug/kg/day for T4 and T3 respectively were found (Broome, Hays & Turrel, 1987).

Up to 60 % of circulating T3 is produced from peripheral tissue monodeiodination of T4 in the dog (Belshaw, Barandes, Becker & Berman, 1974) and a similar situation is thought to occur in the cat.

Overall control of thyroid hormone production is provided by a negative feedback mechanism of circulating T4 and T3 on thyrotropin releasing hormone (TRH) from the hypothalamus and TSH from the anterior pituitary. Peripheral conversion of T4 to T3 may be autoregulated and is altered in iodine deficiency and in a variety of other pathological states (Nicoloff, 1986). Since T3 is the more metabolically active hormone, this mechanism may serve to maintain euthyroidism in the face of a low or a high serum T4 concentration. Such a mechanism may be responsible in part for the maintenance of normal circulating T3 concentrations when T4 concentrations are only mildly elevated in hyperthyroid cats, or when circulating T4 concentrations are markedly suppressed as during carbimazole therapy (Peterson *et al.*, 1983b; Mooney, Thoday & Doxey, 1992a; Thoday & Mooney, 1992). T3 toxicosis, an elevated circulating T3 concentration, accompanied by a normal circulating T4 concentration, which has been described in man has not been described in the cat (Peterson *et al.*, 1983b; Thoday & Mooney, 1992). Given this, in the cat the measurement of circulating T3 concentrations in isolation gives a less meaningful estimate of thyroid function than the study of circulating T4 concentrations alone.

Transport of thyroxine within the circulation

Thyroid hormones are water-insoluble lipophilic compounds. In humans, their ability to circulate in plasma depends primarily on binding by specific binding proteins, thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA) and by albumin itself. Less importantly, T4 may bind to certain lipoproteins and other minor transport hormones. These transport hormones have been reviewed in detail by Robbins (1991). The major fraction of T4 (approximately 70 %) is bound to TBG, a trace inter-alpha-globulin. It is the least abundant of the three major transport proteins, having a concentration of approximately 15 mg/l in human plasma, but has an extremely high affinity for T4. In normal plasma, about one fourth of the TBG molecules contain T4. TBPA is more abundant than TBG, having a concentration of approximately 250 mg/l in plasma. The affinity constant for the T4-TBPA interaction is intermediate between TBG and albumin, such that TBPA transports only about 10 % of the T4 in blood. Albumin is the most abundant protein in human plasma. Although very few albumin molecules carry T4, their high concentration results in the binding of approximately 15 to 20 % of the circulating T4. Compared to TBG, more T4 binding sites of TBPA and albumin remain unoccupied.

Larsson, Pettersson & Carlstrom (1985) attempted to isolate TBG and TBPA from canine serum utilising purification methods developed for the isolation of these proteins in humans. An analogue of human TBPA was found to exist in canine plasma, albeit

migrating in the alpha-2 globulin region. TBG was also present in canine serum and reported to be the major T4 carrier. In addition, T4 was found to bind to albumin and, in equal amounts, to another protein migrating in the alpha-1 region, with both transport proteins showing low affinity but high capacity for T4. The relative distribution of T4 on these proteins at basal T4 concentrations were 60 % for TBG, 17 % for TBPA, 12 % for albumin and 11 % for the alpha-1 protein. Further characterisation of these proteins (Larsson, 1987) showed many similarities between human and canine TBPA with regard to tetrameric structure, microheterogeneity, extinction coefficient, molecular weight, and the concentration in plasma. Partial immunochemical identity between the two was also reported. However, canine TBG, migrating in the beta region, was of lower plasma concentration, and of higher molecular weight than human TBG and showed no immunochemical resemblance to this protein. Evidence was also presented that the previously unidentified T4-binding protein in the alpha-1 globulin region was the high density lipoprotein (HDL), HDL_2 and in the pre-beta region, very low density lipoprotein (VLDL). TBG in the dog is not completely saturated until the circulating T4 concentration is approximately 150 nmol/l, while the other serum proteins are virtually never saturated (Larsson, Pettersson & Carlstrom, 1985).

The isolation and identification of feline plasma thyroid hormone binding proteins have been reported. Larsson, Pettersson & Carlstrom (1985) identified albumin and TBPA, albeit migrating in a different direction to human TBPA, as the major T4 binding proteins but failed to identify TBG in the cat. Thoday (1986) reported that T4 was bound to albumin and to a protein migrating in the alpha-1/alpha-2 globulin fraction although this had a lower affinity, capacity or concentration in plasma than human TBG.

Over 99 % of T4 circulating in human plasma is protein bound, while a small fraction of approximately 0.03 % is unbound or free (Woeber, 1986). Robbins & Rall (1957) initially proposed that protein binding functioned as a mechanism for retention of thyroxine in a fluid compartment, but that the bound hormone was an inactive form, whereas the free or unbound component was responsible for the actions of the hormone. However, Pardridge (1981) presented experimental evidence showing that single-pass organ uptake of hormone may exceed the free fraction; this suggests protein-mediated hormone uptake and that certain binding proteins may serve to distribute hormone to specific tissues. Despite these challenges it is usually accepted that the free fraction of T4 is metabolically active, while the protein bound fraction serves to restrict hormone availability to tissue sites of metabolism and disposal and to modulate the effects of alterations in T4 secretion or administration (Nicoloff, 1986).

In the dog, since the overall concentration and binding affinity of the binding proteins is lower, the total (protein-bound and free) T4 concentration is lower, the unbound or free T4 fraction is higher and hormone turnover more rapid than in man. The free T4 fraction in the dog has been variably estimated as 0.103 to 0.189 % (Furth, Becker, Nunez & Reid, 1968), 0.155 to 0.303 % (Refetoff, Robin & Fang, 1970) and

0.09 ±(standard error of the mean (sem)) 0.003 % (Ferguson & Peterson, 1992). Given the higher free T4 fraction, absolute concentrations of free T4 are similar in dog and man. Indeed, it has been suggested that the free T4 concentration in warm blooded vertebrates is more constant than any other parameter of thyroid function despite variations in the nature and capacity of serum T4 binding proteins (Refetoff, Robin & Fang, 1970). A similar situation is thought to exist in the cat as in the dog, but there are few references to free T4 fractions in this species. Thoday (1990a) reported that the free T4 fraction ranged from 0.083 to 0.105 % in cats but further details were not provided. Hays, Turrel & Broome (1988) reported the mean ±sd free T4 fraction measured in five euthyroid cats as 0.057 ±0.009 %.

Laboratory assessment of circulating thyroxine concentrations

Measurement of total and free circulating concentrations of T4 are now carried out routinely in human medicine, and most methods have been used in the cat.

Circulating total thyroxine concentrations

Measurement of the plasma protein-bound iodine (PBI) concentration was for many years the mainstay as an index of circulating T4 concentrations in man but this method is now of mere historic interest. The method relied on the fact that the iodine present in T4 comprises 80 to 90 % of the total plasma iodine concentration. However, this assay was subject to spurious results in patients whose thyroids were secreting large quantities of iodoproteins and in those who had received large quantities of exogenous iodine in organic or inorganic form. The plasma butanolol-extractable iodine (BEI) concentration assay was developed in an attempt to overcome some of the limitations of the PBI test by increasing the specificity for T4, but was beset by similar problems. Such methods have been employed in the dog. However, the dog has plasma iodide concentrations 10 to 20 times higher than human plasma, and this constitutes approximately one-third of the total PBI (Belshaw, Cooper & Becker, 1975; Belshaw, 1983). Preliminary data in the cat suggest a similar plasma iodide concentration (Peterson & Ferguson, 1989a). Thus PBI determinations are unsatisfactory indices of T4 concentrations in small animals.

The development of the competitive protein-binding displacement assay (CPBA) offered many advantages over PBI determination for T4 in human subjects. T4 contained in an ethanol extract of the patient's serum competes with tracer T4 for binding to TBG in a standard dilute solution of pooled human serum. Apart from the fact that the ethanol extraction step is time consuming, such assays are not easily adaptable to either the dog or cat because of the differences in concentration and affinity of TBG in the dog (Larsson, 1987) and the lack of TBG in the cat (Larsson, Pettersson & Carlstrom, 1985).

There is no doubt that the development of a radioimmunoassay (RIA) system for the measurement of circulating insulin concentrations in humans revolutionised the study of hormones (Yalow & Berson, 1960). The method, using specific insulin antibodies and a radioactive ligand, was found to offer unprecedented specificity, sensitivity, precision and practicality, and was exploited by Chopra in 1972 to measure total T4 concentrations. The principles behind the assays have been reviewed previously (Mooney, 1990). The analyte reacts with a specific antibody in the presence of a radiolabelled analyte. The labelled analyte is distributed between the antibody bound and free forms in a proportional manner to the non-labelled analyte. Following separation of the antibody bound radiolabelled analyte, and by comparison with an appropriate standard, the concentration of the test analyte can be interpolated.

Since the first description of the total T4 RIA, numerous kit methods have been developed commercially and are routinely used in most human laboratories. In addition, using the same principles, ELISA kits have been developed to avoid the use of radioactive ligands but the sensitivity of RIAs remains unequalled. It is recommended that reference ranges be determined for each kit method used in each laboratory. As discussed previously, total T4 concentrations are higher in humans than either dogs or cats. In general, typical serum total T4 concentrations vary between approximately 60 and 140 nmol/l in healthy euthyroid adult humans (Stockigt, 1991).

Measurements of circulating total T4 concentrations using RIAs have been reported in the cat since 1978 (Reap, Cass & Hightower, 1978). Some authors have recommended that standards be prepared in hormone free feline serum to minimise differences in the nature and concentrations of binding proteins between the standards and the samples (Peterson, Keene, Ferguson & Pipers, 1982; Hoenig & Ferguson, 1983; Thoday, Seth & Elton, 1984). In addition, optimisation of each reagent used in the assay has been recommended to ensure an assay response appropriate to the type of sample and the concentration range of interest, and to provide a consistent response in the face of mild variations in conditions (Thoday, Seth & Elton, 1984). However, such recommendations are expensive and time consuming to implement and it is generally accepted that kits designed for use with human serum or plasma are applicable in the cat once the assay is modified to allow for the measurement of the lower circulating total T4 concentrations in this species. This generally involves the use of standards of a lower concentration than those provided in the kit simply by dilution of those already provided. In addition, each RIA system should be fully validated with regard to specificity, sensitivity, accuracy and precision to ensure reliability (Midgley, Niswender & Rebar, 1969; Hunter, 1978). As with medical laboratories, each veterinary laboratory should determine its own reference range for the particular species in question.

A review of the measurement of total T4 concentrations in cats has been presented previously (Mooney, 1990). Despite the variations in techniques and methods used, the reference ranges for serum total T4 concentrations in healthy euthyroid cats, with few

exceptions, tend to lie between 10 and 60 nmol/l. Thoday, Seth & Elton (1984) measured serum total T4 concentrations in 318 healthy cats ranging in age from four months to 13 years. Total T4 concentrations in both males and females tended to decrease until approximately five years of age and then increase again. For any given age, females and neutered females tended to have higher serum total T4 concentrations than males and neutered males. Animals living in the same environment had similar total T4 concentrations although it was not possible to differentiate between genetic and environmental effects for T4. Peterson & Gamble (1990) found no difference in total T4 concentrations between young and older cats.

The measurement of circulating total T4 concentrations reflects the concentration of hormone bound to the binding proteins, while the minute concentration of free hormone makes an insignificant contribution to the total (Toft, Campbell & Seth, 1981). As a consequence, changes in measured total T4 concentrations can occur in response to changes in the concentration and/or affinity of the binding proteins. In humans, most notable among these would be changes that occur in pregnancy, hereditary TBG excess or deficiency, therapy with certain drugs and non-thyroidal illness. Factors of importance to the cat are reviewed in Chapter 5.

Circulating free thyroxine concentrations

Despite the challenges of Pardridge (1981), the diagnostic value of free thyroid hormone concentrations has not been seriously questioned. Such measurements obviate the inherent errors arising from a reliance on serum total thyroid hormone concentrations particularly if the relationship between circulating total and free hormone concentration is disturbed, for example, when concentrations of binding proteins are abnormal, when autoantibodies directed against thyroid hormones exist in the circulation and when endogenous or exogenous competitors for protein binding are present (Ekins, 1985).

For many years in human medicine, there has been a reliance on the indirect and rather crude free T4 index (FTI) which provides an approximate indication of circulating free T4 concentrations by correcting total T4 concentrations for changes in serum binding protein concentrations. The calculation of the FTI involves measurement of the circulating total T4 concentration, and the correction factor is estimated by one of a variety of assays, collectively termed thyroid hormone (T4 or T3) uptake tests (THUT). Alternatively, TBG can be measured by immunoassay and a total T4 : TBG ratio calculated (Seth & Beckett, 1985). Both of these approaches yield a quantity correlating reasonably closely with circulating free T4 concentrations (Ekins, 1985). The FTI has been of value in the correct interpretation of serum total T4 concentrations when serum concentrations of TBG are mildly abnormal as during administration of oestrogen, and in pregnancy and genetic disturbances of TBG synthesis. However, the FTI has two main disadvantages. It is cumbersome and costly to perform and there is a greater probability of error implicit in the conduct of two separate and methodologically

unrelated measurements (Ekins, 1985). The FTI has been shown to provide unreliable and low results in patients hospitalised for non-thyroidal illness in whom an additional diagnosis of hypothyroidism could have been considered (Chopra, Solomon, Hepner & Morgenstein, 1979a). Despite these problems the FTI is often measured and is still considered to be useful to experienced thyroidologists (Alexander, 1986) but has largely been replaced by direct methods of determining free T4 concentrations. In veterinary medicine, because of the differences in serum thyroid hormone binding proteins compared to man, the FTI is not a reliable indicator of free T4 concentrations and any studies must rely on the direct methods currently available.

There are three principal methods for directly measuring circulating free T4 concentrations:

1. Equilibrium dialysis
2. Ultrafiltration
3. Direct immunoassay

Equilibrium dialysis and ultrafiltration are considered to be the "gold standard" or reference techniques by which other techniques are compared (Ekins, 1985). The problems associated with each of these techniques for the measurement of circulating free T4 concentrations in non-thyroidal illness are reviewed in more detail in Chapter 6.

1. ***Equilibrium dialysis*** relies on bringing the serum (the "dialysand") into contact with a buffer (the "dialysate"), the two being separated by a membrane permeable to free hormone and other small molecules but not to serum proteins responsible for hormone binding. As free hormone molecules pass through the membrane, there is a net dissociation of bound hormone restoring thermodynamic equilibrium throughout the system. At the time of equilibrium, the free hormone concentration on both sides of the membrane will be identical. The free hormone concentration in the dialysate can be measured either directly by a sensitive RIA or indirectly by first measuring the total hormone concentration by RIA and then the fraction that appears in the dialysate, relying on radioactively labelled hormone initially introduced into the test sample. The second of these two techniques was originally developed by Sterling & Hegedus (1962). Minor problems associated with this indirect technique have been related to certain buffering salts and preservatives in the dialysate which affect the free T4 concentration. However, the major problem associated with this method has derived from the impurities present in the radiolabelled hormones used to measure the free fraction. Sterling & Brenner (1966) attempted to overcome or correct the problem of iodide contamination by the use of magnesium precipitation. However, even when freshly purified tracer is used the indirect method is fraught with radioiodide contamination problems. This is apparently due to photochemical deiodination of tracer T4 which occurs at body temperature during the incubation required to reach dialysis equilibrium (Nelson & Weiss, 1985). Thus, the results obtained by the indirect approach have tended to be artefactually raised (Ekins, 1985).

Direct dialysis measures free T4 concentrations by a sensitive RIA applied to the dialysate. This was originally developed by Ellis & Ekins (1973) in an attempt to avoid errors arising from the impurity of radioactive preparations employed in the more conventional methods. Although this proved to be a breakthrough in equilibrium dialysis techniques, it was not widely or routinely used in clinical laboratories because it was costly and technically demanding. Helenius & Liewendahl (1983) later devised a new and practical modification of the technique which proved to be analytically accurate, sensitive, reproducible, suitable for routine use in the clinical laboratory and, since the dialysis cell design permitted serial production, relatively inexpensive. The serum sample was diluted with buffer prior to dialysis but the free T4 concentration was found to be independent of dilution in the dialysis step for sera from healthy subjects, patients with non-thyroidal illness and subjects with abnormal values for TBG when dilutions of 1 : 5, 1 : 20 and 1 : 55 were tested. Dilution of serum samples introduces an element of inaccuracy into the measurement of free hormone concentrations but, particularly in the case of T4, the magnitude of the error is relatively unimportant and therefore permissible (Ekins, 1985). However, if non-dialysable protein binding inhibitors, postulated to occur in non-thyroidal illness, are present in the serum, the effect of sample dilution may become important. If the effect of an inhibitor is dependent on its concentration, then dilution of serum would diminish inhibition resulting in increased protein binding and decreased free T4 concentrations. Thus in the study of Helenius & Liewendahl (1983), the negligible effect of dilution on serum free T4 concentrations in serum from healthy, euthyroid subjects was expected but was not expected in the serum from patients with non-thyroidal illness. By contrast, Nelson & Weiss (1985) using a similar technique, found that progressive dilution of serum (1 : 2 to 1 : 1024) from patients with non-thyroidal illness resulted in a progressive decrease in free T4 concentrations, similar to that induced by the addition of salicylates to normal sera, known inhibitors of T4 binding to serum proteins, providing evidence for the presence of a circulating inhibitor(s) of T4 binding in non-thyroidal illness. The discrepancy between the studies of Helenius & Liewendahl (1983) and Nelson & Weiss (1985) may have arisen as a result of patient selection since in the latter study patients were specifically selected for both low serum total T4 concentrations but normal TBG concentrations. In addition, it may have been the result of the wider range of dilutions studied since individual results from these patients displayed wide variations in the dilution-induced fall in free T4 concentrations.

Nelson & Tomei (1988) designed a re-usable dialysis cell and a complex buffer, with which undiluted serum samples could be dialysed with minimal changes in their serum matrix, following which free T4 concentrations could be measured by a sensitive RIA. The dialysate buffer was designed to approximate the composition of a protein-free ultrafiltrate of normal human serum, at least for those compounds present in serum at a concentration of greater than or equal to 1 mmol/l, except that glucose was omitted and preservatives included. Equilibrium was achieved in 14 hours at 37^{o}C but assay

incubations were routinely performed for 16 to 18 hours to ensure measurement on the plateau of the time-response curve. To study intra-assay precision, a single normal serum pool was dialysed in 57 cells. The mean free T4 concentration was 19.31 pmol/l with a coefficient of variation (c.v.) of 5.3 %. A second normal serum pool was run in one dialysis cell per day on 57 different days over four months. The mean concentration was 23.17 pmol/l with a c.v. of 6.9 %. A pool of dialysates with a mean concentration of 18.03 pmol/l was similarly assayed in these latter 57 assays with a c.v. of 7.4 % suggesting that the dialysis step adds little to the variability of the results. Repeated freeze-thaw cycles, up to a maximum of 10, had no effect on serum free T4 concentrations. The working range of the assay was 2.57 - 164.74 pmol/l. Using this method serum free T4 concentrations were found to be normal in individuals whose hyperthyroxinaemia was caused by increased T4 binding to serum proteins. More importantly, the method separated the patients with hypothyroxinaemia of non-thyroidal illness or hypothyroxinaemia of TBG deficiency from those with hypothyroidism, whether it was primary or central. Although the dialysis step increased the time and effort required to perform a free T4 estimation, the method was recommended as adaptable to routine clinical laboratory use, if made available as a kit procedure. This has recently been adapted by Nichols Institute Diagnostics as a kit designed for routine clinical laboratory use with single use dialysis cells.

2. ***Ultrafiltration*** is occasionally adopted as an alternative to dialysis to isolate the fluid compartment containing the unbound hormone (Schussler & Plager, 1967) but like most equilibrium dialysis methods is both too time consuming and cumbersome for routine use in the clinical laboratory.

3. ***Free hormone immunoassays*** rely on the fact that when an antibody is introduced into serum, the resulting occupancy of antibody binding sites reflects the free hormone concentration in the surrounding medium (Ekins, 1985). Free T4 assays require a high-affinity T4 antibody, which has to be used at a very low concentration in order to minimise inter-sample bias deriving from variable depletion of the protein-bound pool (Ekins, 1985). Three main RIA methods have been commercially exploited with varying degrees of success, namely the "labelled hormone antibody uptake" method, the "labelled hormone, back titration" technique and the "labelled analogue" approach. Techniques that use nonradioactive detection systems (chemiluminometric and fluorometric systems) are also available based on the same principles but similar pitfalls apply (Stockigt, 1991).

The labelled-hormone antibody uptake approach is similar to the traditional FTI methods since it involves the measurement of the total T4 concentration in the sample and the fraction of the hormone which binds to exogenous antibody. The method essentially depends upon labelling the serum pool with radiolabelled hormone at a (diluted) specific activity which can be calculated from the total T4 concentration. By measuring the amount of radiolabelled hormone bound to antibody, and with knowledge

of its specific activity, the occupancy of antibody sites can be inferred. Using standards of known free hormone concentration, determined by dialysis or ultrafiltration techniques, a dose response curve can be constructed relating antibody occupancy to free T4 concentration. This method is available in kit form, marketed as Immophase by Corning. Controversy surrounded the original introduction of this kit since an incorrect procedure for the calculation of assay results had been adopted. Despite rectification of this problem, spurious results have been found in patients with non-thyroidal illness. Thus, the method has not gained widespread use.

The labelled-hormone, back titration method, often called the "two-step" RIA relies on incubation of the test serum with insolubilised antibody to which some of the free T4 is adsorbed, followed by removal of the serum and therefore any interfering substances and subsequent exposure of the antibody preparation to labelled hormone. During this second incubation stage, labelled material is bound to residual, unoccupied, antibody binding sites and excess label is then washed away. The bound label is thus inversely proportional to the original free T4 concentration of the test sample. Two separate and sequential steps are obligatory in such a technique to avoid any possible interaction of labelled hormone with serum protein binding sites. Although established by Ekins (1985) using antibody coupled to Sephadex particles, it was independently developed by Clinical Assays marketed as the Gamma-Coat two step RIA (Bayer & McDougall, 1980). Ekins (1985) reported that the major disadvantage of this method is its liability to drift because of difficulty ensuring that the timing of the sequential incubation and washing steps is maintained from one sample to another. This has been confirmed using a non-isotopic adaptation (Beckett, Ratcliffe, Chapman, Wu, Rae, Gow & Toft, 1990). Use of an antibody of low affinity will also result in a loss of precision, and may be an additional source of error in results obtained with commercial kits based on this principle (Ekins, 1985). However, despite such problems and although technically demanding because of the two steps involved, the validity of these kits has never been seriously questioned (Alexander, 1986).

The labelled hormone analogue or one step assay approach held the most promise of all the free hormone immunoassays but has been fraught with the most difficulties. The principle is based on a modification of the two step technique, but obviates the two-stage sequential incubation by use of a labelled hormone analogue. This analogue is in principle, totally unreactive with endogenous binding proteins but retains the ability to cross react against native hormone for antibody binding sites. The prototype was the Amerlex free T4 RIA (Amersham Corporation) first reported by Midgley & Wilkins (1980). There is no doubt that this method provides reliable results in euthyroidism, hyperthyroidism and hypothyroidism even when compared to equilibrium dialysis (Stockigt, De Garis, Csicsmann, Barlow, White & Hurley, 1981; Gow, Kellett, Toft & Beckett, 1985; Liewendahl, Mahonen, Tikanoja, Helenius, Turula & Valimaki, 1986). Expected results are also obtained during pregnancy (Ekins, 1985) and in other cases

where there are only minor alterations in TBG concentrations (Beckett *et al.*, 1990). However since its inception it has been clearly shown that spuriously elevated results are obtained in cases of familial euthyroid T4-excess, where T4 is abnormally bound to albumin. Apparently low results are found in patients with non-thyroidal illness using this assay and variable results occur when circulating thyroid autoantibodies are present (Stockigt *et al.*, 1981; Byfield, Lalloz, Pearce & Himsworth, 1983). It is therefore clear that despite the manufacturers' assurances, T4 analogues are not entirely free but can bind to serum proteins and other interfering substances and binding competitors within the test sample (Ekins, 1985; Wilkins, Midgley & Barron, 1985). Indeed, the correlation of low albumin and free T4 concentrations as assessed by analogue methods suggest that these methods actually measure albumin bound T4 rather than free T4 itself (Stockigt, Stevens, White & Barlow, 1983; Csako, Zweig, Benson & Ruddel, 1987). These findings have led to the suggestion that analogue methods should not be used by diagnostic laboratories (Alexander, 1986).

A minidialysis-type free hormone immunoassay kit (Liquisol, Damon Diagnostics) has also been developed. This method uses nylon microcapsules containing T4 antibody. The microcapsules have pore sizes similar to dialysis tubing. Free T4 in the patients serum or standard enters the microcapsule and displaces radiolabelled T4 from the antibody. Since spurious results have been found in patients with non-thyroidal illness using this method, it has not gained widespread use.

Absolute circulating free T4 concentrations in healthy adult humans tend to vary between 10 and 40 pmol/l although it is recommended that each laboratory determine its own reference range.

Given the similarity in absolute circulating free T4 concentrations between species (Refetoff, Robin & Fang, 1970) methods for determination of free T4 concentrations in humans do not have to be modified for use with cat serum. However, as with total T4 assays each should be validated and a reference range determined by each individual laboratory. There are few reports on the measurement of circulating free T4 concentrations in healthy euthyroid cats. Hays, Turrel & Broome (1988) used the equilibrium dialysis method of Sterling & Brenner (1966) to measure the free T4 fraction in five healthy cats. Further details and absolute concentrations were not reported. Ferguson, Peterson & Nachreiner (1989) measured serum free T4 concentrations in 28 healthy cats using unspecified equilibrium dialysis and direct RIA methods. The RIA was identified as an analogue kit method (Magic free T4, Ciba Corning Diagnostics) by Refsal, Nachreiner, Stein, Currigan, Zendel & Thacker (1991). The mean $\pm$sd concentration for free T4 was 20.4 $\pm$11.1 pmol/l by equilibrium dialysis and 23.9 $\pm$5.8 pmol/l by direct RIA. As compared to equilibrium dialysis, the direct RIA tended to overestimate free T4 concentrations in the low normal range while underestimating values in the higher range. Further details of assay methodologies were not provided. Sparkes, Jones, Gruffydd-Jones & Walker (1991) measured serum free T4

concentrations in 13 healthy cats on 31 occasions using an analogue RIA (Amerlex-M, Amersham International). The mean ±sd free T4 concentration was 10.0 ±3.0 pmol/l with a range of 3.8 to 15.1 pmol/l. The assay had a sensitivity of 0.4 pmol/l and was fully validated for use in cats by demonstration of hormone recovery, parallelism studies and interassay reproducibility, although further details were not provided. The authors did not comment on the apparently low values of free T4 obtained. A reference range of 10 to 25 pmol/l for serum free T4 concentrations using the same kit method has also been reported (Jones *et al.*, 1991).

Refsal *et al.* (1991) are the only workers to report on the validation of a direct free T4 analogue RIA (Magic free T4, Ciba Corning Diagnostics) for use with cat serum. The reported laboratory reference range for healthy cats was 12 to 36 pmol/l. The mean ±sd free T4 concentration as measured in four healthy cats was 28.3 ±5.6 pmol/l. The assay protocol provided by the manufacturer was not changed. The sensitivity of the assay, defined as the calculated concentration 2sd below the total specific binding (using 11 duplicate measurements of the 0 standard) was 0.8 pmol/l. Two pools of feline serum were diluted in phosphate buffered saline solution with 0.1 % gelatin (4 : 1, 1 : 2 and 1 : 4). The recovery rate of expected values was 110, 117 and 109 %, respectively. However, when the same pools of feline serum were diluted (10 : 1, 5 : 1, 4 : 1, 1 : 2, and 1 : 4) in the supplied human 0 standard, the recovery of expected values was 80, 76, 74, 57 and 46 % showing a lack of dilutional parallelism. This was not considered surprising, since serum binding proteins differ significantly between the cat and humans and the assay was designed specifically for use with human serum. When repeated measurements were performed on feline serum pools with mean free T4 concentrations of 16.1 and 44.9 pmol/l, the intra-assay c.v. over 10 assays was 4.3 and 9.6 %, respectively and the interassay c.v. over 10 assays was 7.3 and 7.2 %, respectively. A comparison was made between this assay and a direct equilibrium dialysis kit method (Nichols Institute Diagnostics) using 30 samples from hyperthyroid cats or cats with non-thyroidal illnesses. Individual values were not reported. The correlation of the results was 0.90. The regression coefficient was 0.44 and the y-intercept was 6.4 (differing from 0 at $P = 0.089$). Absolute concentrations were reported to differ between the two methods, but relative ranking of the results was similar.

4.2 INTRODUCTION

The free hormone hypothesis, proposed by Robbins & Rall (1957), suggested that it was the free or unbound fraction of a circulating hormone that was available to tissues and therefore metabolically active. Although this theory has been disputed by some workers on the basis of experimental evidence (Pardridge, 1981), it is generally accepted that the concentration of free T4 in serum is a more reliable indicator of thyroid function than that of total hormone taken alone. Despite this, controversy surrounds certain methods currently available for determination of serum free T4 concentrations in man. To date, equilibrium dialysis or ultrafiltration techniques are theoretically the most reliable methods considered as "gold standards" by which other methods are compared (Ekins, 1985).

Despite the frequency of thyroid disease in the cat, there are few reports on the measurement of free T4 concentrations in this species (Hays, Turrel & Broome, 1988; Ferguson, Peterson & Nachreiner, 1989; Thoday, 1990a; Jones *et al.*, 1991; Refsal *et al.*, 1991; Sparkes *et al.*, 1991). In the majority of the studies commercial analogue kit methods have been used (Ferguson, Peterson & Nachreiner, 1989; Jones *et al.*, 1991; Refsal *et al.*, 1991; Sparkes *et al.*, 1991). This chapter describes the validation of two assays, for the measurement of total and free T4 concentrations respectively in feline serum, the latter using a direct equilibrium dialysis kit method. The results from a series of healthy euthyroid cats are presented and provide a basis for comparison with T4 concentrations in both feline hyperthyroidism and cats with non-thyroidal illness.

4.3 MATERIALS AND METHODS

Serum total and free thyroxine assays

Serum total T4 concentrations were measured using a liquid phase pre-precipitated antibody RIA kit (Gamma-B T4, ImmunoDiagnostic Systems Limited). The materials provided with the kit included seven standards prepared in 1 ml horse serum at concentrations of 0, 25, 50, 100, 150, 200 and 300 nmol/l, one vial of ^{125}I-T4 (radioactive count < 110 kilo Becquerel (kBq) per vial), one vial of pre-precipitated sheep anti-T4 and one vial of assay buffer of sufficient volume to allow the assay of 41 samples in duplicate per standard curve. Prior to routine use, to increase the range of the assay, the 25 nmol/l standard was diluted 1:1 with the 0 standard resulting in an additional standard of 12.5 nmol/l. All reagents, standards and samples were allowed to come to room temperature and mixed by repeated inversion prior to use. All standards and samples were assayed in duplicate in labelled 12 x 75 mm polystyrene tubes (Sarstedt) as follows;

1. 25 ul of each standard or sample was added to appropriately labelled tubes. 25 ul of 0 standard was added to non-specific binding (NSB) tubes.

2. 200 ul of ^{125}I-T4 was added to all tubes including two additional tubes set aside as total counts (TC).

3. 500 ul T4 antiserum complex was added to all tubes except the TC and NSB tubes. 500 ul assay buffer was added to the NSB tubes.

4. Each tube was vortexed gently and incubated in a water bath at 37 $\pm$2^{o}C for between 20 and 25 minutes.

5. All tubes were then centrifuged at 1500 g for 20 minutes.

6. The tubes were decanted, drained and blotted on a pad of absorbent paper.

7. All tubes were counted in an auto gamma counter (Packard Cobra II, Packard Instrument Company) for 60 seconds.

8. The concentrations of samples were extrapolated from the standard curve using a four parameter logit-log model (SAS Immunoassay Program 632014, P.R. Edwards).

9. Within sample errors outwith the range were rejected and the samples re-assayed.

Free T4 concentrations were measured using a direct equilibrium dialysis kit method (Catalog #40-2210, Nichols Institute Diagnostics). The materials provided with the kit included 40 single use Nelson Dialysis cells (patent pending), one vial of dialysis buffer, 100 anti-T4 coated tubes, one vial ^{125}I-T4 solution (radioactive count < 370 kBq), 1 vial wash solution concentrate, six lyophilised standards at concentrations of 0, 3.60, 8.24, 21.88, 55.34 and 128.70 pmol/l and two lyophilised controls of known concentration. The standards, controls and wash solution required reconstitution prior to use. Each kit contained materials of sufficient number or volume to assay 38 samples. All reagents, standards, controls and samples were allowed to come to room temperature prior to use and assayed as follows;

1. The dialysis cells were separated into the dialysate vial and membrane cylinder and labelled.

2. 2.4 ml dialysis buffer was pipetted into each dialysate vial and the membrane cylinder reinserted.

3. 200 ul control or sample was pipetted into the membrane cylinder. The standards were not dialysed.

4. Each dialysis cell was individually sealed with Parafilm and incubated at 37 $\pm$0.5^{o}C for between 16 and 18 hours in a dry heat incubator.

5. The membrane cylinders were immediately removed and discarded and the dialysates allowed to come to room temperature prior to RIA.

6. Anti-T4 coated tubes were labelled in duplicate together with two 12 x 75 mm polystyrene tubes (Sarstedt) as TC tubes.

7. 800 ul of each standard or dialysate was pipetted in duplicate into the appropriately labelled tubes.

8. 50 ul of the ^{125}I-T4 solution was added to each tube.

9. All tubes were vortexed and incubated in a water bath at 37 $\pm$0.5^{o}C for 3 hours.
10. All tubes, except the TC, were washed twice by dispensing 2.0 ml of the working wash solution into each tube and completely decanting the liquid from each tube.
11. All tubes were counted in the gamma counter for 60 seconds and the data treated as for the total T4 assay.

Validation of assays for determination of total and free thyroxine concentrations in feline serum

To assess the reliability of the assays for use with feline serum, both were fully validated with regard to specificity, sensitivity, precision and where appropriate, accuracy.

Specificity

To ensure specificity for the hormone to be measured, inhibition curves produced by serial dilutions of serum were compared to that produced by the standard solutions for parallelism. For the total T4 assay, serum obtained from a hyperthyroid cat was diluted 1:1.25, 1:1.56, 1:2.34, 1:3.52, 1:5.27, 1:7.91, 1:11.87 and 1:17.80 with hormone-free serum. This hormone-free serum was prepared from pooled feline serum using the anion exchange method described by Salter (1979) and modified for use with cat serum by Thoday, Seth & Elton (1984). For the free T4 assay, serum from a hyperthyroid cat was diluted 1:2, 1:4, 1:8 and 1:16 with hormone-free serum prior to the dialysis procedure. The extent of cross reactivity for chemically similar substances was assessed by the kit manufacturers.

Sensitivity

For both the total and free T4 assays, the detection limit was calculated as the concentration corresponding to the mean minus 1sd of the 0 standard.

Precision and drift

For the total T4 assay, low, medium and high pools were included in each assay. The medium pool was prepared from healthy cat serum and the low pool from this by dilution with hormone-free feline serum. The high pool was prepared from hyperthyroid cat serum. The medium pool was assayed repeatedly in one assay to calculate intra-assay precision. The low, medium and high pools were included at the beginning of each assay to calculate the interassay precision. In addition, the low, medium and high pools were placed at the beginning and end of the assay over four assays to calculate drift.

For the free T4 assay, medium and high pools were included in each assay. The medium pool was prepared from healthy cat serum and the high pool from hyperthyroid cat serum. The medium pool was assayed repeatedly, incorporating the dialysis step

each time, to calculate intra-assay precision. The medium and high pools were included at the beginning of each dialysis and assay run to calculate interassay precision. In addition, the medium pool was placed at the end of one assay to estimate drift.

Each pool was stored at -20^{o}C in aliquots of 0.5 ml to avoid repeated freeze/thaw cycles.

Accuracy

For the total T4 assay accuracy was assessed by determining the recovery of known amounts of T4 added to feline serum with endogenous hormone concentrations in the low (hormone-free serum), medium (prepared from healthy cat serum) and high (prepared from hyperthyroid cat serum) end of the reference range. A nominal 1 mmol/l T4 solution was prepared by dissolving 88.9 mg (formula weight 888.9) of L-thyroxine sodium salt pentahydrate crystalline (T2126, Sigma Chemical Company Limited) in 100 ml of a 1:1 propylene glycol : distilled water solution, adjusted to pH 9.0 by the dropwise addition of a 1 molar sodium hydroxide solution. The concentration of this solution was checked spectrophotometrically and found to have a concentration of 0.85 mmol/l. This solution was sequentially diluted in barbitone buffer (pH 8.6), such that the addition of 30 ul to 1.0 ml of test serum increased the concentration by 24.7 nmol/l. The per cent thyroid hormone recovery was calculated from the equation;

$$\frac{(\text{Concentration of hormone found} - \text{Endogenous hormone concentration}) \times 100}{\text{Concentration of hormone added}}$$

The recovery test used for assessing accuracy could not be applied to the free T4 assay. As an alternative, the accuracy of the method itself was assessed by assaying the two quality control samples supplied with each kit. These contained T4 lyophilised in a protein matrix with 0.1% sodium azide and required reconstitution with distilled water prior to use.

Clinical material

Blood samples were obtained from a series of healthy cats and stored as described in Chapter 2, until required for assay. These healthy animals were being sampled for FeLV, FIV and/or FCoV antibody titres either routinely as adults prior to mating, as kittens prior to rehoming or because of contact with a cat known to have a viral related illness.

4.4 RESULTS

Results of the validation methods for the total and free thyroxine assays

Specificity

The results of the parallel studies are presented in Tables 3 and 4.

Sample	Observed concentration	Expected concentration	% Observed/Expected
Undiluted	261.13		
Diluted 1: 1.25	194.36	208.90	93.04
1: 1.56	153.25	167.12	91.70
1: 2.34	102.44	111.42	91.94
1: 3.52	71.46	74.28	96.20
1: 5.27	48.03	49.52	97.00
1: 7.91	33.64	33.01	101.91
1:11.87	27.69	22.01	125.81
1:17.80	16.82	14.67	114.66
Mean ±sd			101.53 ±12.37

Table 3. The observed and expected concentrations of total thyroxine in a sample of hyperthyroid cat serum serially diluted with hormone-free feline serum. All concentrations are expressed in nmol/l.

Sample	Observed concentration	Expected concentration	% Observed/Expected
Undiluted	231.80		
Diluted 1: 2	94.10	115.90	81.19
1: 4	56.14	57.95	96.88
1: 8	26.58	28.98	91.73
1:16	14.97	14.89	100.64
Mean ±sd			92.61 ±8.44

Table 4. The observed and expected free thyroxine concentrations in a sample of hyperthyroid cat serum serially diluted with hormone-free feline serum. All concentrations are expressed in pmol/l.

For both assays, cross reactivity data were provided by the manufacturers. For the total T4 assay, these were 1.4 % for rT3, 0.02 % for T3 and < 0.01 % for 3',5'-diiodo-L-thyronine, 3'-iodo-L-thyronine, phenytoin, phenylbutazone, sodium salicylate and o-acetylsalicylic acid. For the free T4 assay, these were 0.044 % for D-T4, 0.005 % for T3, DL-T3 and 3-iodo-L-thyronine, 0.0036 % for rT3, 0.001 % for 3,5'-diiodo-L-thyronine and 0 % for aspirin, sodium-salicylate, phenylbutazone and 5,5-diphenylhydantoin.

Sensitivity

For the total T4 assay, the detection limit, calculated over five assays, was always less than 2.00 nmol/l (range; 0.23 - 1.81 nmol/l). Any concentrations below this were arbitrarily defined as 2.00 nmol/l for statistical analyses.

For the free T4 assay, the detection limit, calculated over six assays, was always less than 1.00 pmol/l (range; 0.10 - 0.72 pmol/l). Any concentrations below this were arbitrarily defined as 1.00 pmol/l for statistical analyses.

Precision and drift

The results from which data on precision and drift were calculated are presented in Appendices 4, 5, 6 and 7.

For the total T4 assay, the medium pool was assayed 20 times within one assay resulting in an intra-assay c.v. of 6.48 % (mean $\pm$sd; 30.33 $\pm$1.96 nmol/l). For the free T4 assay, the medium pool was assayed 10 times within one assay resulting in an intra-assay c.v. of 8.48 % (mean $\pm$sd; 24.02 $\pm$2.04 pmol/l).

For the total T4 assay, the interassay c.v. for the low, medium and high pools over 14 assays were 9.60 % (mean $\pm$sd; 16.09 $\pm$1.54 nmol/l), 8.94 % (mean $\pm$sd; 33.01 $\pm$2.95 nmol/l) and 5.64 % (mean $\pm$sd; 153.51 $\pm$8.65 nmol/l), respectively. There was no evidence of drift ($P > 0.05$) as assessed by Student's t test on pools placed at the beginning and end of a run over four assays. For the free T4 assay, the inter-assay c.v. for the medium and high pools over six and five assays respectively, were 16.01 % (mean $\pm$sd; 26.20 $\pm$4.19 pmol/l) and 13.03 % (mean $\pm$sd; 131.91 $\pm$17.18 pmol/l). In one assay, the medium pool was placed at the beginning and end of the run with results of 26.32 and 25.33 pmol/L, respectively.

Accuracy

The results from which data on accuracy were calculated are presented in Appendices 8 and 9.

The mean $\pm$sd recovery for the total T4 assay was 99.82 $\pm$10.48 %. For the free T4 assay, the supplied quality control samples fell within the expected target ranges for each assay, respectively.

Serum total and free thyroxine concentrations in healthy cats

These results are detailed in Appendix 10 and summarised in Table 5.

The mean ±sd age of the 50 healthy cats in which circulating total T4 concentrations were determined was 4.5 ±3.6 years (range; 0.08 - 14.0 years). Thirty-five cats were domestic short haired, the remainder being pedigree animals of various breeds. Twenty-five animals were male (17 castrated, eight entire) and 25 were female (13 ovariohysterectomised, 12 entire). Serum free T4 concentrations were determined in 38 of these animals aged between 0.08 and 14.0 years (mean ±sd; 4.14 ±3.17 years). This group consisted of 20 male (14 castrated, six entire) and 18 female (10 ovariohysterectomised, eight entire) animals; 24 were domestic short-haired and the remainder were pedigree animals. In the remaining 12 animals, an inadequate volume of serum was available to measure both total and free T4 concentrations. The free T4 fraction, expressed as a percentage, was determined from the serum total and corresponding free T4 concentration.

The reference ranges for serum total and free T4 concentrations were calculated as the mean ±2sd and were 10.75 - 41.25 nmol/l and 8.14 - 41.45 pmol/l, respectively.

	Total T4 (nmol/l)	Free T4 (pmol/l)	% free T4 fraction
n	50	38	38
Mean	26.00	24.79	0.10
sd	7.62	8.33	0.06
Minimum	11.67	6.63	0.04
1st quartile	20.72	19.52	0.08
Median	26.60	23.88	0.09
3rd quartile	31.37	29.76	0.12
Maximum	44.33	47.72	0.37

Table 5. Summary statistics of serum total and free thyroxine (T4) concentrations and free T4 fractions in healthy cats.

4.5 DISCUSSION

The total and free T4 assays were easy to use and readily adaptable for routine application in a clinical laboratory. The free T4 assay was more time consuming because of the dialysis step, a larger volume of serum was required (200 ul compared to 50 ul for

a total T4 determination) and was approximately three times more expensive than a total T4 estimation. Modification of the total T4 assay to include a low standard of 12.5 nmol/l introduced a potential dilutional error into the standard curve, but this drawback was outweighed by the benefits in measuring the lower total T4 concentrations in the cat compared to man.

The results of the validation studies were considered to be adequate to excellent. Data on specificity for T4 were provided by the respective manufacturers and avoided the expense and time involved in their determination. The results of the inhibition curves for both total and free T4 in feline serum were parallel to their respective standard curves, and confirmed that the assays were specific for human and feline T4. The sensitivity (detection limit) of an assay, defined as that concentration which can be distinguished from zero with a stated degree of probability can be evaluated using a variety of techniques (Midgley, Niswender & Rebar, 1969; Hunter, 1978). The most common method is to determine the concentration corresponding to the mean $\pm$2sd of replicate measurements of the 0 standard or the lowest value that can be distinguished from 0 at the 95 % confidence limit. Some workers consider it more accurate to report the detection limits of an assay as the lowest and the highest standards (Thoday, 1986). However, this limits the range of an assay and computerised methods of curve fitting are adept at extrapolating concentrations below or above these confines. In both assays used here, the sensitivity for each assay run was calculated by the computer programme as the concentration corresponding to the mean $\pm$1sd of the 0 standard. This is perhaps an overestimation of the sensitivity, but the arbitrary definition of a concentration that always exceeded this limit was considered to overcome this problem. The sensitivity of 2.00 nmol/l for the total T4 assay was the same figure as that reported by Sparkes *et al.* (1991) using the same kit method. For the free T4 assay, the sensitivity of 1.00 pmol/l was similar to that of 1.93 pmol/l reported by the manufacturers, which was itself an improvement on the higher concentration of 2.57 pmol/l reported for the prototype (Nelson & Tomei, 1988).

In RIAs precision is considered adequate if the intra-assay c.v. is between 5 and 10 % and the interassay c.v. between 8 and 15 % (Hunter, 1978). The intra-assay c.v.'s of 6.48 and 8.48 % for the respective medium pools used in the total and free T4 assays were therefore excellent. The interassay c.v.'s for the low, medium and high pools in the total T4 assay and the high pool in the free T4 assay were below 15 % and were therefore also considered adequate. The interassay c.v. for the medium pool in the free T4 assay was slightly higher at 16.01 %. However, given the two steps (dialysis and RIA) for this assay and that calculations were made over six assay runs only, this c.v. was felt to be acceptable. The lack of drift calculated over four assay runs for the total T4 assay was reassuring, since the time for completion of an assay run was less than 3 hours. Drift was not considered to be as great a potential problem for the free T4 assay,

because of the long incubation periods, but the placement of a medium pool at the beginning and end of one assay run revealed no significant difference.

Accuracy in a hormone RIA is usually considered adequate if the recovery of known amounts of added hormone is between 80 and 120 % (Thoday, 1986). The accuracy (mean ±sd; 99.8 ±10.5 %) of the total T4 assay was therefore excellent, even when individual results over the low, medium and high end of the standard curve were examined. Accuracy was impossible to determine for the free T4 assay, because free T4 concentrations depend both on the quantity of T4 added and on the affinity and amount of binding proteins present in the sample. However, the fact that the external quality control samples fell within their respective expected values suggested that the assay itself was working adequately.

The use of this equilibrium dialysis method for the measurement of circulating free T4 concentrations in healthy, euthyroid cats is unique in feline medicine. As in man (Ekins, 1975), it could be considered the "gold standard" by which other techniques are compared. The measurement of corresponding total and free T4 concentrations and the subsequent calculation of the free T4 fraction contributes greatly to our hitherto inadequate knowledge of these parameters in cats. The reference range for serum total T4 concentrations in healthy euthyroid cats of 10.75 to 41.25 nmol/l, was similar to previous reports (Mooney, 1990). The reference range of 8.14 to 41.45 pmol/l with a mean ±sd of 24.79 ±8.33 for serum free T4 concentrations in healthy cats supports the premise of Refetoff, Robin & Fang (1970) that absolute free T4 concentrations are similar in all vertebrate species. This range resembles that reported by Ferguson, Peterson & Nachreiner (1989) and Refsal *et al.* (1991) who used equilibrium dialysis and the Magic Free T4 analogue RIA. These similarities are not unexpected since the results for free T4 concentrations in healthy adult humans tend to be similar whether using equilibrium dialysis and direct immunoassays (Stockigt *et al.*, 1981; Gow *et al.*, 1985; Liewendahl *et al.*, 1986). However, the low reference range values for the Amerlex-M free T4 analogue kit method reported for cats (Jones *et al.*, 1991; Sparkes *et al.*, 1991) are inexplicable. The properties of feline thyroid hormone binding proteins may invalidate an assay essentially optimised for use with human serum containing TBG and there are no reports comparing this kit with any other method in cats. Five unspecified analogue RIA's were used to measure free T4 concentrations in 15 healthy dogs and, compared to results obtained by equilibrium dialysis, the concentrations found were significantly lower (Montgomery, Nelson, Ferguson & Feldman, 1991). The results of free T4 concentrations measured by analogue RIA in any species should therefore be viewed with circumspection.

The calculated mean ±sd free T4 fraction of 0.10 ±0.06 % confirms previous expectations (Peterson & Ferguson, 1989a) and is essentially similar to the unspecified reports of Thoday (1990a) and the results obtained in five healthy cats reported by Hays, Turrel & Broome (1988). The results are also similar to those reported for the dog

(Furth *et al.*, 1968; Refetoff, Robin & Fang, 1970; Ferguson & Peterson, 1992). Thus, in the cat it can conclusively be said that circulating total T4 concentrations are lower, the free T4 fraction is higher but that circulating free T4 concentrations are similar to those reported in man.

This study therefore provides a basis upon which the effect of hyperthyroidism and non-thyroidal illness on circulating total and free T4 concentrations can be evaluated. In addition, it identifies areas where further study might be fruitful. For example, a comparison may now be made between analogue methods and equilibrium dialysis in the measurement of circulating free T4 concentrations. Moreover, assessments of the effects of age, sex, breed and environment on circulating free T4 concentrations in cats, which were not carried out due to the small population size in this study, can now be envisaged.

CHAPTER 5
THE EFFECT OF NON-THYROIDAL ILLNESS ON SERUM TOTAL THYROXINE CONCENTRATIONS

5.1 LITERATURE REVIEW

A brief introduction to illness and endocrine status

A variety of non-thyroidal illnesses are known to have a profound effect on serum thyroid hormone concentrations in humans who have no apparent intrinsic thyroid disease (Nicoloff & LoPresti, 1991). Alterations also occur in the dog (Ferguson, 1988). More recent investigations suggest similar effects in the cat (Peterson & Gamble, 1990). Virtually any illness is capable of such effects. However in humans, these alterations usually occur in association with several other systemic hormonal responses. These include reductions in circulating insulin-like growth factors, gonadotropin and sex hormone concentrations and increases in serum adrenocorticotropin hormone (ACTH) and cortisol concentrations. Experimentally, administration of cytokines (interferons, interleukins and tumour necrosis factor) produce the characteristic features of illness such as fever and leucocyte responses, as well as the endocrine alterations (Nicoloff & LoPresti, 1991). It has therefore been suggested that changes in thyroid hormone concentrations should be viewed as a part of a coordinated systemic response to illness that involves both the immune and the endocrine systems.

The following review will concentrate on the effect of illness on total thyroid hormone concentrations in humans, the dog and the cat. A more detailed review of the effect of illness on serum free T4 concentrations is presented in Chapter 6.

The effect of illness on serum thyroid hormone concentrations in man

In humans, there are two major and distinct thyroid hormone responses in the face of illness. The first, and more common of the two, is associated with a depression of serum total and free T3 concentrations with normal or near-normal serum TSH and total and free T4 concentrations. This is termed the "low T3 state", often coined the "euthyroid sick syndrome" (Wartofsky & Burman, 1982). The second syndrome is associated with a depression of both serum T3 and total T4 concentrations and is termed the "low T4 state of medical illness" (Wartofsky & Burman, 1982).

The low triiodothyronine state

A variety of illnesses are associated with the low T3 state and include chronic starvation or malnutrition, diabetes mellitus, chronic illnesses and certain renal and hepatic diseases. In one study, 70 % of hospitalised patients studied had depressed serum total T3 concentrations (Bermudez, Surks & Oppenheimer, 1975). The decrease in serum T3 concentrations is roughly related to the severity of the disease (Chopra, Hershman, Pardridge & Nicoloff, 1983).

The underlying mechanism of the low T3 state is a reduction in or impairment of, the peripheral conversion of T4 to T3 (Vagenakis, Portnay, O'Brian, Rudolph, Arky, Ingbar & Braverman, 1977; Faber, Thomsen, Lumholtz, Kirkegaard, Siersbaek-Nielsen & Friis, 1981; Kaptein, Robinson, Grieb & Nicoloff, 1982a). In healthy individuals, the major route of T4 disposal is by 5'- and 5-deiodination producing T3 and rT3, respectively (Nicoloff, 1986). In non-thyroidal illness, serum total T3 concentrations are reduced while serum rT3 are reciprocally elevated and the fraction of T4 metabolism accounted for by the combined T3 and rT3 production is reduced (Chopra *et al.*, 1983). This is primarily due to reduced T3 production, since production of rT3 remains relatively constant (Faber *et al.*, 1981; Kaptein *et al.*, 1982a; Chopra *et al.*, 1983; LoPresti, Gray & Nicoloff, 1991). Thus, the increased serum concentrations of rT3 appear to be a result of reduced clearance rather than increased production (LoPresti, Gray & Nicoloff, 1991). A unifying explanation would be inhibition of the 5'-deiodinase enzyme responsible both for T3 production and clearance of rT3 to its less iodinated metabolites (Wartofsky & Burman, 1982). However, there are no substantiated reports of such inhibition. The accounting gap in T4 disposal suggests that another metabolite of T4 is formed as an alternative and possibly diverting pathway. Such an alternative pathway has not yet been identified.

Serum TSH concentrations are usually normal in non-thyroidal illness (Wartofsky & Burman, 1982). Various explanations have been advanced for the failure of the pituitary to increase TSH production and secretion in response to the low serum T3 concentrations of non-thyroidal illness. The normal serum T4 concentrations coupled with the particularly active type II 5'-deiodinating system in the pituitary, which may not be inhibited by illness, may compensate for reduced circulating T3 concentrations (Utiger, 1980; Bacci, Schussler & Kaplan, 1982). A lowering of the "setpoint" about which serum thyroid hormone concentrations regulate TSH secretion has been suggested (Utiger, 1980). An alternative explanation is that the stress of illness can inhibit pituitary TSH secretion, thereby preventing the expected response. Bacci, Schussler & Kaplan (1982) found that during the recovery phase of non-thyroidal illness, the mean serum concentration of TSH in 41 patients was significantly higher than in both healthy patients and the same patients during the acute stage of the illness. During this stage serum concentrations of T3 remained depressed, albeit higher than during the acute stage of the illness. The reappearance of the T3-dependent negative feedback suggested alleviation of stress inhibition of pituitary TSH secretion. Serum TSH concentrations during the recovery phase of the illness were negatively correlated with both total and free T3 but less strongly correlated with total T4. The negative effect of total T4 was presumably due to a positive correlation found between serum total T4 and total T3 concentrations. There was no negative correlation between serum TSH and free T4 concentrations. Thus, T3 was the predominant stimulus to TSH secretion during recovery from non-thyroidal illness. The authors reported that in general, the serum concentrations of TSH found in the recovery phase were lower than those of hypothyroid patients with primary

hypothyroidism and similar serum total T3 concentrations. This may have been due to the persistence of some degree of stress inhibition. In the same study in contrast to the findings in patients that survived, serum TSH concentrations did not increase in 22 patients that died. An elevated serum TSH concentration could therefore be viewed as a favourable prognostic sign. A variety of hormones and peptides inhibit TSH and TRH secretion and are secreted in response to stress. These include cortisol, dopamine, growth hormone, opiate peptides, somatostatin and cholecystokinin (Chopra *et al.*, 1983).

Apart from low serum concentrations of T3, affected patients have no other biochemical or clinical features classically associated with hypothyroidism. Utiger (1980) argued that since administration of T3 during fasting resulted in increased urinary excretion of nitrogen, including 3-methyl-histidine, an indicator of muscle breakdown, the reduced T3 production of illness and fasting would result in conservation of muscle and perhaps other protein. Decreased T3 production could also result in a decreased rate of oxygen consumption, substrate utilisation and various other catabolic processes. Utiger (1980) therefore concluded that the low T3 state is metabolically protective and a beneficial adaptation to illness in man.

The low thyroxine state

Both serum total T4 and T3 concentrations are depressed in acute and severe illnesses. Decreased serum total T4 concentrations are not as common as decreased serum total T3 concentrations. In one study, while 68 % of 195 patients requiring intensive medical therapy had decreased serum total T3 concentrations, only 43 % had decreased serum total T4 concentrations (Kaptein, Weiner, Robinson, Wheeler & Nicoloff, 1982b). A continuum of changes probably extends from patients with mild illnesses and the low T3 state, to critically ill patients who have the low T4 state of medical illness (Nicoloff & LoPresti, 1991). The time required from transition from one to the other is presumably dependent on the progression of the illness itself.

Patients with the low T4 state of medical illness are not considered to be hypothyroid. Serum TSH concentrations are usually normal compared to the elevated concentrations seen in primary thyroid failure (Chopra *et al.*, 1983). Thus, the low T4 state may be due to a reduction in T4 secretion by the thyroid gland as a consequence of either secondary or tertiary hypothyroidism. However, serum free T4 concentrations, measured by the gold standard techniques of equilibrium dialysis or ultrafiltration, are usually normal (Chopra *et al.*, 1983). T4 production rates in non-thyroidal illness are also normal (Kaptein, Grieb, Spencer, Wheeler & Nicoloff, 1981a). In addition, T4 therapy in non-thyroidal illness is not beneficial. Brent & Hershman (1986) treated 23 severely ill patients with T4. This treatment resulted in rapid normalisation of serum T4 concentrations. Serum T3 concentrations remained depressed, rT3 concentrations increased and TSH concentrations were suppressed. Survival was not improved. The authors concluded that, by inhibiting TSH secretion, T4 treatment may suppress an

important mechanism for normalisation of thyroid function during recovery (Bacci, Schussler & Kaplan, 1982).

Marked increases in the serum free fraction of T4 occur in non-thyroidal illness. In addition, kinetic data of T4 metabolism in non-thyroidal illness have revealed an increased metabolic clearance rate and fractional catabolic rate, analogous to euthyroid patients with low serum concentrations of TBG (Kaptein *et al.*, 1981a, 1982a). However, immunoassayable serum TBG concentrations in affected patients are normal or only minimally reduced (Kaptein *et al.*, 1981a, 1982a). Thus, the major cause of reduced serum total T4 concentrations is an acquired defect in which the binding of T4 to serum proteins is altered. In both of these studies, an impairment of extravascular T4 binding was apparent, which may have been the result of a common factor (Kaptein *et al.*, 1981a, 1982a).

Several studies have suggested that a substance is released during illness which inhibits T4 binding to serum proteins, although this is controversial. Chopra, Teco, Nguyen & Solomon (1979b) were the first authors to demonstrate the existence of a non-dialysable inhibitor in non-thyroidal illness. There was a smaller decrease or an actual increase in the dialysable fraction of T4 when sera from sick patients were added to pooled normal serum, as compared to a decrease when normal sera were added. The inhibitor was either an immunoglobulin M (IgM) antibody, an immune complex with IgM, or a substance that shares with IgM several physicochemical and antigenic characteristics. However, in another study, although the existence of a non-ultrafiltrable inhibitor was confirmed, it was neither rT3 nor an immunoglobulin (Woeber & Maddux, 1981). The discrepancy in these results may have been the result of different methodologies. Oppenheimer, Schwartz, Mariash & Kaiser (1982) also reported the existence of an inhibitor of serum protein binding which was not readily dialysable but which could not be identified with any immunoglobulin fraction. Homogenates of extrathyroidal tissue of patients with non-thyroidal illness and extrathyroidal tissue of healthy rats contained an inhibitor of protein binding similar to the serum inhibitor (Chopra, Solomon, Teco & Eisenberg, 1982). These authors suggested that the inhibitor may leak into the circulation during illness. Subsequently, a simple, sensitive, rapid and precise competitive ligand binding assay was putatively developed for the measurement of the thyroid hormone binding inhibitor in serum and extrathyroidal tissues (Chopra, Huang, Hurd, Beredo & Solomon, 1984). In this study, the inhibitor was found to be associated with a lipid moiety, several fatty acids, particularly oleic and linoleic acids, being candidates, with the small intestine as a potent source of the inhibitor. In accord with this, *in vitro* studies with oleic acid suggest it may be an important inhibitor in non-thyroidal illness (Chopra, Huang, Solomon, Chaudhuri & Teco, 1986). In contrast with these reports, Mendel, Laughton, McMahon & Cavalieri (1991) failed to find evidence of an inhibitor of T4 to serum binding proteins in non-thyroidal illness. Mixtures of sera from 111 sick patients with a pool of normal sera yielded free T4 fractions predicted from the law of mass action without assuming the presence of an inhibitor. These

authors suggested that in previous reports, evidence for the presence of an inhibitor may have been the result of heparin (used to keep intrarterial lines patent) in the sera or the failure to select normal sera with a low triglyceride concentration leading to *in vitro* generation of free fatty acids. The authors do conclude however, that irreversible inhibitors of binding indistinguishable from altered affinities of the binding proteins may be present in non-thyroidal illness. An alternative and plausible explanation for the low serum total T4 concentrations could be the presence of desialylated TBG which is immunoreactive but has a low affinity for T4 (Mendel *et al.*, 1991).

Although the physiological significance of the low T4 state remains unknown an intriguing and important aspect is its use as a prognostic indicator (Chopra *et al.*, 1983). Slag, Morley, Elson, Crowson, Nuttall & Shafer (1981a) reported subnormal serum total T4 concentrations in 22% of 84 patients admitted to an intensive care unit. An initial serum total T4 concentration of less than 38.61 nmol/l was correlated with 84 % mortality, a concentration of 38.61 to 64.35 nmol/l with 50 % mortality while only 15 % of patients with reference range serum total T4 concentrations died. In the same study, the predictive value of low serum total T3 concentrations was poor. A more detailed study of the predictive value of altered thyroid hormone parameters was subsequently reported by Kaptein *et al.* (1982b). Decreased serum total T4 concentrations had the highest correlation with mortality ($P < 0.001$) and correctly predicted outcome in 70 % of patients. Other thyroid hormone indices, including total T3, T3UT, FTI and the free T4 fraction, correlated with total T4 and did not contribute independently to prediction accuracy. Seventy-five patients with nadir serum total T4 concentrations below 38.61 nmol/l were also studied. In these patients only an increased free T4 fraction was found to correlate significantly with mortality and correctly predicted outcome in 67 % of survivors and 65 % of non-survivors. If a circulating inhibitor of T4 binding is present in non-thyroidal illness, the extent of the impaired binding would appear to reflect the severity of the disease state and explain the relationship between decreased serum total T4 concentrations, the increased free T4 fraction and mortality (Kaptein *et al.*, 1982b).

Miscellaneous states

Most patients with non-thyroidal illness present with either the low T3 or low T4 state. However, several variants can occur. Chronic renal disease and the nephrotic syndrome result in a unique response since serum rT3 concentrations do not increase as serum total T3 concentrations decrease (Wartofsky & Burman, 1982; Nicoloff & LoPresti, 1991). This is probably due to failure of rT3 clearance rates to slow as they do in other non-thyroidal illnesses. Circumstantial evidence suggests that a circulating factor, possibly increased parathormone concentrations, may play a role (Nicoloff & LoPresti, 1991). Non-thyroidal illness may also lead to hyperthyroxinaemia (proportionate increments in circulating total T4 and T3) due to acquired TBG excess (Borst, Eil & Burman, 1983). This effect most commonly occurs in patients with infectious hepatitis, chronic active hepatitis, and primary biliary cirrhosis (Borst, Eil & Burman, 1983; Nicoloff & LoPresti,

1991). The hepatocellular damage in these disorders may cause leakage of excessive amounts of TBG into the circulation or increased hepatocyte TBG synthesis (Borst, Eil & Burman, 1983; Nicoloff & LoPresti, 1991). There may also be discharge of hepatic stores of T4 (Nicoloff & LoPresti, 1991). Elevated serum total T4 concentrations with normal total T3 concentrations can also occur, presumably due to decreased extrathyroidal metabolism of T4 (Gavin, Rosenthal & Cavalieri, 1979; Borst, Eil & Burman, 1983). Such patients are difficult to differentiate from hyperthyroid patients with concurrent illnesses and depressed serum total T3 concentrations.

A variety of drugs are also capable of altering circulating thyroid hormone concentrations (Wenzel, 1981; Wartofsky & Burman, 1982; Borst, Eil & Burman, 1983). Most notable are the effects of endogenous or exogenous glucocorticoids. These hormones inhibit serum thyroid hormone protein binding, peripheral conversion of T4 to T3, and suppress both basal and TRH-stimulated TSH release (Wartofsky & Burman, 1982).

The effect of illness on serum thyroid hormone concentrations in the dog

In the dog fasting, non-thyroidal illness and drug administration are known to affect serum total thyroid hormone concentrations. As in man, these effects complicate the diagnosis of hypothyroidism, a common disease in the dog. In an intensive care unit, almost all dogs (numbers unspecified) had serum total T4 and T3 concentrations below the reference range and approximately half had undetectable concentrations (Ferguson, 1988). However, there are notable differences in the pattern of thyroid hormone changes between man and the dog.

In contrast to the situation in humans, a two week period of fasting in the dog was associated with a decrease in serum total T3, but unchanged serum total T4 and rT3 concentrations (deBruijne, Altszuler, Hampshire, Visser & Hackeng, 1981). Laurberg & Boye (1984) similarly found that fasting was associated with a pronounced decrease in serum total T3 but a slight decrease in serum total T4 and rT3 concentrations. The absence of increased serum rT3 concentrations may be related to the dog's resistance to the development of ketosis during fasting (deBruijne *et al.*, 1981).

Spontaneous hyperadrenocorticism has a profound effect on circulating thyroid hormone concentrations. Serum total T4 and T3 concentrations were measured in a series of 102 dogs with untreated hyperadrenocorticism (Peterson, Ferguson, Kintzer & Drucker, 1984a). Mean serum total T4 and T3 concentrations were significantly ($P < 0.001$) decreased compared to healthy dogs. Serum total T4 and T3 concentrations were below the reference range in 58 (57 %) and 53 (52 %) affected dogs, respectively. Forty-two of the dogs had both decreased serum total T4 and T3 concentrations, 16 had decreased total T4 alone and 11 had decreased total T3 alone. Twenty dogs were evaluated after successful treatment when there was a significant increase in serum total T4 and T3 concentrations. A TSH stimulation test was performed in 22 additional dogs with hyperadrenocorticism. In these animals both basal and post-TSH serum total T4

concentrations were significantly ($P < 0.01$) lower than in healthy animals. To examine the possible mechanisms involved, serum free T4 (by equilibrium dialysis) and rT3 concentrations were subsequently measured in 42 dogs (Ferguson & Peterson, 1992). The mean free T4 fraction was significantly higher ($P < 0.001$) in affected dogs suggesting reduced serum thyroid hormone binding. However, despite the high free T4 fraction, absolute serum free T4 concentrations were significantly ($P < 0.001$) lower in the dogs with hyperadrenocorticism. The mean serum rT3 concentration, measured in 23 dogs with this disease was also significantly ($P < 0.001$) lower compared to healthy controls. The authors concluded that inhibition of peripheral 5'-deiodination of T4 is not a predominant feature of chronic glucocorticoid excess in the dog. Suppression of pituitary TSH secretion may play the major role in the alterations of serum thyroid hormone concentrations in spontaneous hyperadrenocorticism in the dog (Ferguson & Peterson, 1992). By contrast, acute doses of glucocorticoids are associated with minimal changes in serum total T4 concentrations, while serum total T3 concentrations are depressed and rT3 increased. These findings are suggestive of 5'-deiodination inhibition (Woltz, Thompson, Kemppainen, Munnell & Lorenz, 1983; Laurberg & Boye, 1984).

Larsson (1987) measured serum concentrations of total T4, T3 and rT3 in 35 dogs with hepatic disease, 31 dogs with renal disease, 22 dogs with diabetes mellitus and 40 dogs with spontaneous hyperadrenocorticism. Elevated concentrations of serum rT3 were found in all illnesses except hyperadrenocorticism. Significantly depressed serum total T3 concentrations were not found in any disease, while elevated concentrations were found in diabetes mellitus. Depressed serum total T4 concentrations were common. The mean serum total T4 was markedly depressed and serum TBPA concentrations subnormal in the patients with renal disease. The elevated circulating concentrations of T3 found in dogs with diabetes mellitus have not been explained. In another study of six dogs with diabetes mellitus, basal serum concentrations of total T4 and T3 were essentially normal although the T4 response to TSH administration was blunted (Gosselin, Capen, Martin & Targowski, 1980). Peterson & Ferguson (1989a) observed subnormal concentrations of both serum total T4 and T3 concentrations in uncontrolled diabetic ketoacidosis and suggested this may reflect the severity of the disease. These authors also studied 16 dogs with chronic renal disease. Subnormal serum total T4 concentrations were found in 14 animals, and four of these had undetectable concentrations. Most of the dogs had depressed serum total T3 but elevated serum rT3 concentrations. Elevated serum concentrations of both serum total T4 and T3 can occur in obese dogs (Gosselin *et al.*, 1980).

The estimation of serum total T4 concentrations is useful as a prognostic indicator in humans (Slag *et al.*, 1981a; Kaptein *et al.*, 1982b). Serum total T4 concentrations are frequently depressed by non-thyroidal illness in the dog. However, there are no conclusive reports evaluating the correlation between the degree of suppression, severity of illness and subsequent mortality in this species.

The effect of illness on serum thyroid hormone concentrations in the cat

There is only one report detailing the effect of non-thyroidal illness on serum thyroid hormone concentrations in the cat. Peterson & Gamble (1990) measured serum total T4 concentrations in a series of 494 cats with a variety of illnesses and a group of 156 healthy cats. The sick cats ranged in age from one to 24 years (mean ±sd, 12.6 ±3.6 years). The various categories of disease included renal failure (n = 128), diabetes mellitus (n = 37), congestive heart failure (n = 27), systemic neoplasia (n = 34), focal neoplasia (n = 44), hepatic disease (n = 26), inflammatory bowel disease (n = 23), inflammatory pulmonary disease (n = 17), miscellaneous disorders (n = 79) and undiagnosed disease (n = 79). The cats were allotted to groups on the basis of severity of illness (i.e. mild (n = 107), moderate (n = 244) and severe (n = 143)) and also outcome (i.e. lived (n = 371), died (n = 29) or euthanased (n = 94)). The sick cats had significantly ($P < 0.001$) lower serum total T4 concentrations than healthy controls. Mean serum total T4 concentrations were lowest in cats with diabetes mellitus (10.5 ±11.1 nmol/l), hepatopathy (10.8 ±7.6 nmol/l), renal failure (12.3 ±8.4 nmol/l) and systemic neoplasia (12.6 ±9.1 nmol/l). The percentage of cats with serum total T4 concentrations below 10.0 nmol/l was highest in diabetes mellitus (59 %), hepatopathy (54 %), renal failure (48 %) and systemic neoplasia (41 %). By contrast serum total T4 concentrations were minimally affected by focal neoplasia, since only 4 % had values below the reference range. There was also a significant decrease in serum total T4 concentrations from healthy cats through those with mild (mean ±sd, 21.3 ±6.8 nmol/l), moderate (14.8 ±8.1 nmol/l) and severe (6.5 ±5.8 nmol/l) illnesses. The differences in serum total T4 concentrations between the various disease categories was almost entirely attributable to the severity of the disease. Serum total T4 concentrations in the cats that died (mean ±sd, 7.8 ±9.8 nmol/l), or were euthanased (10.0 ±7.0 nmol/l) were significantly ($P < 0.001$) lower than in those cats that survived (15.2 ±8.8 nmol/l). Of the sick cats with low (less than 10.0 nmol/l) serum total T4 concentrations (n = 182), 41 % died or were euthanased. By contrast, only 20 % of the cats (n = 201) with low normal (10.0 - 20.0 nmol/l) and 7 % of the 111 cats with normal (20.0 - 50.0 nmol/l) serum total T4 concentrations died or were euthanased.

In this study, the presence or absence of palpable goitre was recorded in the sick cats and a comparison made of their respective serum total T4 concentrations. This is addressed in more detail in Chapter 7.

There is only one report on the effect of specific drug therapy on thyroid function in healthy cats (Peterson & Ferguson, 1989b). In eight healthy cats, an immunosuppressive dose of prednisone (10 mg/cat) was injected intramuscularly, followed 24 hours later by a repository subcutaneous injection of methylprednisolone acetate (20 mg/cat). There was a significant ($P < 0.004$) decrease in serum total T4 concentrations from a mean ±sd of 15.4 ±3.1 nmol/l to 8.0 ±2.2 nmol/l, 24 hours after injection of prednisone. When retested one and two weeks later (after injection of the methylprednisolone acetate), the serum total T4 concentrations of 14.2 ±7.1 and 17.3

$\pm$7.1 nmol/l, respectively were not significantly different from the pre-treatment values. Thus, an immunosuppressive dose of glucocorticoids acutely depresses serum total T4 concentrations, but a standard dose of methylprednisolone acetate has no detectable effect.

5. INTRODUCTION

Non-thyroidal illness in both man and the dog is capable of suppressing serum total thyroid hormone concentrations often to levels indistinguishable from those exhibited in hypothyroid patients (Wartofsky & Burman, 1982; Kaptein *et al.* 1982b; Chopra *et al.* 1983; Peterson *et al.*, 1984a; Larssen, 1987; Ferguson, 1988, Peterson & Ferguson, 1989a; Nicoloff & LoPresti, 1991). There is only one report of the effect of non-thyroidal illness on thyroid hormone concentrations in the cat (Peterson & Gamble, 1990). In this study only serum total T4 concentrations were measured and were found to be significantly depressed in ill cats. The present study was designed to attempt to both corroborate these results and to establish a data base for further studies on the possible mechanisms involved.

5. MATERIALS AND METHODS

Clinical material

The case material comprised a series of 76 sick geriatric cats referred to Glasgow University Small Animal Hospital. Thirty-seven were female (33 ovariohysterectomised, four entire) and 39 were male (37 castrated, two entire). They ranged in age from 5.0 to 20.0 years with a mean $\pm$sd of 10.1 $\pm$3.6 years. Fifty-eight of the cats were either domestic short (n = 54) or long (n= 4) haired; the remaining cats represented four other breeds Siamese (n = 12), Persian (n = 3), British Blue (n = 2) and Birman (n = 1). In each case, a diagnosis of a non-thyroidal illness was made as described in Chapter 2. Most of these cats form part of the survey detailed in Chapter 3.

In addition, 31 younger sick cats were included in this study. Seven were female (five ovariohysterectomised, two entire) and 24 were male (20 castrated, four entire). They ranged in age from 0.5 to 4.5 years with a mean $\pm$sd of 2.4 $\pm$1.3 years. Twenty-two of the cats were either domestic short (n = 19) or long (n = 3) haired; the remainder were Persian (n = 4), Siamese (n = 2), Burmese (n = 2) and Devon Rex (n = 1). A diagnosis of a non-thyroidal illness was made using the same criteria as for the older cats.

The healthy cats in which serum total T4 concentrations were measured for comparison purposes are described in Chapter 4.

Estimation of serum total thyroxine concentrations

At the time of initial presentation a serum sample from each case was stored as described in Chapter 2, until required for assay. Serum total T4 concentrations were measured as described in Chapter 4.

Statistical analyses

The Mann Whitney U test was used for all statistical analyses.

5.4 RESULTS

Serum total thyroxine concentrations in sick cats

These results are detailed in Appendix 11 and summarised in Table 6.

There was no significant difference ($P > 0.05$) in the serum total T4 concentrations between the geriatric and young sick cats. These data were therefore grouped together for future analyses.

Only two sick cats had serum total T4 values (43.77 and 45.33 nmol/l) exceeding the reference range (10.75 - 41.25 nmol/l) but were less than 3 sds from reference mean (48.87 nmol/l). Neither of the cats were severely ill. One (case number 114861) was mildly hypokalaemic after a period of anorexia of undetermined cause, while the other (case number 117989) was suffering recurrent vomiting as a result of mild congenital pyloric stenosis. There were no clinical features of thyrotoxicosis apparent in either animal and both cats remained healthy after treatment for follow-up periods of two years. The next highest value was 33.51 nmol/l.

Comparison of serum total thyroxine concentrations in sick and healthy cats

Serum total T4 concentrations were significantly ($P < 0.001$) lower in the sick cats compared to the healthy controls (mean $\pm$sd, 26.00 $\pm$7.62; reference range, 10.75 - 41.25 nmol/l)(Figure 5).

Outcome of illness compared to serum total T4 concentrations in sick cats

These data are summarised in Table 7.

There was a significant difference ($P < 0.001$) in the serum total T4 concentrations in those cats that died or required euthanasia ($n = 49$) compared to those that remained alive ($n = 58$) (Figure 6). In 40 cases (82 %) the cats died or were euthanased at the time of initial presentation, hospitalisation or shortly afterwards. When the time to euthanasia was delayed, this was a result of owners refusing this option or because attempted treatment failed. There was only one cat (case number 114769) with a serum total T4 concentration as low as 2.00 nmol/l that survived. This cat was suffering from a protein-losing nephropathy.

The mortality of the cats associated with different serum total T4 concentrations is presented in Figure 7. The overall mortality of the 107 cats was 46%, and ranged from 83 % in cats with serum total T4 concentrations less than 5.00 nmol/l to 20 % in cats with concentrations of greater than 25.00 nmol/l. Thirty-nine cats with serum total T4 concentrations of less than or equal to 20 nmol/l died or were euthanased, representing a mortality of 56% in this group. Ten cats with serum total T4 concentrations greater than 20 nmol/l died or were euthanased, representing a mortality of 27 %.

	Geriatric	Young	Geriatric and young
n	76	31	107
Mean	17.65	16.62	17.35
sd	8.14	9.39	8.49
Minimum	2.00	2.00	2.00
1st quartile	12.01	9.65	11.68
Median	17.50	17.96	17.68
3rd quartile	22.81	24.19	22.95
Maximum	45.33	31.39	45.33

Table 6. Summary statistics of serum total thyroxine (T4) concentrations (nmol/l) in geriatric cats, young cats and both geriatric and young cats grouped together. These cats were suffering from a variety of non-thyroidal illnesses.

	ALIVE	DEAD
n	58	49
Mean	20.29	13.87
sd	8.01	7.76
Minimum	2.00	2.00
1st quartile	15.53	8.97
Median	19.94	13.33
3rd quartile	23.59	19.97
Maximum	45.33	28.23

Table 7. Summary statistics of serum total thyroxine concentrations (nmol/l) in cats that remained alive, or required euthanasia or died as a direct result of their illnesses.

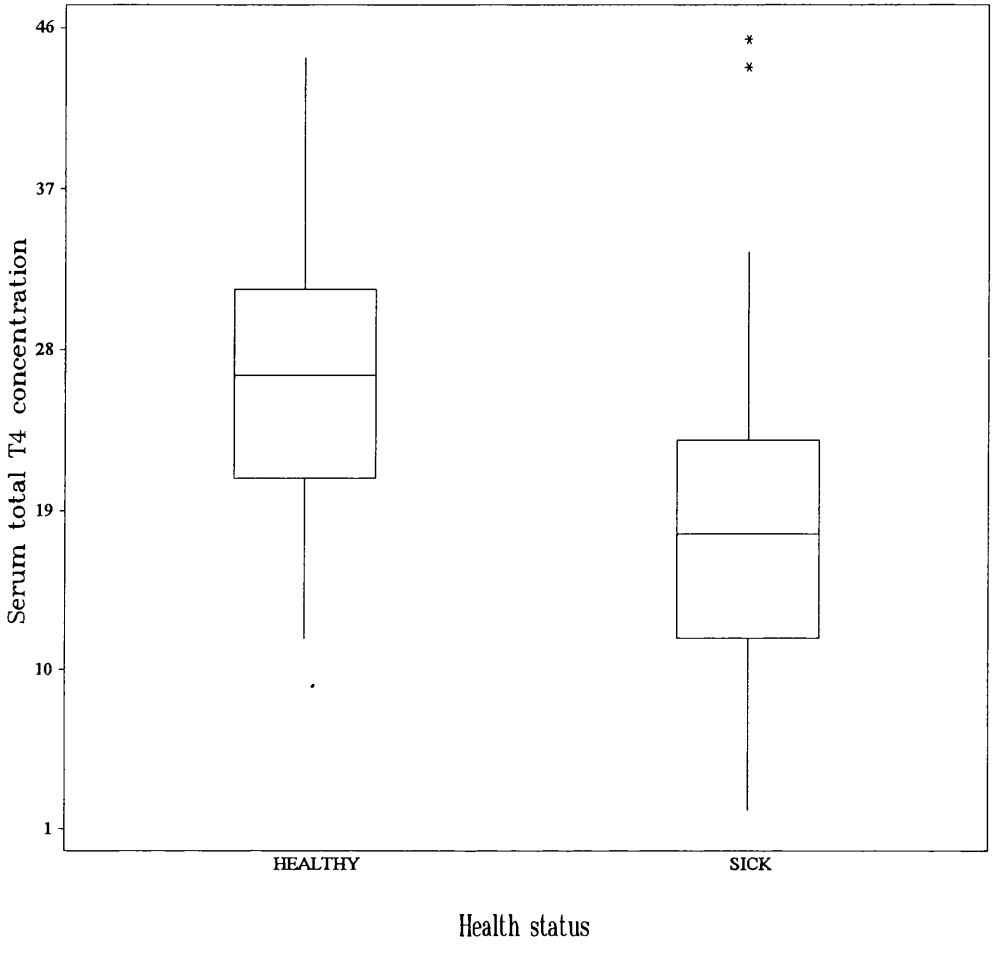


Figure 5. Box and whisker plot of serum total thyroxine (T4) concentrations in 50 healthy and 107 sick cats. The box encloses the middle half of the data and is bisected by a line representing the median. The whiskers represent the range of typical values. Possible outliers are represented by * and represent values from case numbers 114861 and 117989. There are no probable outliers.

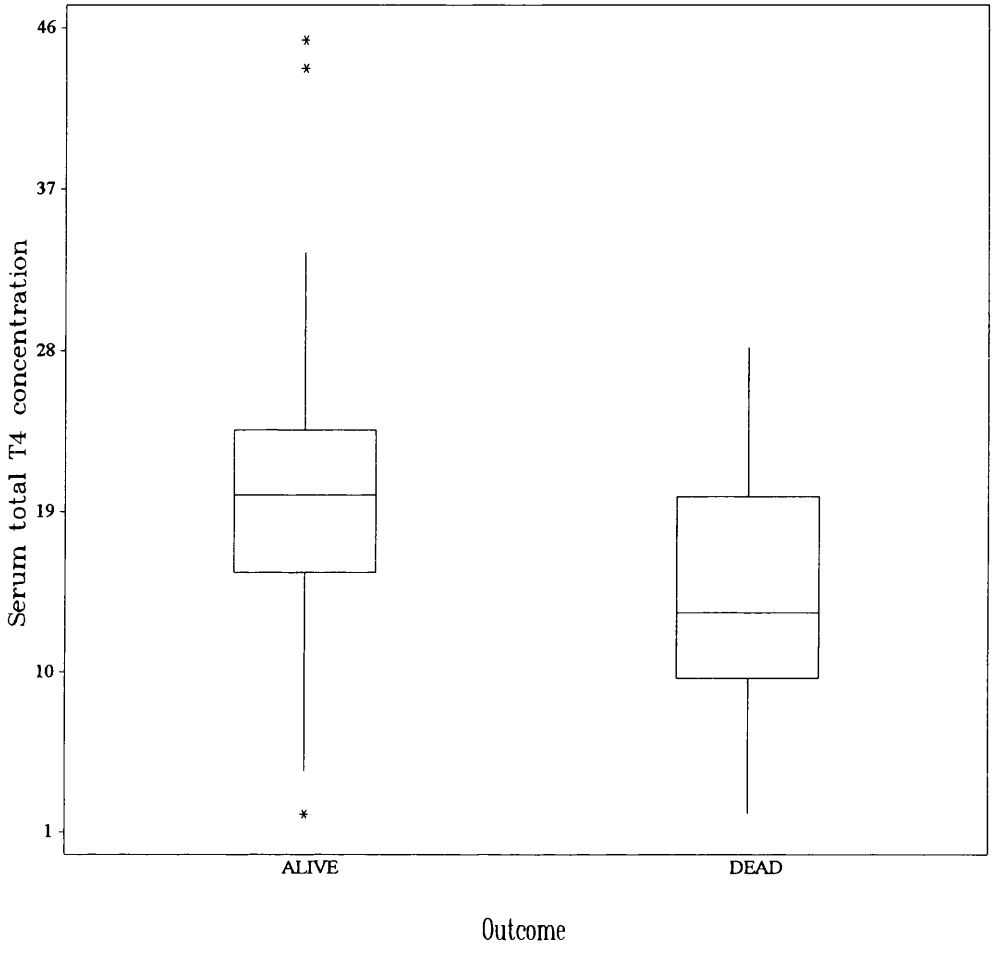


Figure 6. Box and whisker plot of serum total thyroxine (T4) concentrations in 58 sick cats that remained alive and 49 cats that died or required euthanasia as a direct result of their illnesses. The box encloses the middle half of the data and is bisected by a line representing the median. The whiskers represent the range of typical values. Possible outliers are represented by * and represent values from case numbers 114861 and 117989 (high) and 114769 (low). There are no probable outliers.

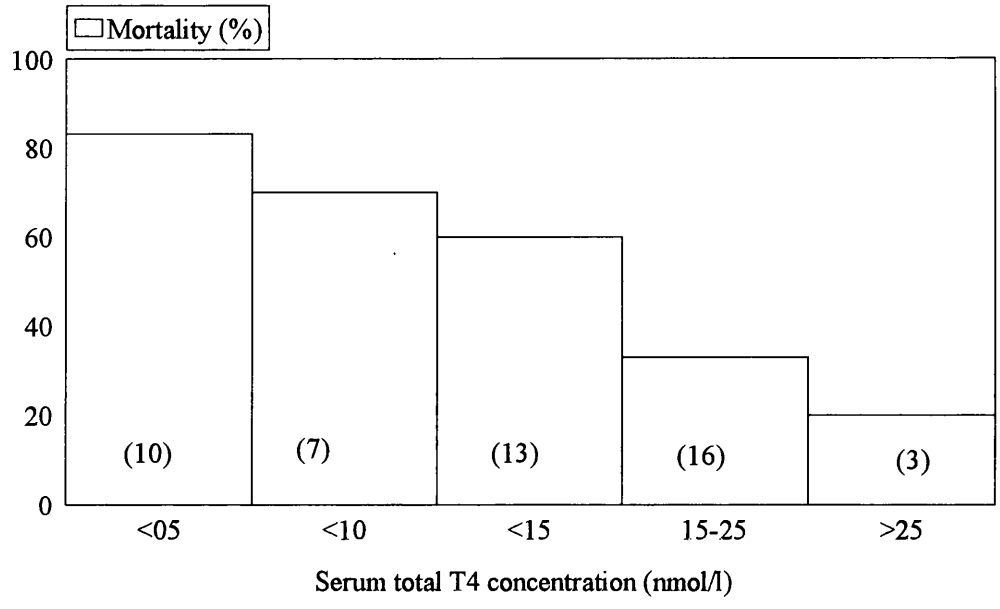


Figure 7. The relationship of mortality to serum total thyroxine (T4) concentrations in 107 cats with a variety of non-thyroidal illnesses. The number of cats which died or were euthanased are depicted in parentheses.

5.5 DISCUSSION

These results are in agreement with those of Peterson & Gamble (1990) confirming that the presence of non-thyroidal illness has a profound effect on depressing circulating total T4 concentrations in the cat. In contrast to their study, the cats were not allocated to groups based on the disease category or severity of the illness. This was due to the smaller number of cases (107 versus 494) examined and the existence of many categories with few cases. However, the two cats with the highest serum total T4 concentrations were considered to be the least clinically ill, one recovering from an unknown illness and one suffering congenital pyloric stenosis. Their total T4 concentrations exceeded the reference range. It could be argued that, contrary to the clinical evidence, they were hyperthyroid. However, the serum total T4 values of these two cats did not exceed three sds from the reference mean, a value which has been suggested to differentiate hyperthyroid cats from the small number of healthy animals with elevated results (Turrel, Feldman, Hays & Hornof, 1984). Peterson & Gamble (1990) reported that all of the high outlying serum total T4 concentrations occurred in the sick cats with palpable goitre. A small number of these animals subsequently were known to develop overt hyperthyroidism. However, in the two cats of the present study, goitre was not palpable and hyperthyroidism did not subsequently develop in either animal.

In this study the inverse correlation between mortality and serum total T4 concentrations is similar to that found in humans (Kaptein *et al.*, 1982b). This could be viewed as an alternative way of assessing severity of illness since cats that die or are euthanased are most likely to be severely ill. This was certainly true in the study presented here. The significantly decreased serum total T4 concentrations in the cats that died or were euthanased compared to those that survived was therefore expected. Only one cat that survived had a serum total T4 concentration at the lower limit of detection. Although initially surprising, this cat was severely hypoproteinaemic and the results can therefore be explained. As suggested by Peterson & Gamble (1990) this study confirms the value of serum total T4 measurements as a prognostic indicator in sick cats.

Neither this study nor that of Peterson & Gamble (1990) specifically addressed the possibility of serum total T4 concentrations increasing as the cats recover from ill-health. Although likely, since the decline in serum total T4 concentrations is related to the severity of illness rather than the specific disease category, this needs to be addressed.

A criticism of this study is the failure to age match the healthy controls (mean $\pm$sd, 4.5 $\pm$3.6 years) to the sick cats, the majority being in excess of 5 years. Since 1986, obtaining reference values from healthy cats requires Home Office licensing and the healthy cats used in this study were being bled for other reasons. Controlling for age was therefore impossible. In the study by Peterson & Gamble (1990) the sick cats were also significantly older than the healthy controls. However, they analysed the serum total T4 concentrations in the healthy group according to age and found no significant difference between young and old cats. Thoday, Seth and Elton (1984) reported that serum total T4 concentrations varied significantly with age in 318 cats ranging from 4 months to 13 years, but mean serum total T4 concentrations decreased in cats greater than 5 years and then increased rather than decreasing. In addition, in the present study there was no significant difference in serum total T4 concentrations in the sick cats less than 5 years compared to those greater than 5 years. This suggests that the effect of illness is greater than any putative effect(s) of age.

The frequency with which serum total T4 concentrations are depressed in sick cats suggests that, as in the dog, the low T4 state of medical illness is more common than in humans (Ferguson, 1988; Kaptein *et al.*, 1982b). Further investigations are required to determine if serum total T3 concentrations are also depressed as they are in humans (Nicoloff & LoPresti, 1991). The mechanisms involved in depressing serum total T4 concentrations warrant further investigation. The low circulating total T4 concentrations could be the result of decreased thyroid hormone production due to suppressed TSH secretion, as they are in spontaneous hyperadrenocorticism in dogs (Ferguson & Peterson, 1992). If such is the case, serum free T4 concentrations would be decreased. If circulating binding inhibitors were present in sick cats as suggested in humans (Chopra *et al.*, 1979b; Woeber & Maddux, 1981; Oppenheimer *et al.*, 1982; Chopra *et al.*, 1982, 1984), the serum T4 fraction would be increased. These questions are addressed in Chapter 6 by measurement of serum free T4 concentrations in affected cats.

CHAPTER 6
THE EFFECT OF NON-THYROIDAL ILLNESS ON SERUM FREE THYROXINE CONCENTRATIONS

6.1 LITERATURE REVIEW

General Introduction

While non-thyroidal illness profoundly depresses serum total T4 concentrations in man, controversy surrounds its effect on serum free T4 concentrations. This controversy reflects the variety of methods available for measurement of circulating free T4 concentrations (reviewed in Chapter 4). The effect of a variety of non-thyroidal illnesses on serum free T4 concentrations in the dog have been reported but the results must be examined critically with regard to the assay methods used. There is a paucity of reports on the effect of non-thyroidal illness on serum free T4 concentrations in the cat.

The effect of illness on serum free thyroxine concentrations in man

A variety of methods are used for measuring serum free T4 concentrations. The principles of the methods are reviewed in Chapter 4. In assessing the validity of commercial kit methods, the results obtained should be compared to the reference techniques of equilibrium dialysis or ultrafiltration (Ekins, 1985).

Non-analogue kit methods

Serum free T4 concentrations were measured using a labelled-hormone antibody uptake RIA method (Immophase, Corning) in 35 euthyroid patients (Chopra, Van Herle, Teco & Nguyen, 1980). These patients were hospitalised for a variety of non-thyroidal illnesses. Twenty-four of the patients had serum total T4 concentrations within, while 11 had concentrations below the reference range. The reference range for serum free T4 concentrations was established using samples from 32 euthyroid, healthy subjects (mean $\pm$sd, 21.36 $\pm$5.15 pmol/l; range, 13.51 - 35.39 pmol/l). Serum free T4 concentrations were within the reference range in all but three of the 24 patients with reference range total T4 concentrations. In one patient, the serum free T4 concentration was just below the reference range at 12.23 pmol/l, while in the remaining two, the values were clearly subnormal at 10.30 pmol/l each. The mean serum free T4 concentration in this group (17.63 pmol/l) was slightly, but significantly ($P < 0.01$), lower than the reference mean. Serum free T4 concentrations were within the reference range in only five of the 11 patients with subreference range serum total T4 concentrations. Values were below the reference range in the remaining six patients (range, 6.82 - 12.87 pmol/l). The mean serum free T4 concentration in these 11 patients (12.87 pmol/l) was significantly ($P < 0.001$) lower than the reference mean.

Serum free T4 concentrations were also measured by these workers using the equilibrium dialysis technique of Sterling & Brenner (1966). In 32 euthyroid patients, the mean ±sd serum free T4 concentration was 29.60 ±6.31 pmol/l (reference range, 18.40 - 40.80 pmol/l). The mean free T4 concentrations of 89.83 pmol/l and 46.33 pmol/l in the sick patients with either reference range or subnormal serum total T4 concentrations respectively were significantly ($P < 0.001$ and $P < 0.02$, respectively) higher than the reference mean. The mean free T4 fractions of 0.11 and 0.12 % were higher than that of euthyroid patients (0.03 %). In the nine patients with low serum free T4 concentrations by RIA, these values were usually within or above the reference range by equilibrium dialysis. In one case, the serum free T4 concentration was below the reference range using both techniques of measurement. The correlation between serum free T4 concentrations as measured by RIA and equilibrium dialysis was low ($r = 0.32$). The authors concluded that serum free T4 concentrations as measured by this RIA technique would not serve as an appropriate sole test of thyroid status in the presence of non-thyroidal illness. In addition, they suggested that the high serum free T4 concentrations found by the dialysis method may be related to the putative non-dialysable binding inhibitors of non-thyroidal illness (Chopra *et al.*, 1979b).

Kaptein, MacIntyre, Weiner, Spencer & Nicoloff (1981b) evaluated three different methods of serum free T4 estimation in a series of 14 healthy subjects and 26 sick and 16 hypothyroid patients who had comparable serum total T4 concentrations. Serum free T4 concentrations were measured by equilibrium dialysis using the method of Sterling & Brenner (1966) with purification of the tracer before and after dialysis, a labelled-hormone, back titration kit method (GammaCoat Two Step Free T4, Clinical Assays), a microencapsulated antibody kit method (Liquisol, Damon Diagnostics) and the Immophase kit. In sick patients with total T4 concentrations less than 38.61 nmol/l (reference range, 64.35 - 154.44 nmol/l) ($n = 9$), only the serum free T4 concentrations measured by equilibrium dialysis (mean ±sem, 30.89 ±6.45 pmol/l) and the GammaCoat kit (18.02 ±6.45 pmol/l) were similar to the concentrations in healthy subjects (mean ±sem, 29.60 ±1.29 and 15.44 ±1.29 pmol/l, respectively). All of these values were therefore significantly higher ($P > 0.01$ in each case) than those of the corresponding hypothyroid patients. Serum free T4 concentrations as measured by the Liquisol and Immophase kits (mean ±sem, 7.72 ±1.29 and 6.45 ±1.29, respectively) were significantly ($P < 0.001$ in each case) lower than in healthy patients (mean ±sem, 25.74 ±1.29 and 20.59 ±1.29 pmol/l, respectively) and were similar to those exhibited by the corresponding group of hypothyroid patients. In patients with serum total T4 concentrations between 38.61 and 64.35 nmol/l ($n = 7$), only the serum free T4 concentrations measured by equilibrium dialysis (mean ±sem, 30.89 ±5.15 pmol/l) and the GammaCoat kit (23.17 ±3.86 pmol/l) were similar to euthyroid patients and therefore significantly ($P < 0.025$ and $P < 0.01$, respectively) higher than those of hypothyroid patients. Serum free T4 concentrations as measured by the Liquisol and Immophase kits (mean ±sem, 18.02 ±2.57 and 12.87 ±1.29, respectively) were significantly ($P < 0.001$

and $P < 0.025$, respectively) lower than healthy subjects and similar to those exhibited by the corresponding group of hypothyroid patients. In the patients with reference range serum total T4 concentrations (n = 10), serum free T4 concentrations by equilibrium dialysis were significantly ($P < 0.025$) higher (mean ±sem, 41.18 ± 3.86 pmol/l) than the healthy controls. Similarly, the results by the GammaCoat kit (mean ±sem, 23.17 ±2.57 pmol/l) were also significantly ($P > 0.025$) higher than in the healthy subjects The results were significantly ($P < 0.001$) lower using the Immophase kit (mean ±sem, 15.44 ±1.29 pmol/l) but similar using the Liquisol kit (25.74 ±1.29 pmol/l) than in the healthy controls. In summary, taking all sick patients together serum free T4 concentrations were below the reference range in three (11.5 %) patients by equilibrium dialysis, two (7.7 %) patients by the GammaCoat kit, 19 (73.1 %) patients by the Immophase kit and 14 (53.8 %) patients by the Liquisol kit. Serum free T4 concentrations were above the reference range in seven (26.9 %) patients by equilibrium dialysis and six (23.1 %) by the GammaCoat kit. The results for the Immophase kit are therefore similar to the report of Chopra *et al.* (1980). The authors do not comment on the discrepancy between results of the equilibrium dialysis values in their study and the higher values in the study of Chopra *et al.* (1980). This may be related to the tracer purification technique used by Kaptein *et al.* (1981b), but which was not specified by Chopra *et al.* (1980). Radioiodide contamination may artefactually raise values in indirect equilibrium dialysis (Ekins, 1985). Differences in patient selection may have also played a role.

Similar results using the Immophase kit have been reported by Slag, Morley, Elson, Labrosse, Crowson, Nuttall & Shafer (1981b). Serum free T4 concentrations were measured in 20 critically ill patients with serum total T4 concentrations below 64.35 nmol/l and another 65 ill patients with normal serum total T4 concentrations (> 64.35 nmol/l). The serum free T4 concentrations in both groups (mean ±sd, 12.36 ±0.90 and 20.59 ±0.64 pmol/l, respectively) were significantly ($P < 0.001$ and $P < 0.01$, respectively) lower than the concentrations in 35 euthyroid subjects (mean ±sd, 23.17 ±0.77 pmol/l). The mean ±sd serum free T4 concentration of 13.64 ±1.02 pmol/l in the patients with low serum total thyroxine concentrations measured using the GammaCoat kit method was similar to that of 12.10 ±0.77 pmol/l in the healthy subjects. However, the value of 16.47 ±0.64 pmol/l was slightly and significantly ($P < 0.001$) higher in the patients with serum total T4 concentrations above 64.35 nmol/l compared with the healthy subjects. This confirms the results of Kaptein *et al.* (1981b). In addition, the mean ±sd (15.83 ±1.02 pmol/l) serum free T4 concentration measured using the Liquisol kit in the patients with low serum total T4 concentrations was similar to that of 14.16 ±0.64 pmol/l in the control group. In the second group of patients using the latter kit method, the serum free T4 concentrations were significantly ($P < 0.01$) higher (mean ±sd, 19.31 ± 0.77 pmol/l) than in the healthy controls. These results are in contrast to the previous report but may reflect the lower mean of 14.16 pmol/l in the healthy controls compared to that of 25.74 pmol/l reported by Kaptein *et al.* (1981b). Free T4 concentrations were also measured in this study by equilibrium dialysis using an organic

anion exchange resin. The values were significantly ($P < 0.01$) lower in the patients with low serum total T4 concentrations than in the healthy patients (mean $\pm$sd, 11.20 $\pm$1.29 and 16.73 $\pm$1.03 pmol/l, respectively (reference range, 7.72 - 25.74 pmol/l)) and slightly but not significantly higher (mean $\pm$sd, 27.03 $\pm$2.57 pmol/l) in the patients with normal serum total T4 concentrations. The differences between these results and those of Kaptein *et al.* (1981b) presumably reflect the different methodologies and/or patient selection. There was little or no correlation ($r = 0.05 - 0.33$) between equilibrium dialysis and the kit methods.

Melmed, Geola, Reed, Pekary, Park & Hershman (1982) also compared these three kit methods with equilibrium dialysis and found comparable results to those of Chopra *et al.* (1980) and Kaptein *et al.* (1981b). Fourteen patients admitted to a medical intensive care unit were studied. These patients had serum total T4 concentrations below 64.35 nmol/l. In addition, 13 patients with chronic liver disease and 32 patients with renal failure were studied. Serum total T4 concentrations were below the reference range in 46 and 31 % of these patients, respectively. Serum free T4 concentrations were measured by equilibrium dialysis (Sterling & Brenner, 1966) and the Liquisol, Immophase and GammaCoat kit methods. Each of the three groups of patients had a high proportion of low values for the dialysable free T4 concentration; six (43 %) critically ill, three (23 %) liver and three (9 %) renal patients. Since a similar equilibrium dialysis technique to that of Chopra *et al.* (1980) was used the higher proportion of low values in the critically ill group may reflect the small numbers of patients in this category in both studies. The means in each group were significantly ($P < 0.01$) lower than for healthy controls. Using the GammaCoat kit, four (29 %) critically ill patients had low serum free T4 concentrations and the mean value was significantly ($P < 0.01$) lower than for healthy controls. The difference between this and the study of Kaptein *et al.* (1981b) may again reflect patient selection. None of the hepatic patients and two (6 %) renal patients had low serum free T4 concentrations. As in the study of Kaptein *et al.* (1981b), a high proportion ($n = 6$ (10.2 %)) of elevated values were found with this kit. When free T4 concentrations were measured using the Immophase kit, all of the critically ill, 10 (77 %) of the liver and 24 (75 %) of the renal patients had low values indistinguishable from those exhibited by a group of hypothyroid patients ($n = 20$). Free T4 concentrations were measured using the Liquisol kit; four (29 %) of the critically ill group, two (15 %) of the liver group and 10 (31 %) of the renal group had values below the reference range. The mean value of the critically ill group was significantly ($P < 0.01$) lower than that of healthy patients. However, using this kit only 50 % of the hypothyroid patients had serum free T4 concentrations below the reference range compared to 95 to 100 % using the other methods. One value was actually above the reference range. The results for the Immophase and Liquisol kits in sick patients are therefore essentially similar to those of Kaptein *et al.* (1981b).

Thus based on these reports, the GammaCoat kit method appears to be the most reliable for measuring serum free T4 concentrations in non-thyroidal illness yielding

results usually comparable to those of equilibrium dialysis. However, conflicting reports on the values obtained with this kit are apparent. This may be due to differences in patient selection but suggests that none of these assays are entirely satisfactory for assessing serum free T4 concentrations in non-thyroidal illness.

Analogue kit methods

Stockigt *et al.* (1981) assessed the recently introduced Amerlex (Amersham) free T4 kit method in a series of 14 critically ill patients selected for low serum concentrations of total T4 (< 55 nmol/l) and serum free T4 concentrations within the reference range as assessed by equilibrium dialysis using the technique of Sterling & Brenner (1966). Serum free T4 concentrations assessed by the Amerlex kit (mean ±sd 6.5 ±2.0 pmol/l) were significantly ($P < 0.001$) lower than those in 15 healthy patients (mean ±sd, 17.0 ±2.0 pmol/l). Serum binding of the labelled analogue was evaluated by dextran-charcoal separation which suggested that binding correlated directly with prealbumin concentration. Further studies with purified prealbumin diluted in buffer showed dose-dependent binding of labelled analogue. Subsequently the Amerlex kit was assessed in patients with hereditary analbuminaemia and familial dysalbuminaemic hyperthyroxinaemia (Stockigt *et al.*, 1983) and gave spuriously low and high values for free T4, respectively. This study suggested that the analogue binds to albumin. In their previous publication, albumin contamination and not prealbumin was responsible for the binding (Stockigt *et al.*, 1983).

After the introduction of the Amerlex kit, several similar kits were marketed adopting different (and usually unspecified) analogues, separation systems, and methods of standardisation. Within the category of non-thyroidal illness, these kits could give varying results. In 40 patients with critical non-thyroidal illness, serum free T4 concentrations measured using two analogue kit methods (Amerlex Free T4, Amersham; GammaCoat One Step Free T4, Clinical Assays) correctly identified only 17 and 16 respectively as euthyroid (Waud, Chan, Drew, Oropeza, Sucupira, Scheinen, Garrison, Mayo, Taylor, Stem, Graham, Coyle, Niebyl & Wagner, 1983). The remaining values were within the hypothyroid range. Further details were reported by Chan, Waud, Taylor, Stem, Drew, Oropeza & Sucupira (1983). In the 40 patients the mean serum total T4 concentration was approximately half the mean value for euthyroid patients. Both free T4 assays gave similar results (mean ±sd, 8.62 ±5.28 pmol/l; reference range, 8.75 - 23.12 pmol/l (Amerlex) and 8.11 ±6.44 pmol/l; reference range, 9.00 - 25.74 pmol/l (GammaCoat)). By contrast, in 25 of the patients, serum free T4 concentrations as measured by equilibrium dialysis were usually within the reference range (mean ±sd, 17.89 ±12.36 pmol/l; reference range 12.87 - 29.60 pmol/l). Two values (8 %) were above and 4 (16 %) were below the reference range. As in previous reports (Chopra *et al.*, 1980; Kaptein *et al.*, 1981b; Slag *et al.*, 1981b; Melmed *et al.*,1982), serum free T4 concentrations measured using a labelled-hormone, back titration kit method

(GammaCoat Two Step Free T4, Clinical Assays) were more comparable to equilibrium dialysis (mean ±sd, 15.83 ±8.75 pmol/l; reference range, 9.00 - 25.74 pmol/l).

Despite the similarity between the Amerlex and GammaCoat analogue kits, other kit methods have been found to give different results (Gow *et al.*, 1985). Two groups of patients with non-thyroidal illness were studied: 39 patients from a general medical ward and 36 patients with renal failure undergoing dialysis treatment. All of these patients had reference range serum TSH concentrations, but 28 and 67 % of serum total T4 concentrations were below the reference range in each group, respectively. Five kits were compared: Amerlex and Amerlex-M (Amersham), Becton-Dickinson (Becton Dickinson), Coat-A-Count (Diagnostic Products) and Magic Free T4 (Corning). The Amerlex-M kit contained the same analogue and standards as the Amerlex kit but involved magnetic separation rather than centrifugation. Many patients were found to have serum free T4 concentrations below the reference range. In the medical patients the number of values below the reference range were 4 (10 %) by Amerlex and Amerlex-M, 7 (18 %) by Becton-Dickinson, 9 (23 %) by Coat-A-Count and 7 (18 %) by Magic Free T4. In the renal patients, these values were 24 (67 %) by Amerlex and Amerlex-M, 30 (83 %) by Becton-Dickinson, 26 (72 %) by Coat-A-Count and 29 (81 %) by Magic Free T4. Thus the least number of low values were obtained with the Amerlex and Amerlex-M kits.

Csako *et al.* (1987) further evaluated several kit methods in 82 patients with non-thyroidal illness and compared the results with serum albumin concentrations. The patients were classified as having reference range serum total T4 and T3 concentrations (Group 1, n = 22), low total T3 concentrations (Group 2, n = 37) and both low total T4 and T3 concentrations (Group 3, n = 23). The kits evaluated were Amerlex, GammaCoat one step and Coat-A-Count and results were compared with equilibrium dialysis. Euthyroid (n = 26) and Group 1 patients had similar serum free T4 concentrations and none were below the reference range. In turn, 24 to 30 % of Group 2 and 61 to 74 % of Group 3 patients had subnormal serum free T4 concentrations assessed by the kit methods. By contrast, 46 % of Group 2 patients exhibited high serum free T4 concentrations by equilibrium dialysis but there were no subnormal values in either group. The albumin concentration was subnormal more often in Group 1 patients than in healthy controls. Group 2 patients revealed greater numbers of subnormal albumin concentrations and all of the Group 3 patients had abnormally low concentrations. Using previously established correlations the serum free T4 concentrations were corrected for albumin. The results with all techniques were unchanged by this correction in healthy and Group 1 patients. By contrast, in Group 2 and 3, after correction for albumin, almost all of the formerly low serum free T4 concentrations assessed by analogue RIAs became normal. This study indicates that albumin-dependence often is responsible for the falsely low free T4 values obtained with analogue kit methods confirming preliminary reports (Stockigt *et al.*, 1981, 1983).

Given this albumin dependence, several manufacturers attempted to improve the analogue RIA systems by adding binding blocker(s) that chemically inhibit(s) the analogue-albumin interaction. Two of these improved kits (Amerlex-M and Coat-A-Count) were assessed in 79 patients with nonthyroidal illness and compared to results obtained by ultrafiltration (Konno, Hirokawa, Tsuji, Hagiwara, Taguchi, Murakami & Taguchi (1989). Eight (10.1 %) patients had subnormal serum free T4 concentrations as assessed by ultrafiltration, 29 (37.2 %) by Amerlex-M and 19 (33.3 %) by Coat-A-Count. The possible influence of thyroid hormone binding protein (TBG, albumin and TBPA) concentrations on these results were studied. Serum free T4 concentrations by ultrafiltration were independent of the concentrations of these binding proteins ($r = -0.031 - 0.160$, $n = 79$, $P > 0.05$). Amerlex-M free T4 concentrations correlated with albumin ($r = 0.491$, $n = 78$, $P < 0.001$) and TBG ($r = 0.250$, $P < 0.05$), but not with TBPA. Coat-A-Count free T4 concentrations also correlated with TBG ($r = 0.636$, $n = 57$, $P < 0.001$), but not with TBPA and albumin concentrations. The falsely low free T4 concentrations with Coat-A-Count were largely found in patients with the low T4 state where serum TBG concentrations were reduced. The low values with Amerlex-M were found in the low T3 and low T4 states where albumin concentrations were reduced. The kit manufacturers did not supply data on the concentrations of blockers used in the kits. Thus, the authors suggested that the effect of the binding blockers depends on their concentration and in excess could possibly disrupt T4-TBG binding. There was no correlation with serum free T4 concentrations measured by ultrafiltration and oleic acid (a putative binding inhibitor (Chopra *et al.*, 1986)) concentrations. This does not exclude the possible involvement of other substances which could interfere with T4 binding in non-thyroidal illness. In this study mathematically calculated circulating free T4 concentrations correlated with serum TBG concentrations. By correcting for an altered binding constant of TBG, there was a closer agreement between calculated serum free T4 concentrations and those obtained by ultrafiltration. The dependence of the calculated values on serum TBG concentrations was then lost. This suggests that the binding affinity of TBG in non-thyroidal illness is altered as has since been reported elsewhere (Mendel *et al.*, 1991).

Taken together, it is clear from these reports that analogue kit methods are inadequate for the measurement of serum free T4 concentrations in the presence of non-thyroidal illness. Although the problem appears to lie with the analogues used, it is possible that the serum dilution inherent in the method may be an additional source of error (Nelson & Weiss, 1985).

Equilibrium dialysis and ultrafiltration

Despite equilibrium dialysis and ultrafiltration being considered as the gold standard techniques for measurement of serum free T4 concentrations, Ekins (1985) has identified several problems inherent with various methods. These were reviewed in Chapter 4, and

included in particular, problems with tracer purification with indirect equilibrium dialysis and falsely low values in non-thyroidal illness if dilution of serum was involved.

In the above studies when equilibrium dialysis or ultrafiltration were used, serum free T4 concentrations were usually within or above the respective reference ranges. Occasionally, values were depressed particularly if corresponding total T4 values were markedly depressed. For the most part indirect equilibrium dialysis techniques were used which also involved dilution of serum samples. Surks, Hupart, Pan & Shapiro (1988) compared serum free T4 concentrations in 29 critically ill patients as measured by ultrafiltration and equilibrium dialysis. The ultrafiltration technique involved minimal dilution of serum and careful chromatographic purification of tracer T4 in the ultrafiltrate. An indirect equilibrium dialysis technique involving serum dilution was used (Oppenheimer, Squef, Surks & Hauer, 1963). The mean serum free T4 concentration by ultrafiltration was not significantly different between the sick patients and 12 healthy controls (mean $\pm$sem, 34.7 $\pm$7.6 and 22.7 $\pm$1.5 pmol/l, respectively. The mean serum free T4 concentration by equilibrium dialysis was also not significantly different between the sick and healthy patients (mean $\pm$sem, 45.3 $\pm$5.1 and 31.0 $\pm$0.9 pmol/l, respectively). Free T4 values were higher when determined by equilibrium dialysis but there was an excellent correlation with ultrafiltration ($r = 0.84$, $P < 0.001$). In patients with serum total T4 concentrations greater than 40 nmol/l ($n = 20$), the serum free T4 concentrations were either within or above the reference range. In patients with serum total T4 concentrations below 40 nmol/l ($n = 9$), the serum free T4 concentrations were either within or more rarely above the reference range. Occasionally in this group serum free T4 concentrations were just below the reference range (one (11.1 %) value by ultrafiltration and two (22.2 %) values by equilibrium dialysis), but these values were always higher than those exhibited by hypothyroid patients ($n = 7$). However, the mean serum free T4 concentrations by ultrafiltration or equilibrium dialysis (mean $\pm$sem, 19.4 $\pm$3.9 and 27.1 $\pm$6.5 pmol/l, respectively) was not significantly different from the control group. In total, seven (24.3 %) values were above and one (3.4 %) value below the reference range by ultrafiltration and 10 (34.5 %) values were above and two (6.9 %) below the reference range by equilibrium dialysis. The discrepancy between this report and those above (Chopra *et al.*, 1980; Kaptein *et al.*, 1981b; Slag *et al.*, 1981b; Melmed *et al.*, 1982; Chan *et al.*, 1983) could be related to dialysis tubing, buffer constituents, factor of dilution or other technical factors (Surks *et al.*, 1988). Since the results were comparable using equilibrium dialysis and ultrafiltration which involved minimal sample dilution and maximal tracer purification, the former could therefore be considered an accurate technique (Surks *et al.*, 1988).

Using a similar ultrafiltration technique Faber, Kirkegaard, Rasmussen, Westh, Busch-Sorensen & Jensen (1987) reported comparable results to those of Surks *et al.* (1988). Serum free T4 concentrations were measured in 34 critically ill patients and did not differ from values in healthy patients (numbers not reported). One value (2.9 %) was above and one below the reference range. By contrast, ill patients treated with dopamine

(known to suppress TSH concentrations) had significantly lower serum free T4 concentrations. The authors concluded that any reports where serum free T4 concentrations are significantly reduced in the presence of non-thyroidal illness should be interpreted with caution.

A direct equilibrium dialysis method eliminates the need for adding radiolabelled tracer to the serum sample before dialysis, thereby eliminating the need for tracer purification steps. Theoretically it is therefore more accurate than any indirect technique (Ekins, 1985). Helenius and Liewendahl (1983) measured serum free T4 concentrations in 33 sick patients with low circulating T3 and high rT3 concentrations using an indirect equilibrium dialysis technique. The mean serum free T4 concentration was significantly ($P < 0.001$) higher in the sick patients compared with 40 healthy euthyroid subjects (mean $\pm$sd, 26.5 $\pm$12.3 and 15.1 $\pm$3.0 pmol/l, respectively). The patients were divided into two groups. Group 1 ($n = 20$) had normal serum total T4 concentrations, although the mean value was lower than for healthy controls and Group 2 ($n = 13$) had values below 60 nmol/l. Serum free T4 concentrations remained significantly higher compared to the healthy controls in Group 1 (mean $\pm$sd, 31.1 $\pm$12.3 pmol/l) but were not significantly different in Group 2 (mean $\pm$sd, 19.4 $\pm$8.6 pmol/l). The reference range for serum free T4 concentrations was defined as the mean $\pm$2sd and was 9.1 to 21.1 pmol/l. Thus, 14 (70 %) values in Group 1 and two (15.4 %) values in Group 2 were above the reference range. There were no values below the reference range. Compared to this equilibrium dialysis method, conflicting results were obtained using two analogue (Amerlex and GammaCoat) and two non-analogue (Liquisol and Immophase) kits. The authors suggested the observed increases in circulating free T4 concentrations correlated well with the possible existence of hormone binding inhibitors. The higher values in sick patients compared to the values in the patients studied by Surks *et al.* (1988) presumably reflect differences in patient selection.

The method of Helenius and Liewendahl (1983) involved dilution of samples. The serum free T4 concentrations were independent of dilution in sick patients when dilutions of 1 : 5 to 1 : 55 were tested. However, using a similar dialysis technique Nelson & Weiss (1985) found a marked dilutional effect in serum from sick patients. Thus, Nelson & Tomei (1988) used a direct dialysis method involving minimal dilution of serum samples. Twenty-seven sick patients with the low T4 state of medical illness were studied. The serum total T4 concentrations ranged from less than 6.45 to 61.78 nmol/l. The serum free T4 concentrations were within the reference range in most subjects. One (3.7 %) patient had an elevated serum free T4 concentration (45.05 pmol/l; reference range, 10.30-34.75 pmol/l). Three (11.1 %) patients had a serum T4 concentration at the lower limit of the reference range.

Thus, of the various techniques, apparently the most accurate results have been obtained using the untrafiltration technique of Faber *et al.* (1987) and Surks *et al.* (1988) and the direct dialysis technique of Nelson & Tomei (1988).

The effect of illness on serum free thyroxine concentrations in the dog

Montgomery *et al.* (1991) compared five analogue free T4 assays (manufacturers unidentified) which used magnetic separation (A and B) or antibody coated tubes (C, D and E) with results of indirect equilibrium dialysis. Dogs were classified as healthy (n = 15), hypothyroid (n = 11), or euthyroid with hyperadrenocorticism (n = 11) megaoesophagus (n = 4), hypercholesterolaemia/hypoalbuminaemia (n = 9) and obesity (n = 3). These classifications were based on history, clinical examination, results of biochemical analyses and TSH stimulation testing, requirement for T4 replacement therapy and additional, but unspecified, diagnostic tests where necessary. In the healthy dogs the mean (±sd) serum free T4 concentrations were consistently lower with the analogue RIAs (A, 10 ±4; B, 18 ±5; C, 12 ±5; D, 17 ±6 and E, 5 ±1 pmol/l) compared to equilibrium dialysis (26 ±12 pmol/l). A similar trend was reported to occur in the presence of non-thyroidal illness. Comparably low results between equilibrium dialysis and the analogue RIAs were found in hypothyroidism. There was a significant correlation between equilibrium dialysis and each of the RIA systems ($r = 0.85$-0.91, $P < 0.001$ in each case) in healthy dogs. When all dogs were evaluated, a significant correlation was only found between equilibrium dialysis and systems A ($r = 0.39$, $P < 0.002$) and C ($r = 0.50$, $P < 0.001$). Accuracy in assessing thyroid gland function was defined as hypothyroid dogs with positive test results and euthyroid dogs with negative test results over all dogs tested. Accuracy was 0.91 with equilibrium dialysis, 0.71 with system A, 0.65 with system B, 0.71 with system C, 0.78 with system D and 0.75 with system E. It is difficult to interpret the effect of non-thyroidal illness on serum free T4 concentrations from this study since actual values were not reported. However, from the accuracy studies, it would seem that serum free T4 concentrations have a tendency to be low in non-thyroidal illness when assessed by analogue assays but not by equilibrium dialysis, similar to the situation in man.

Nelson, Ihle, Feldman & Bottoms (1991) measured serum free T4 concentrations in 59 euthyroid dogs with dermatopathy (n = 38) or concurrent illnesses (n = 21) and compared the results to those obtained in euthyroid, healthy (n = 62) and hypothyroid dogs (n = 51). An analogue RIA system was used which contained thyroid hormone binding blockers (Diagnostic Products). From this report it is difficult to conclude the effects of non-thyroidal illness on serum free T4 concentrations. Dogs with a peripheral neuropathy or hyperadrenocorticism had serum free T4 values that were not significantly different from hypothyroid dogs. However, serum total T4 values between these dogs were also similar. In the remaining sick dogs serum free and total T4 concentrations differed significantly, in the same direction, from those in hypothyroid dogs. Thus, it is likely that the free T4 assay was not giving a true circulating free T4 concentration, but rather reflected the total T4 concentration.

Ferguson and Peterson (1992) studied serum free T4 concentrations in a series of 42 dogs with hyperadrenocorticism using an indirect equilibrium dialysis technique.

Since glucocorticoids have specific effects on functional thyroid status, which may differ from the effects of other illnesses, this study was previously reviewed in Chapter 5.

The effect of illness on serum free thyroxine concentrations in the cat

There is only one report which mentions the assessment of serum free T4 concentrations in sick cats. As part of a study designed to assess the value of the T3 suppression test in the diagnosis of hyperthyroidism Refsal *et al.* (1991) included 23 cats with a variety of non-thyroidal illnesses. However, serum total T4 concentrations in these cats were within the reference range (12 - 49 nmol/l) and were not significantly different from those in four healthy controls (mean ±sd, 27.9 ±10.3 and 25.7 ±11.1 nmol/l, respectively). Serum free T4 concentrations were measured using an analogue RIA kit (Magic Free T4, Corning). Serum free T4 concentrations in these cats were within the reference range (12-36 pmol/l) and were not significantly different from those in four healthy controls (mean ±sd, 21.7 ±5.4 and 28.3 ±5.6 pmol/l, respectively). The authors mention that a comparison was made using this RIA system and a direct dialysis technique (Nichols Institute Diagnostics). This is addressed in Chapter 4.

6.2 INTRODUCTION

Despite the known effects of non-thyroidal illness in suppressing circulating total T4 concentrations in cats (Peterson & Gamble, 1990) there are no conclusive reports on its possible effects on corresponding free T4 concentrations. To date, it has been assumed that, as in man, serum free T4 concentrations are essentially normal and the animals euthyroid. Given the wide range of commercial kit methods applicable to the measurement of serum free T4 concentrations in man and their increasing use in veterinary laboratories, such reports are likely to be made in the near future. However, these kit methods frequently produce erroneous results in humans and should be compared to the gold standard techniques of equilibrium dialysis or ultrafiltration (Ekins, 1985). This study was designed to assess circulating free T4 concentrations in sick cats, where serum total T4 concentrations were known to be depressed. An equilibrium dialysis technique was used in order both to provide more accurate results and to establish a data-base with which other techniques could be compared.

6.3 MATERIALS AND METHODS

Clinical Material

The case material comprised 67 of the sick geriatric cats described in Chapter 5. Thirty-three were female (29 ovariohysterectomised, four entire) and 34 were male (32 castrated, two entire). They ranged in age from 5.0 to 20.0 years with a mean $\pm$sd of 10.2 $\pm$3.7 years. Fifty of the cats were either domestic short (n = 47) or long (n = 3) haired; the remaining cats represented four other breeds: Siamese (n = 11), Persian (n = 3), British Blue (n = 2) and Birman (n = 1).

In addition, the 31 sick younger cats described in Chapter 5 were included.

The healthy cats used for comparison purposes are those described in Chapter 4.

Estimation of serum free thyroxine concentrations

At the time of initial presentation a serum sample from each case was stored as described in Chapter 2, until required for assay. Serum free T4 concentrations were measured as described in Chapter 4. The free T4 fraction, expressed as a percentage, was determined from the serum free and corresponding total T4 concentration (Chapter 5).

Statistical analyses

The Mann Whitney U test was used for all statistical purposes.

6.4 RESULTS

Serum free thyroxine concentrations in sick cats

These results are detailed in Appendix 11 and summarised in Table 8.

There was no significant difference ($P > 0.05$) in the serum free T4 concentration between the geriatric and young sick cats. These data were therefore grouped together for future analyses.

	Geriatric	Young	Geriatric and young
n	67	31	98
Mean	27.71	27.67	27.70
sd	13.91	12.89	13.53
Minimum	8.45	1.52	1.52
1st quartile	19.04	22.05	19.62
Median	25.08	24.59	24.95
3rd quartile	33.29	33.57	33.36
Maximum	75.54	62.31	75.54

Table 8. Summary statistics of serum free thyroxine (T4) concentrations (pmol/l) in geriatric cats, young cats and both geriatric and young cats grouped together. These cats were suffering from a variety of non-thyroidal illnesses.

Serum free T4 concentrations were within the reference range (8.14 - 41.45 pmol/l) for healthy cats in 83 (84.7 %) of the 98 sick cats.

Three cats (3.1 %) had serum free T4 concentrations below the reference range. These concentrations of 6.85 (case number 116330), 4.06 (case number 117268) and 1.52 (case number 114584) pmol/l corresponded to serum total T4 values of 2.00, 2.89 and 2.00 nmol/l respectively, all markedly below the reference range (10.75 - 41.25 nmol/l). Two of these cats died and one was euthanased as a result of their illnesses. In the remaining cats with serum total T4 values below the reference range (n = 18), corresponding serum free T4 concentrations were either within (n = 16), or above (n = 2), the reference range.

Twelve cats (12.2 %) had serum free T4 concentrations above the reference range, but in only six (6.1 %) were these values greater than 3 sds from the reference mean (49.78 pmol/l). These concentrations of 75.54 (case number 113929), 75.31 (case number 113235), 70.84 (case number 110531), 62.31 (case number 114049), 55.08 (case number 116128), 51.34 (case number 115485) pmol/l corresponded to serum total T4 concentrations of 22.12, 15.62, 16.16, 17.82, 15.07 and 10.03 nmol/l, respectively.

Five of the serum total T4 concentrations were within the reference range, while the remaining value was just below the reference range.

The calculated free T4 fraction in sick cats

These results are detailed in Appendix 11 and summarised in Table 9.

	Geriatric	Young	Geriatric and young
n	67	31	98
Mean	0.20	0.32	0.24
sd	0.15	0.48	0.30
Minimum	0.06	0.06	0.06
1st quartile	0.10	0.10	0.10
Median	0.14	0.14	0.14
3rd quartile	0.21	0.34	0.24
Maximum	0.82	2.01	2.10

Table 9. Summary statistics of the calculated free thyroxine (T4) fraction (%) in geriatric cats, young cats and both geriatric and young cats grouped together. These cats were suffering from a variety of non-thyroidal illnesses

There was no significant difference ($P > 0.05$) in the calculated free T4 fraction (%) between the geriatric and young sick cats. These data were therefore grouped together for future analyses.

The five highest values (range, 0.74 - 2.10 %) occurred in cats (case numbers, 113701, 114769, 115950, 115953, 119282) with serum free T4 concentrations (range, 14.86 - 42.06 pmol/l) either within (n = 4), or above (n = 1), the reference range. Corresponding serum total T4 concentrations (range, 2.00 - 4.64 nmol/l) were all below the reference range. Four (80 %) of these cats died. The one surviving cat (case number, 114769) was suffering from a protein-losing nephropathy.

The next six highest values (range, 0.45 - 0.59 %) occurred in cats (case numbers 109212, 110531, 113235, 114854, 115449, 115485) with serum free T4 concentrations (range, 33.57 - 75.34 pmol/l) either within (n = 3), or above (n = 3) the reference range. Corresponding serum total T4 concentrations (range, 4.37 - 16.16 nmol/l) were either

within the low end or below the reference range. Four (67 %) of these cats died or were euthanased as a result of their illnesses.

In the remaining cats (n = 87), the free T4 fraction range from 0.06 to 0.39 %. Thirty-seven (43 %) died or were euthanased as result of their illnesses.

Comparison of serum free thyroxine concentrations in sick and healthy cats.

There was no significant difference ($P > 0.05$) in the serum free T4 concentrations between the sick and healthy cats (mean $\pm$sd, 24.79 $\pm$8.33; reference range, 8.14 - 41.45 pmol/l) (Figure 8).

There was no significant ($P > 0.05$ in each case) difference in the serum free T4 concentrations in cats with corresponding serum total T4 concentrations within (mean $\pm$sd, 28.73 $\pm$13.60 pmol/l, n = 77), or below (mean $\pm$sd, 23.93 $\pm$12.92 pmol/l, n = 21), the reference range and the healthy cats.

The calculated free T4 fraction was significantly ($P < 0.001$) higher in the sick cats compared with the healthy controls (mean $\pm$sd, 0.10 $\pm$0.06; range, 0.04 - 0.37 %) (Figure 9).

In comparing the results between the healthy and sick cats two notable results occurred in the healthy animals. Cat number 5 had a serum free T4 concentration of 47.72 pmol/l, a serum total T4 concentration of 12.94 nmol/l and a calculated free T4 fraction of 0.37 %. The next highest free T4 concentration was 41.38 pmol/l. Cat number 3 had a serum free T4 fraction of 0.24 %, corresponding to a serum free T4 concentration of 30.51 pmol/l and a serum total T4 concentration of 12.50 nmol/l. The next highest serum free T4 fraction was 0.18 %.

6.5 DISCUSSION

The equilibrium dialysis technique used in this study eliminated the need to add radiolabelled tracer T4 to the serum sample before dialysis, thereby eliminating the need for tracer purification steps and its associated problems (Ekins, 1985). In addition, minimal serum dilution was involved. Sample dilution disturbs the concentration of hormone binding inhibitors which may be present in non-thyroidal illness, thereby artefactually decreasing serum free T4 concentrations (Ekins, 1985).

The serum free T4 concentrations in the cats with non-thyroidal illness were comparable with values in human patients where ultrafiltration of undiluted serum (Faber *et al.*, 1987; Surks *et al.*, 1988) or a similar equilibrium dialysis technique was used (Nelson & Tomei, 1988). In these human studies, serum free T4 concentrations were not significantly different in sick compared to healthy patients even when serum total T4 concentrations were markedly depressed as was found in the cats presented here. This provides evidence that euthyroidism is maintained in sick cats despite suppressed circulating total T4 concentrations. Consequently, and as expected, the calculated free T4 fraction was elevated in these cats.

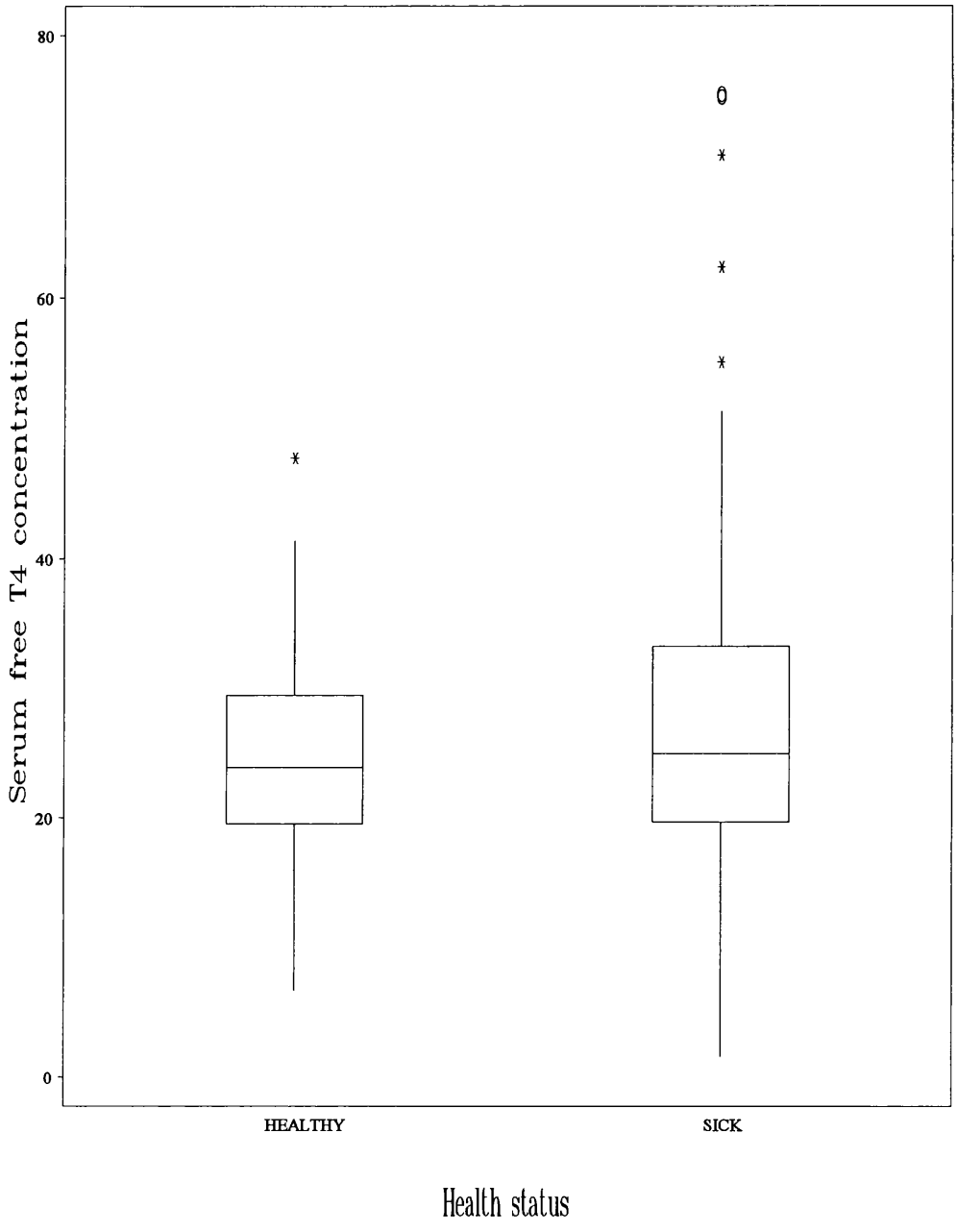


Figure 8. Box and whisker plot of serum free thyroxine (T4) concentrations in 38 healthy and 98 sick cats. The box encloses the middle half of the data and is bisected by a line representing the median. The whiskers represent the range of typical values. Possible outliers are represented by * and represent values from cat number 5 (healthy) and case numbers 110531, 114049 and 116128 (sick). Probable outliers are represented by 0 and represent values from case numbers 113235 and 113929.

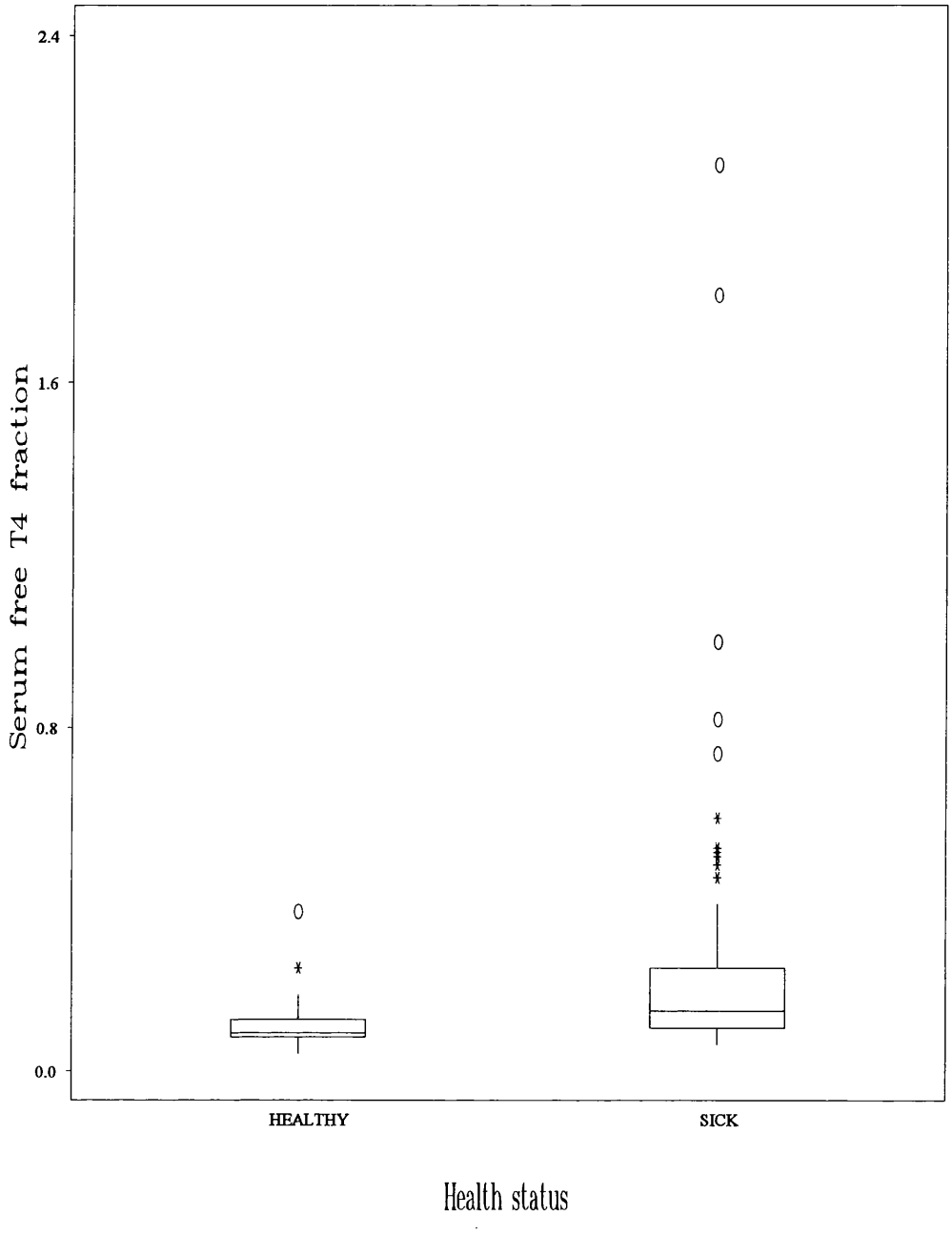


Figure 9. Box and whisker plot of the calculated serum free thyroxine (T4) fraction (%) in 38 healthy and 98 sick cats. The box encloses the middle half of the data and is bisected by a line representing the median. The whiskers represent the range of typical values. Possible outliers are represented by * and represent values from cat number 3 (healthy) and case numbers 109212, 110531, 113235, 114854, 115449 and 115485 (sick). Probable outliers are represented by 0 and represent values from cat number 5 (healthy) and case numbers 113701, 114769, 115950, 115953, 119282.

In man, elevated serum free T4 concentrations have been found in 24.5 % of 29 and 2.9 % of 34 patients with non-thyroidal diseases as estimated by ultrafiltration (Faber *et al.*, 1987; Surks *et al.*, 1988) and 3.7 % of 27 patients as estimated by equilibrium dialysis (Nelson & Tomei, 1988). Of the cats studied here, 12 (12.2 %) had elevated serum free T4 concentrations. As in humans (Surks *et al.*, 1988), these values tended to correspond to reference range serum total T4 concentrations. The biological consequences of elevated circulating free T4 concentrations in sick human patients are unclear (Tibaldi & Surks, 1985). Such consequences remain unclear in the cat.

Three (3.1 %) cats had serum free T4 concentrations below the reference range whereas in humans using the same equilibrium dialysis technique none were found (Nelson & Tomei, 1988). Although every attempt was made to measure serum free T4 concentrations in the cats before treatment had commenced, there was no control over possible drug therapy prior to referral. It was therefore possible that in these three cats, drugs (in particular glucocorticoids) known to suppress circulating serum total T4, TSH and free T4 concentrations were administered prior to referral (Wartofsky & Burman, 1982; Peterson & Ferguson, 1989b; Ferguson & Peterson, 1992).

Since TBG does not exist in the cat (Larsson, Pettersson & Carlstrom, 1985; Thoday, 1986), alterations in TBG binding capacity or affinity (Konno *et al.*, 1989; Mendel *et al.*, 1991) could not explain the results presented. A more plausible explanation for the low total T4 concentrations, normal or high free T4 concentrations and the elevated free T4 fraction seen in the sick cats would be the presence of serum protein binding inhibitors as suggested in humans (Chopra *et al.*, 1979b; Woeber & Maddux, 1981; Oppenheimer *et al.*, 1982; Chopra *et al.*, 1982, 1984, 1986). Since this premise remains controversial in humans, the cat can now be viewed as an appropriate model for further investigation of the possible mechanisms involved.

There are no substantiated reports on the use of this equilibrium dialysis technique in healthy or in sick cats. However, in the report of Refsal *et al.* (1991) where the technique was briefly mentioned, no problems were highlighted. In addition, Ferguson (1994) also briefly mentions its use in the dog where its accuracy was virtually identical to that of indirect tracer equilibrium dialysis. The serum samples used in the present study were frozen and thawed prior to analysis. No studies on the effect of freeze/thaw cycles were carried out. However, using human serum, values for free T4 were not altered by 10 freeze-thaw cycles (Nelson & Tomei, 1988).

As in the study of serum total T4 concentrations (Chapter 5), it was impossible to age match the sick cats and healthy controls. However, there was no significant difference in the serum free T4 concentrations or calculated free T4 fraction between the sick cats less than five and those in excess of five years of age.

All but one of the cats with the five highest free T4 fractions died or were euthanased as result of their illnesses. The cat that survived was suffering from a protein-losing nephropathy, was severely hypoproteinaemic and the markedly elevated free T4 fraction is therefore easily explainable. Four of the cats with the next six highest

free T4 fractions also died. This resulted in a mortality rate of 73 % for the cats with high outlying values, compared to a mortality of 43 % in the remaining cats. In humans with markedly depressed serum total T4 concentrations, an increased free T4 fraction was found to correlate significantly with mortality and contribute to prediction accuracy (Kaptein *et al.*, 1982b). From the results of the present study, a similar situation appears to exist in the cat. Thus, the likelihood of a cat dying or requiring euthanasia increases as the serum total T4 concentration decreases, particularly if the free T4 fraction is elevated.

The unusual results found in the healthy cats are interesting. In the two cases with the highest free T4 fractions and either normal or slightly elevated serum free T4 concentrations, the corresponding serum total T4 concentrations were within the low end of the reference range. These cats were classified as healthy on the basis of history and full clinical examination. Their results were highlighted as outliers and were similar to those found in the sick cats. It is thus possible that they were subclinically ill. Measurement of these three parameters in a larger group of healthy cats may prove to be a useful adjunct to physical examination in the assessment of health status.

In dogs, as in cats, the low T4 state appears to be a common response to illness (Larsson, 1987; Ferguson, 1988). It is therefore probable that dogs exhibit a similar serum free T4 response in illness to that found in the cats in this study. Hypothyroidism is a common disease of dogs (Peterson & Ferguson, 1989a) but commercial free T4 analogue methods are largely incapable of separating hypothyroidism from non-thyroidal illness (Montgomery *et al.*, 1991). It is therefore possible that this equilibrium dialysis technique may be more accurate in diagnosing hypothyroidism in the dog and would obviate the need for either dynamic TRH or TSH stimulation tests. In the cat, hypothyroidism is extremely rare (Peterson, Randolph & Mooney, 1994) and an accurate test for its diagnosis is not so frequently required. However, feline hyperthyroidism is common (Peterson, 1984; Thoday & Mooney, 1992) and usually diagnosed on the basis of a single serum total T4 concentration. Since serum free T4 concentrations, as measured by equilibrium dialysis were occasionally elevated in cats with non-thyroidal illness, this test may not be as accurate in confirming a diagnosis of hyperthyroidism. This issue is addressed in Chapter 7.

CHAPTER 7
SERUM FREE THYROXINE CONCENTRATIONS IN HYPERTHYROID CATS AND ITS VALUE AS A DIAGNOSTIC TEST

7.1 LITERATURE REVIEW

Circulating thyroid hormone concentrations in hyperthyroid humans

Typically in thyrotoxicosis, serum total T4 and T3 concentrations are elevated (Larsen, 1972). In this study, serum total T4 concentrations averaged about 2.5 times the reference mean, whereas serum total T3 concentrations averaged 4.5 times the reference mean. Thus, there is a relative greater overproduction of T3 than of T4 in this disease. Since T3 is produced within the thyroid gland and peripherally from monodeiodination of T4, the increase could arise from either source. However, in hyperthyroidism, peripheral production of T3 contributes only 40 to 50 % to the T3 pool, compared to 85 to 90 % in euthyroid individuals (Abuid & Larsen, 1974). Thus, the ratio of T3 to T4 in thyroid secretion is increased in hyperthyroidism. There are two possible mechanisms by which this occurs (Larsen, 1986). Studies of thyroglobulin isolated from the thyroid glands of patients with Graves' disease show that there is an increase in the ratio of T3 to T4 that is independent of iodine content (Izumi & Larsen, 1977). Secondly, the activity of a T4 5'-deiodinase present in thyroid tissue, has been reported to be enhanced in hyperthyroidism (Ishii, Inada, Tanaka, Mashio, Naito, Nishikawa & Imura, 1981).

It has been estimated that from 3 to 6 % of all patients with untreated hyperthyroidism have T3 toxicosis (Medeiros-Neto, 1986). Serum T3 but not T4 concentrations are elevated in affected patients. This syndrome is more common in iodine deficient areas. Most untreated patients with this syndrome progress spontaneously to conventional hyperthyroidism with elevated circulating concentrations of both T3 and T4.

Rarely, patients with hyperthyroidism have elevated serum total T4 concentrations but reference range serum total T3 concentrations (Gavin, Rosenthal & Cavalieri, 1979). Such patients are usually suffering from severe concurrent non-thyroidal diseases and presumably peripheral production of T3 is inhibited. These individuals must be distinguished from sick euthyroid patients with isolated T4 hyperthyroxinaemia (Gavin, Rosenthal & Cavalieri, 1979; Borst, Eil & Burman, 1983).

Severe concurrent illnesses may also affect serum total T4 concentrations in hyperthyroid individuals. Lum, Kaptein & Nicoloff (1983) measured serum total T4 and T3 concentrations in four hyperthyroid patients with concurrent diabetic ketoacidosis, myocardial infarction, fulminant hepatitis or bacterial pneumonia. In all four patients, serum total T4 and T3 concentrations were either within or below the reference range. In two patients that survived serum total T4 and T3 concentrations increased into the thyrotoxic range in association with clinical improvement of the non-thyroidal illness. In

the two patients who died serum total T4 and T3 concentrations remained depressed or decreased during the terminal phase of the illness.

When serum total T4 concentrations are increased in hyperthyroidism, the concentration of free T4 is disproportionately increased (Woeber, 1986). This is related, in part, to the relative saturation of binding sites in plasma proteins by the excessive total concentration of T4 (Woeber, 1986). However, Inada & Sterling (1967) reported that subnormal concentrations of TBG, and possibly TBPA, may be additional factors. T4 regulation of TBG synthesis has been studied in a continuous cell culture line of Rhesus monkey hepatocarcinoma cells (Gershengorn, Glinoer, Fox & Robbins, 1976). At physiological or lower concentrations of T4, TBG production was enhanced. At higher T4 concentrations, TBG production was decreased. This suggests that thyroid hormones can regulate the synthesis of their major transport proteins. If such a situation exists in humans, measurement of circulating free T4 concentrations ought to be a more sensitive test for the diagnosis of hyperthyroidism. In addition, the degree of hyperthyroidism is related to circulating free and not total T4 concentrations.

Measurement of serum free T4 concentrations may also be of benefit in hyperthyroid patients with severe concurrent illnesses. Lum, Kaptein & Nicoloff (1983) measured serum free T4 concentrations, by equilibrium dialysis, in three hyperthyroid patients with severe concurrent non-thyroidal diseases and associated depression of serum total T4 concentrations. In two of these patients, the serum free T4 concentrations were diagnostically elevated.

Circulating total thyroid hormone concentrations in hyperthyroid cats

Elevated basal serum total thyroid hormone concentrations are the biochemical hallmark of feline hyperthyroidism. Compared to humans, serum total T3 concentrations are not as consistently elevated in thyrotoxic cats. In the first large series of 131 hyperthyroid cats reported (Peterson *et al.* 1983b), the mean serum total T3 concentration was approximately nine times the reference mean. The mean serum total T4 concentration was approximately seven times the reference mean. However, serum total T3 concentrations were within the reference range in four animals (3 %), while serum total T4 concentrations were above the reference range in all cases. In the largest series of 126 hyperthyroid cats studied in the United Kingdom (Thoday & Mooney, 1992), 11 of 122 (9 %) cats had serum total T3 concentrations within the reference range. In all 125 cases where serum total T4 concentrations were measured the values exceeded three sds from the reference mean. This value has been suggested by Turrell *et al.* (1984) as a basis for differentiating hyperthyroid animals from the small number of healthy individuals with serum total T4 concentrations above the reference range. In a later report by Broussard & Peterson (1993), the changes in clinical and laboratory findings of 202 hyperthyroid cats diagnosed between 1982 and 1992 were compared to the study of Peterson *et al.* (1983b). The serum total T3 concentrations were within the reference range in 29 % of the cases. In such cases serum total T4 concentrations were only

marginally elevated (Peterson *et al.* 1983b; Thoday & Mooney, 1992). These cats are usually mildly hyperthyroid but the effect of concurrent non-thyroidal illness on depressing serum total T3 concentrations may play a role in a small number of cases (Thoday & Mooney, 1992). Broussard & Peterson (1993) concluded that the higher prevalence of reference range serum total T3 concentrations in their study was related to earlier diagnosis or the inclusion of a population of mildly affected cats which previously would not have been tested. It is likely that serum total T3 concentrations would increase into the thyrotoxic range if the disorder were allowed to progress untreated (Peterson *et al.*, 1983b). Determination of serum total T4 is therefore of greater diagnostic value than determination of serum total T3 concentrations alone.

Serum total T4 concentrations are elevated in most hyperthyroid cats with a maximum concentration up to 19 times the upper limit of the reference range (Thoday & Mooney, 1992). Broussard & Peterson (1993) found that 2 % of their cats had reference range serum total T4 concentrations as compared to none in the earlier study of Peterson *et al.* (1983b). This suggests that serum total T4 concentrations may not be diagnostically elevated in cases of early or mild hyperthyroidism.

Peterson, Graves & Cavanagh (1987) measured serum total T4 concentrations hourly for 10 hours in 14 hyperthyroid cats and daily for 15 days in seven of these cases. The cats were selected because of the presence of a relatively mild hyperthyroid state. During the 10 hour sampling period, the individual c.v. for serum total T4 concentrations from the 14 cats (range, 6.4 - 22.6 %; mean $\pm$sd, 12.0 $\pm$4.8 %) was higher than the intra-assay c.v. of 5.1 %. Three of the cats had mean serum total T4 concentrations (47.62, 50.19 and 50.19 nmol/l) within the reference range (10.30 - 51.48 nmol/l). Of the 10 hourly determinations, five cats had from one to eight values within the reference range. When serum total T4 concentrations were averaged for each sampling time, there was no significant fluctuation in values over the 10 hour period. The times at which the lowest and highest values occurred were extremely variable among individual cats. During the 15 day sampling period, the individual c.v. for serum total T4 concentrations from the seven cats (range, 6.6 - 34.8 %; mean $\pm$sd, 18.4 $\pm$9.3 %) was higher than the intra-assay c.v.. Two of the cats had mean serum total T4 concentrations (48.91 and 50.19 nmol/l) within the reference range. Of the 15 daily determinations, three cats had six to 11 serum total T4 concentrations within the reference range. In two cats, the variations in serum total T4 concentrations were greater during the 15 day period than during the 10 hour sampling period. Serum total T3 concentrations were also measured and as expected there was a greater frequency of values within the reference range. It was concluded that thyroid hormone concentrations are subject to a degree of fluctuation in hyperthyroid cats. In cats with mild disease and only marginally elevated serum total T4 concentrations, fluctuations can occur to values below those diagnostic for hyperthyroidism. Multiple blood samples may therefore be required to confirm a diagnosis of hyperthyroidism. The authors concluded that samples should be collected on different days rather than during the same day. An increase in thyroid hormone

secretion could contribute to increases in circulating thyroid hormone concentrations. However, since the biological half life of T4 is measured in hours (Peterson & Becker, 1983; Broome, Hays & Turrel, 1987; Hays, Broome & Turrel, 1988), it is unlikely that a decrease in thyroid gland secretion could account for the acute decreases reported. The authors suggested that the short-term changes in circulating total T4 concentrations could result from fluctuations in plasma proteins, or from unspecified haemodynamic changes. These theories have yet to be investigated further. The degree of fluctuation is of little diagnostic significance in cats with markedly elevated serum total T4 concentrations (Peterson, Graves & Cavanagh, 1987; Broome, Feldman & Turrel, 1988).

The presence of concurrent non-thyroidal illness may also effect serum total T4 concentrations. In a series of 494 cats with a variety of non-thyroidal illnesses, 63 cats had a palpable thyroid nodule (Peterson & Gamble, 1990). In these cases the mean (±sd) serum total T4 concentration of 21.70 (± 10.4) nmol/l was significantly ($P < 0.001$) higher than in the 431 cats in which a thyroid nodule was not palpated (mean ±sd, 12.70 ±8.10 nmol/l). Of the unspecified number of cats with palpable thyroid nodules that survived, four developed elevated serum total T4 concentrations consistent with overt hyperthyroidism. Of the unspecified number that died or were euthanased, two were necropsied and both had adenomatous hyperplasia of the thyroid gland on histopathological examination. Thus, the authors concluded that at least some cats with palpable thyroid nodules had mild hyperthyroidism, but because of their concurrent illness, serum total T4 concentrations were depressed into the reference range. In addition, these results suggest that following treatment of, or recovery from, the concurrent illness, serum total T4 concentrations will increase into the diagnostic thyrotoxic range. Given that serum total T4 concentrations are depressed in cats with non-thyroidal illness, concurrent hyperthyroidism should be suspected in severely ill cats with serum total T4 concentrations within the medium to high end of the reference range. When cats are markedly hyperthyroid, the presence of severe concurrent non-thyroidal disease appears to have little effect on their serum total T4 concentrations (Thoday & Mooney, 1992).

McLoughlin, DiBartola, Birchard & Day (1993) evaluated the effect of concurrent non-thyroidal illness in a series of 110 hyperthyroid cats. In all cats, a diagnosis of hyperthyroidism was confirmed on the basis of a serum total T4 concentration greater than 51.48 nmol/l, or either a positive radionuclide image of the thyroid gland or histopathological evidence of adenomatous hyperplasia or carcinoma of thyroid tissue obtained by surgical excision or at necropsy. Each cat was also evaluated for systemic non-thyroidal illness during the six months prior to, at the time of, and during the six months following a diagnosis of hyperthyroidism. Diagnosis of non-thyroidal illness was established based on results of physical examination, laboratory screening tests (complete blood count, serum biochemical profile, urinalysis, FeLV and FIV status), thoracic and abdominal radiography, electrocardiography, echocardiography, abdominal ultrasonography, and biopsy and cytological examination or bacterial culture of

appropriate samples where indicated. Non-thyroidal diseases were considered significant if the patient required hospitalisation and specific treatment of that disease. Not all patients were clinically symptomatic for non-thyroidal disease.

Systemic non-thyroidal illness was diagnosed in 39 (35.5 %) of the cats evaluated. The cats were divided into four groups: Group A (n = 68) included all cats with a serum total T4 concentration greater than 51.48 nmol/l without concurrent disease, Group B (n = 28) included all cats with a serum total T4 concentration greater than 51.48 nmol/l but with concurrent disease, Group C (n = 3) included cats with repeated serum total T4 concentrations lower than 51.48 nmol/l without concurrent disease and Group D (n = 11) included all cats with serum total T4 concentrations lower than 51.48 nmol/l but with concurrent disease. Concurrent non-thyroidal illness was diagnosed in 29.1 % (28 of 96 cases) of the hyperthyroid cats with increased serum total T4 concentrations and in 78.5 % (11 of 14 cases) of the cats with reference range serum total T4 concentrations. The mean ±sd (range) serum total T4 concentration was 142.86 ±69.11 (55.34 - 463.32) in Group A, 124.32 ±66.92 (57.92 - 330.76) in Group B, 45.56 ±0.90 (41.06 - 50.58) in Group C and 37.32 ±9.14 (16.60 - 48.91) nmol/l in Group D. Serum total T4 concentrations were significantly decreased in the cats with concurrent illnesses (Groups B and D) compared to those in cats with no concurrent diseases (Groups A and C). Reference range serum total T4 concentrations were observed in 12.7 % (14 of 110 cases) of the hyperthyroid cats, representing 28.2 % (11 of 39 cases) of the hyperthyroid cats with concurrent diseases and 4.2 % (3 of 71 cases) of the hyperthyroid cats with no other diseases. Unfortunately, serum total T4 concentrations after treatment for non-thyroidal illnesses were not examined.

There is only one report concerning the possible effect of drug therapy on serum total T4 concentrations in hyperthyroid cats (Peterson & Ferguson, 1989b). Eight hyperthyroid cats were treated with an immunosuppressive dose of prednisone (10mg/cat) administered intramuscularly. The basal serum total T4 concentrations were elevated (mean ±sd, 76.8 ±44.4 nmol/l), but no significant decrease was detected 24 hours after the administration of prednisone (mean ±sd, 91.9 ±53.7 nmol/l). The major effect of glucocorticoids in the dog appears to be suppression of pituitary TSH (Ferguson & Peterson, 1992). If a similar situation exists in the cat, the results from the hyperthyroid cats are not surprising since TSH production and secretion are chronically suppressed by the elevated circulating thyroid hormone concentrations.

The TSH and TRH stimulation and T3 suppression tests in hyperthyroid cats

Several additional diagnostic tests have been recommended to confirm a diagnosis of hyperthyroidism when serum total T4 concentrations are not diagnostically elevated. These include the TSH and TRH stimulation and the T3 suppression tests (Graves & Peterson, 1990, 1992; Peterson, Randolph & Mooney, 1994).

Peterson *et al.* (1983b) reported the results of TSH stimulation in a group of 11 hyperthyroid cats. The mean serum total T4 concentration of 144.14 nmol/l after

administration of TSH was not significantly different from the mean basal concentration of 127.41 nmol/l. The authors suggested that either the hyperfunctioning glands were secreting thyroid hormones independently of TSH control or that the glands were already producing thyroid hormones at a maximal rate. Although all of the cats had diagnostically elevated basal serum total T4 concentrations, it was suggested that failure to respond to TSH may be a useful confirmatory test in equivocal cases. Feldman & Nelson (1987) reported an overlap in TSH stimulation test results between seven euthyroid and eight hyperthyroid cats although absolute values were not reported. In the largest series of 42 hyperthyroid cats tested (Mooney, 1990; Mooney, Thoday & Doxey, 1992b), there was a significantly lower serum total T4 response to exogenous TSH administration in hyperthyroid cats, confirming the results of Peterson *et al.* (1983b). However, in three cats with equivocal basal serum total T4 concentrations, the response to TSH was indistinguishable from healthy, euthyroid cats. Thus, a failure to respond to TSH can confirm a diagnosis of hyperthyroidism but a normal response cannot exclude it. In addition, it is in equivocal cases, where the test would be of greatest value that a normal response is likely to occur. In the two studies where serum total T3 concentrations were also determined, there was a greater overlap in test results between hyperthyroid and euthyroid cats because of the more variable response in this analyte in the euthyroid animals (Peterson *et al.*, 1983b; Mooney, Thoday & Doxey, 1992b). Additional drawbacks of this test include expense and difficulty in obtaining TSH.

Peterson (1991) reported the results of the TRH stimulation test in a group of 30 healthy, euthyroid and 21 hyperthyroid cats. Serum total T4 and T3 concentrations were determined before and four hours after intravenous administration of TRH (0.1 mg/kg). In the healthy cats, TRH caused a significant ($P < 0.001$) increase in the mean $\pm$sd serum total T4 concentration from 23 $\pm$9.5 nmol/l to 52 $\pm$17 nmol/l, with a percentage T4 increase over basal values of 131 % (range, 61 - 310 %). In the hyperthyroid cats, TRH also caused a significant ($P < 0.03$) increase in serum total T4 concentrations from a mean $\pm$sd of 65 $\pm$22 to 73 $\pm$26 nmol/l, but the mean percentage increase was significantly ($P < 0.001$) less than normal (14.7 %, range, -29 - 81 %). Only one hyperthyroid cat had a serum total T4 increase greater than 60 %, which was the lower limit in the healthy cats. Serum total T3 determinations were reported to be unhelpful because of the greater variability in the two groups, but absolute values were not recorded. These results and others were presented in greater detail by Peterson, Randolph & Mooney (1994) and included 31 healthy cats, 35 hyperthyroid cats and 15 cats with nonthyroidal illnesses. A consistent increase in serum total T4 concentrations, similar to healthy animals was noted in the cats with nonthyroidal illness after administration of TRH. The relative increase (percentage) in serum total T4 concentrations after administration of TRH, and the discriminative function score for total T4 (using an equation derived from canonical discriminant analysis) were the two most sensitive criteria for predicting whether cats were hyperthyroid. A rise in serum total T4 concentrations of less than 50 % was consistent with mild hyperthyroidism, whereas a value of greater than 60 % was only

seen in healthy cats or cats with non-thyroidal disease. Values between 50 and 60 % were considered borderline or equivocal. For the discriminative function score, calculated as 2.2 times the basal total T4 concentration minus the TRH-stimulated total T4 concentration, a D value of greater than 30 was consistent with hyperthyroidism, whereas a value of less than 20 was considered normal. As with the percentage increase in total T4, a value between 20 and 30 was considered ambiguous. The major disadvantage reported for the TRH stimulation test was the frequency of adverse reactions noted immediately after administration. These included salivation, vomiting, tachypnoea and defaecation (Peterson, 1991; Peterson, Randolph & Mooney, 1994).

The T3 suppression test relies on the ability of administered T3 through negative feedback, to decrease T4 production by the thyroid gland. In hyperthyroidism, since excess circulating thyroid hormone concentrations are already suppressing TSH production and secretion, additional T3 has little or no effect on T4 production. Since the serum half life of T4 in the cat is only six to eight hours (Broome, Hays & Turrel, 1987; Hays, Broome & Turrel, 1988), administration of T3 causes a relatively rapid fall in circulating total T4 concentrations. Peterson, Graves and Gamble (1990) evaluated the response of circulating total T4 concentrations to exogenous T3 (liothyronine) administration in 44 clinically healthy cats, 77 cats with hyperthyroidism and 22 cats with non-thyroidal disease. Serum total T4 and T3 concentrations were measured before and two to four hours after administering liothyronine at an oral dosage of 25 ug three times daily for seven doses. A diagnosis of hyperthyroidism was confirmed by the presence of clinical signs, the finding of goitre, high-normal or high serum total T4 concentrations and an adequate response to appropriate treatment. The mean basal serum total T4 and T3 concentrations were significantly higher in the hyperthyroid (53.1 and 1.4 nmol/l) than in the healthy (25.3 and 1.3 nmol/l) and the sick (29.5 and 1.4 nmol/l) cats. Of the 77 cats with hyperthyroidism, 41 (53 %) had basal total T4 concentrations within the reference range and 55 (71 %) also had basal total T3 concentrations within the reference range. After administration of liothyronine, there was a significant ($P < 0.001$) decrease in mean serum total T4 concentrations and a significant ($P < 0.001$) increase in mean serum total T3 concentrations in all three groups of animals. This suggests that in cases of mild hyperthyroidism pituitary TSH secretion has not been completely suppressed. However, the mean serum total T4 concentration fell more markedly in the healthy and sick cats compared to the hyperthyroid animals. The mean ($\pm$sd) post-liothyronine serum total T4 concentration in the hyperthyroid cats (48.5 ($\pm$20.8) nmol/l) was significantly ($P < 0.001$) greater than that of either the healthy (9.5 ($\pm$4.5) nmol/l) or sick (9.9 ($\pm$4.6) nmol/l) cats. No significant difference was noted between the latter two groups. There was no significant difference between mean ($\pm$sd) post-liothyronine serum total T3 concentrations in the hyperthyroid (2.9 ($\pm$0.8) nmol/l), healthy (2.6 ($\pm$1.1) nmol/l) and sick (2.6 ($\pm$1.0) nmol/l) cats. The mean ($\pm$sd) percentage decrease in serum total T4 concentrations after administration of liothyronine in the hyperthyroid cats (9.1 ($\pm$19) %) was significantly ($P < 0.001$) less than the decrease in either the healthy (63

(±15) %) cats or the cats with non-thyroidal diseases (67 (±16) %). Serum total T4 concentrations decreased by more than 35 % in all but one healthy cat and one cat with non-thyroidal disease. Only six of the 77 hyperthyroid cats (7.8 %) had more than a 35 % decrease in serum total T4 concentrations. In the remaining hyperthyroid cats, serum total T4 concentrations decreased marginally or were unchanged in 51, and were increased in 20 cats. All of the cats with hyperthyroidism had post-liothyronine serum total T4 concentrations in excess of 20 nmol/l whereas all of the healthy and sick cats had concentrations less than 20 nmol/l. The presence of hyperthyroidism was therefore confirmed if the post-liothyronine serum total T4 concentration was greater than 20 nmol/l with a percentage decrease of less than 50 %.

Refsal *et al.* (1991) also evaluated the T3 suppression test in ill cats with serum iodothyronine concentrations within the reference range. Serum total and free (by analogue assay) T4 and total T3 concentrations were measured before and two to 12 hours after administering liothyronine at an oral dosage of 15 ug three times daily for six or seven doses. All of the 49 cats tested had clinical signs of variable severity that were compatible with a diagnosis of hyperthyroidism. Twenty-six animals were subsequently confirmed as hyperthyroid either by histopathological confirmation (n = 14) after thyroidectomy or necropsy, or by clinical improvement in response to methimazole administration (n = 12). Three of these cats were subsequently deleted from further evaluation because at the time of the test basal serum total and free T4 concentrations had increased into the thyrotoxic range. Twenty-three cats were classified as non-hyperthyroid on the basis of histopathological confirmation of other diseases by biopsy or at necropsy (n = 12), abnormal diagnostic tests supportive of another illness (n = 8) or spontaneous recovery from the observed clinical signs without treatment (n = 3). The mean ±sd basal serum concentrations of total T4, total T3 and free T4 were 34.3 ±12.7 nmol/l, 0.85 ±0.33 nmol/l and 26.6 ±6.4 pmol/l in the hyperthyroid cats and 27.9 ±10.3 nmol/l, 0.69 ±0.24 nmol/l and 21.7 ±5.4 pmol/l in the non-hyperthyroid cats. The basal serum total T4 concentrations were significantly ($P < 0.05$) higher in the hyperthyroid cats, but there were were no significant differences in the other two variables between the two groups. The mean ±sd post-liothyronine serum concentrations of total T4, total T3 and free T4 were 31.3 ±11.5 nmol/l, 1.60 ±1.19 nmol/l and 25.6 ±6.9 pmol/l in the hyperthyroid cats and 11.7 ±6.4 nmol/l, 0.97 ±0.51 nmol/l and 10.4 ±4.4 pmol/l in the non-hyperthyroid cats. The mean serum total and free T4 concentrations were unchanged in hyperthyroid cats but decreased significantly ($P < 0.001$) in the non-hyperthyroid cats. The mean ±sd percentage decrease in total and free T4 values were 4 ±30 and 2 ±30 % in the hyperthyroid cats and were significantly ($P < 0.001$) lower than the values of 57 ±21 and 50 ±21 % in the non-hyperthyroid cats. However, there was considerable variation within each group. In the hyperthyroid cats, 33 % of post-liothyronine serum total or free T4 concentrations were within the reference range. An additional 50 % of these cats had a 0 to 25 % decrease in these analytes. In 91 % of the

non-hyperthyroid cats, serum total or free T4 concentrations decreased by at least 35 %, with a decrease of over 50 % in half of the cats.

For the stepwise discriminant analysis, the estimation of post-liothyronine serum free T4 concentrations was the single best diagnostic factor, accounting for 64 % of the variation ($P < 0.001$). A two factor model was also identified which included the post-liothyronine serum free T4 concentration ($P < 0.001$) and the percentage decrease in serum total T4 concentrations ($P < 0.05$). This predictive value was calculated as 1.95 plus (0.00378 times % decrease in total T4) minus (0.031 times post-liothyronine serum free T4 concentration). This two factor model accounted for 67.4 % of the experimental variation. The range of values was 0.443 to 1.582 in hyperthyroid cats and 1.417 to 2.220 in cats with other diseases. In the absence of free T4 data, the single variable that best differentiated the two groups was the post-liothyronine serum total T4 concentration, accounting for 53.6 % of the experimental variation ($P < 0.001$). Addition of the percentage decrease in serum total T4 concentrations was of added benefit, accounting for 65.2 % of the experimental variation. Using these two variables, a predictive value was calculated as 1.693 plus (0.0056 times % decrease in total T4) minus (0.017 times post-liothyronine serum total T4 concentration). These values ranged from 0.665 to 1.796 in hyperthyroid cats and 1.382 to 2.185 in cats with other diseases. Three hyperthyroid cats were identified as having false-negative results in all four models. Two non-hyperthyroid cats were classified as having false positive results in all four models. The respective test sensitivity for all four models were comparable at 0.739, 0.826. 0.783 and 0.783. Test specificity was 0.826, 0.913, 0.652 and 0.565. Thus, models incorporating serum free T4 concentrations were better at ruling out hyperthyroidism. The authors pointed out that each laboratory would have to establish its own predictive values (Refsal *et al.*, 1991).

In response to the findings of Refsal *et al.* (1991), Peterson, Randolph & Mooney (1994) evaluated the use of discriminant analysis in the mildly hyperthyroid cats studied by Peterson, Graves and Gamble (1990). This produced a two factor model that included the basal and post-liothyronine serum total T4 concentrations. The equation was calculated as the post-liothyronine serum total T4 concentration minus 0.5 times serum basal total T4 concentration. All cats having a score greater than 8 were hyperthyroid, whereas all cats with a score less than 2 were euthyroid. However, eight hyperthyroid and five non-hyperthyroid cats had scores between 2 and 8. Thus, the post-liothyronine serum total T4 concentration remained the most sensitive means of differentiating the two groups.

Although the T3 suppression test appears to be of more value than either the TSH or TRH stimulation tests, it is expensive to perform. It is a relatively prolonged test (three days), owners are required to give multiple doses of liothyronine and the cats must swallow the tablets. To confirm both owner and cat compliance in administering and taking the drug, serum total T3 concentrations must be measured before and after the test (Peterson, Graves & Gamble, 1990; Peterson, Randolph & Mooney, 1994). In

addition, absorption of liothyronine may be inadequate in cats with gastrointestinal disease (Refsal *et al.*, 1991).

Circulating free thyroid hormone concentrations in hyperthyroid cats

There are relatively few reports on circulating serum free thyroxine concentrations in hyperthyroid cats. Hays, Turrel & Broome (1988) used the equilibrium dialysis technique of Sterling & Brenner (1966) to measure serum free T4 concentrations in six hyperthyroid cats. Absolute serum free T4 values were not reported. However, the mean ±sd free T4 fraction was 0.056 ±0.006 % in the hyperthyroid compared to 0.057 ±0.009 % in five healthy cats. The corresponding values for T3 were 0.46 ±0.09 % and 0.47 ±0.08 %. The authors concluded that in contrast to the situation in humans, the unbound percentage of total T4 and T3 is unchanged in hyperthyroidism. Thus, the degree of hyperthyroidism could be inferred from measurement of circulating total thyroid hormone concentrations alone.

Ferguson, Peterson & Nachreiner (1989) measured serum total and free iodothyronine concentrations in a series of 25 hyperthyroid cats. Serum free thyroxine concentrations were measured by an unspecified equilibrium dialysis technique and a direct RIA. This RIA was identified as an analogue kit method (Magic free T4, Ciba Corning Diagnostics) by Refsal *et al.* (1991). Serum free T3 concentrations were measured by an unspecified direct RIA kit method. Compared to healthy cats (n = 28), cats with hyperthyroidism had significantly higher ($P < 0.001$) mean ±sd serum free T4 concentrations by RIA (80.6 ±40.0 versus 23.9 ±5.8 pmol/l), serum free T4 concentrations by equilibrium dialysis (105.8 ±58.4 versus 20.4 ±11.1 pmol/l) and serum free T3 concentrations by RIA (8.8 ±3.8 versus 1.6 ±7.7 pmol/l). The mean ±sd serum total T4 and T3 concentrations in the hyperthyroid cats were 100.3 ±49.3 and 3.1 ±2.3 nmol/l, respectively. All of the hyperthyroid cats had serum concentrations of total T4 and T3 and free T4 and T3 concentrations by RIA above their respective reference ranges. One cat (4 %) had a serum free T4 concentration by equilibrium dialysis within the reference range. In six hyperthyroid cats undergoing treatment with methimazole, mean ±sd serum concentrations of total T4 (27.5 ±17.4) and free T4 by RIA (29.8 ±11.4 pmol/l) were within their respective reference ranges. In these cats, serum free T4 concentrations by equilibrium dialysis were significantly higher (mean ±sd, 43.7 ±17.1 pmol/l) than in healthy controls. An explanation for this was not offered. In all cats there was a significant correlation between total T4 and free T4 concentrations determined using either RIA ($r = 0.99$) or equilibrium dialysis ($r = 0.93$), as well as between free T4 concentrations determined using the two techniques ($r = 0.97$). Compared to equilibrium dialysis, the RIA technique tended to overestimate free T4 concentrations in the low-normal range and underestimate values in the higher range. The authors concluded that serum free T4 concentrations are elevated in hyperthyroidism. However, since the elevations in free T4 concentrations were directly proportional to those in total T4,

measurement of free T4 concentrations provided no additional diagnostic information over measurement of total T4 concentrations alone.

Refsal *et al.*, (1991) also measured serum free T4 concentrations in hyperthyroid cats using an analogue kit method (Magic free T4, Ciba Corning Diagnostics). However, these cats were specifically chosen because both total and free T4 concentrations were within the reference range. Serum free T4 concentrations in hyperthyroid cats as measured by a direct equilibrium dialysis kit method (Nichols Institute Diagnostics) were mentioned but individual values were not reported (Chapters 4 and 6).

7.1 INTRODUCTION

In the small number of hyperthyroid cats studied to date, serum free T4 concentrations were elevated (Ferguson, Peterson & Nachreiner, 1989). However, to date there are no reports on the comparison of free T4 concentrations in hyperthyroid cats and cats with non-thyroidal illnesses. Since serum free T4 concentrations as measured by equilibrium dialysis, were found to be occasionally elevated in sick cats (Chapter 6), its sole use as a diagnostic test may lead to an overdiagnosis of hyperthyroidism. In addition, Hays, Turrel & Broome (1988) suggested that the degree of hyperthyroidism could be inferred directly from serum total T4 measurements since, in comparison to the situation in humans, the free T4 fraction is unaltered in hyperthyroidism. However, only six hyperthyroid cats were studied by these authors and no data were supplied on the validation of the equilibrium dialysis technique used.

The present study was designed to more clearly evaluate serum free T4 concentrations in hyperthyroid cats and to assess its specificity and sensitivity as a diagnostic test. It was also important to fully assess whether serum free and total T4 concentrations reflected the degree of hyperthyroidism, since this was an important factor used in determining the dose of radioactive iodine subsequently given for treatment (Chapter 8).

7.3 MATERIALS AND METHODS

Clinical material

The case material comprised a series of 95 hyperthyroid cats referred to Glasgow University Small Animal Hospital. Two of the cats had had a bilateral thyroidectomy eight and 36 months previously, respectively. Three cats had had a unilateral thyroidectomy 12, 13 and 18 months previously, respectively. One cat had had two surgical thyroidectomies, the last eight months previously. All of the cats exhibited historical and clinical features consistent with a diagnosis of thyrotoxicosis.

Of these cats, 57 were female (55 ovariohysterectomised and two entire) and 38 were male (all castrated). One cat was of unknown age, but had been in the owner's possession for 10 years. The remaining cats ranged in age from 9.0 to 19.0 years with a mean $\pm$sd of 13.2 $\pm$2.2 years. Ninety-three of the cats were either domestic short (n = 84) or long (n = 9) haired. The remaining two cats were Persians.

Most of these cats form part of the survey detailed in Chapter 3. Fifty cats were subsequently treated with radioactive iodine as described in Chapter 8.

The reference ranges for serum total and free T4 concentrations are detailed in Chapter 4.

The cats suffering non-thyroidal illnesses described in Chapters 5 and 6 were used for comparison purposes.

Estimation of serum total and free thyroxine concentrations

At the time of initial presentation, providing treatment had not commenced, a serum sample was obtained from each cat. Serum total, and if the sample volume allowed, free T4 concentrations were measured and the free T4 fraction calculated as described in Chapter 4.

Statistical analyses

The Mann Whitney U test was used for statistical analyses. Pearson's correlation was used for comparing serum total and corresponding free T4 concentrations. The sensitivity, specificity and efficiency of the serum total and free T4 estimations were calculated as described by Fraser (1986). Sensitivity was calculated as the percentage of true positive results in the hyperthyroid and sick cats. Specificity was calculated as the percentage of true negative results in the hyperthyroid and sick cats. Efficiency was subsequently calculated as the percentage of all results that were true results.

7.4 RESULTS

Serum free thyroxine concentrations in hyperthyroid cats.

Serum total and free T4 concentrations were both measured in 26 of the cats. The data are detailed in Appendix 12 and summarised in Table 10.

	Total T4 (nmol/l)	Free T4 (pmol/l)	% free T4
n	26	26	26
Mean	204.15	233.32	0.11
sd	134.83	177.08	0.04
Minimum	43.83	46.88	0.07
1st quartile	97.64	113.07	0.09
Median	170.06	191.30	0.11
3rd quartile	285.64	259.71	0.12
Maximum	575.57	687.61	0.25

Table 10. Summary statistics of serum total and free thyroxine (T4) concentrations and calculated free T4 fractions in hyperthyroid cats.

None of the cats were suffering any other illness apart from hyperthyroidism. The lowest serum total T4 concentration of 43.83 nmol/l corresponded to the lowest free T4 concentration of 46.88 pmol/l (case number 117880). Both values were above their respective reference ranges (10.75 - 41.25 nmol/l; 8.14 - 41.45 pmol/l) but less than three sds from the reference mean (48.87 nmol/l and 49.78 pmol/l). All of the remaining concentrations were above these two values. The highest serum total T4 concentration of 575.57 nmol/l corresponded to a free T4 concentration of 635.01 pmol/l (case number 119160). The highest serum free T4 concentration of 687.61 pmol/l corresponded to a total T4 concentration of 272.50 nmol/l (case number 117850). This cat consequently had the highest free T4 fraction of 0.25 %. The next highest value was 0.14 %.

The serum free T4 concentrations were significantly ($P < 0.001$) higher in the hyperthyroid compared to the healthy cats. There was no significant ($P > 0.05$) difference in the calculated free T4 fraction between these two groups. There was a highly significant correlation ($r = 0.92$, $P < 0.001$) between the serum total and free T4 concentrations in healthy and hyperthyroid cats taken as one group (Figure 10).

Comparison of total and free thyroxine values for the diagnosis of hyperthyroidism

The serum total T4 concentrations of the 95 hyperthyroid cats ranged from 39.69 to 575.57 nmol/l with a mean $\pm$sd of 164.02 $\pm$102.10 nmol/l. These values were significantly ($P < 0.001$) higher than in the 50 healthy cats. Two cats (case numbers 113704 and 117880) had serum total T4 concentrations above the reference range but less than three sds from the reference mean (48.87 nmol/l). Both cats were considered to be mildly hyperthyroid. One cat (case number 121116) had a serum total T4 concentration of 39.69 nmol/l, within the reference range. This cat was severely ill, suffering from histiocytic lymphoma with associated necrosis and abscessation of the retropharyngeal lymph nodes. The cat was euthanased on humane grounds before treatment of the hyperthyroidism commenced.

In the sick cats with non-thyroidal illnesses (Chapter 5), serum total T4 concentrations were below 48.87 nmol/l in all cases. From Table 11 the sensitivity of serum total T4 estimations for diagnosing hyperthyroidism was 96.9 %. The specificity of this test was 100 % and the efficiency 98.5 %.

In the sick cats (Chapter 6), serum free T4 concentrations were greater than 3 sds above the reference mean in six cases. From Table 12 the sensitivity of serum free T4 estimations for diagnosing hyperthyroidism was 96.2 %. The specificity of this test was 93.9 % and the efficiency 94.4 %.

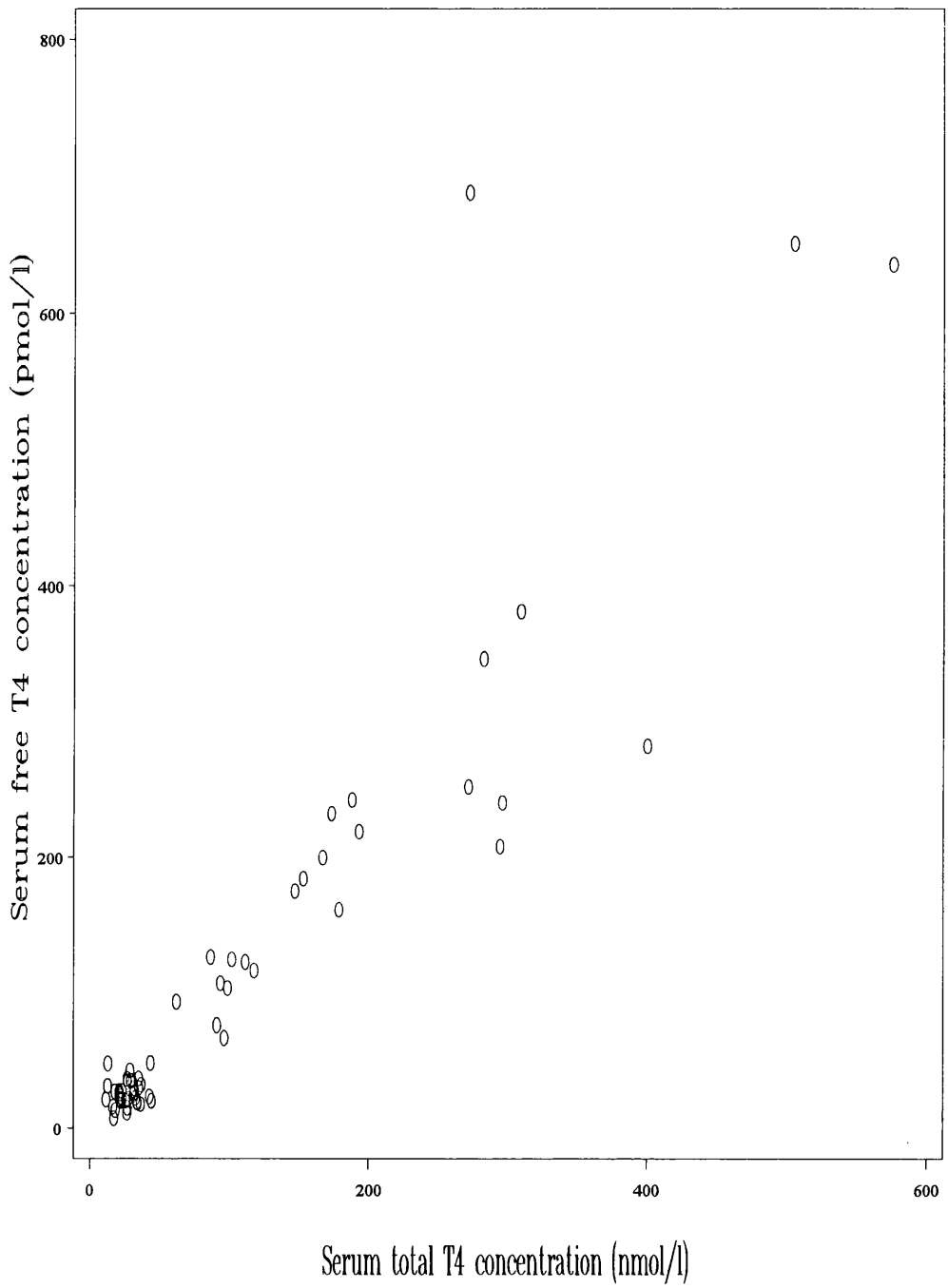


Figure 10. Serum total and free thyroxine (T4) concentrations in 38 healthy euthyroid and 26 hyperthyroid cats.

Disease	Test results		
	Positive	Negative	Totals
Hyperthyroid	92	3	95
Not hyperthyroid	0	107	107
Totals	92	110	202

Table 11. The number of true and false positive and negative results for a diagnosis of hyperthyroidism as assessed by a serum total thyroxine concentration greater than three standard deviations above the reference mean.

Disease	Test results		
	Positive	Negative	Totals
Hyperthyroid	25	1	26
Not hyperthyroid	6	92	98
Totals	31	93	124

Table 12. The number of true and false positive and negative results for a diagnosis of hyperthyroidism as assessed by a serum free thyroxine concentration greater than three standard deviations above the reference mean.

7.5 DISCUSSION

This study confirms previous reports that serum total T4 concentrations are usually elevated in hyperthyroidism (Peterson *et al.* 1983b; Thoday & Mooney, 1992). The mean serum total T4 concentration averaged six times the reference mean comparable to the factor of seven reported by Peterson *et al.* (1983b). This is higher than the factor of 2.5 reported in humans (Larsen, 1972), and presumably reflects the differences in thyroid production rates, serum half lives and protein binding between the two species. These differences require further investigation.

Many of the cats with non-thyroidal illnesses exhibited clinical signs which have been reported in hyperthyroid cats (Peterson *et al.*, 1983b; Thoday & Mooney, 1992). Thus, measurement of serum total T4 concentrations was important in order to eliminate a diagnosis of hyperthyroidism. Using the thyrotoxic limit of a serum total T4 concentration exceeding three sds from the reference mean suggested by Turrel *et al.* (1984), all of these cats were correctly classified as non-hyperthyroid. A small number (3 %) of hyperthyroid cats had serum total T4 concentrations below the thyrotoxic range. As in previous reports, these cats were either suffering mild or early hyperthyroidism or in addition to the hyperthyroidism had severe concurrent non-thyroidal diseases (Peterson *et al.*, 1983b; Peterson, Graves & Cavanagh, 1987; Peterson & Gamble, 1990; Thoday & Mooney, 1992; Broussard & Peterson, 1993; McLoughlin *et al.*, 1993). Thus measurement of serum total T4 concentrations is an extremely specific test with good sensitivity.

In this study, measurement of serum free T4 concentrations did not provide any additional diagnostic information. There was an excellent correlation between serum total and corresponding free T4 concentrations as previously reported (Ferguson, Peterson & Nachreiner, 1989). Thus in one cat with mild hyperthyroidism, both serum total and free T4 concentrations were below the thyrotoxic limit. In only one case was the serum free T4 concentration more elevated than the total T4, but the latter was well within the thyrotoxic range. There was no apparent explanation for this aberrant result.

There was no significant difference between the calculated free T4 fraction in the hyperthyroid and healthy cats. This confirms the preliminary report of Hays, Turrel & Broome (1988). Thus, in hyperthyroid cats it is justifiable to infer the degree of hyperthyroidism from measurement of serum total T4 concentrations alone. Measurement of circulating total T4 is therefore adequate for use in the assessment of a therapeutic dose of radioactive iodine. This contrasts to the situation in humans, where serum free T4 concentrations are more consistently elevated in hyperthyroidism (Woeber, 1986) and again presumably relates to the differences in binding proteins between the two species.

Although measurement of circulating free T4 concentrations proved to be as sensitive a test for the diagnosis of hyperthyroidism in cats, it was less specific. This was related to the finding of elevated serum free T4 concentrations in cats with non-thyroidal

illnesses. Thus as a sole diagnostic test its measurement by equilibrium dialysis would lead to an over diagnosis of hyperthyroidism. It has been suggested that the measurement of serum free T4 concentrations would be of benefit in hyperthyroid cats with concurrent illnesses and serum total T4 concentrations within the reference range (Peterson, Randolph & Mooney, 1994). Only one cat presented here had a serum total T4 concentration within the reference range as a result of a concurrent disease. A serum free T4 concentration was not measured. However, in cats with non-thyroidal illnesses when serum free T4 concentrations were elevated, serum total T4 concentrations tended to be within the reference range. Therefore it is unlikely that estimation of free T4 concentrations in sick hyperthyroid cats would be of benefit.

Estimations of serum total T4 concentrations are less expensive and easier to perform, and more frequently available in veterinary laboratories than free T4 estimations by equilibrium dialysis. This study established that measurement of serum total T4 concentrations is as sensitive a test but more specific than measurement of free T4 concentrations. If serum total T4 concentrations are not diagnostically elevated, but hyperthyroidism is suspected additional diagnostic tests should be considered. To date, the T3 suppression test appears to be of most use (Peterson, Graves and Gamble, 1990; Refsal *et al.*, 1991). Alternatively, in mild cases of hyperthyroidism, serum total T4 concentrations could be measured after a period of several weeks when they should have increased into the thyrotoxic range. Cats with concurrent non-thyroidal illness could be retested on recovery from that illness.

CHAPTER 8
RADIOACTIVE IODINE THERAPY OF FELINE HYPERTHYROIDISM: A SIMPLE METHOD OF DOSE ESTIMATION AND COMPARISON OF INTRAVENOUS AND SUBCUTANEOUS ADMINISTRATION

8.1 LITERATURE REVIEW

Treatment options for hyperthyroidism

The introduction of radioactive iodine, (^{130}Iodine (^{130}I) in 1941 and ^{131}Iodine (^{131}I) in 1946) for the treatment of hyperthyroidism in man proved an historic landmark in medical therapeutics and ushered in the field of nuclear medicine (Hamilton & Lawrence, 1942; Hertz & Roberts, 1942; Hertz & Roberts, 1946). The eight day half life of ^{131}I, compared to 12 hours for ^{130}I, proved superior, as treatment became more predictable. ^{131}I remains the most commonly used isotope in therapeutics today (Solomon, 1986). Since the first confirmed description of feline hyperthyroidism (Cotter, 1979; Peterson, Johnson & Andrews, 1979) and given its similarity to human toxic nodular goitre (Peterson, Becker, Hurley & Ferguson, 1981), administration of radioactive iodine (as ^{131}I) has been recommended as a safe and effective treatment for affected cats (Peterson, Becker, Cahill & Ferguson, 1983a).

Unlike Graves' disease in man, spontaneous remission of feline hyperthyroidism does not occur and the aim of therapy is to control the excessive secretion of the thyroid hormones from affected thyroid tissue by radioablation, surgical thyroidectomy or chronic antithyroid drug administration. The disadvantages of surgical thyroidectomy have been reviewed previously (Mooney, 1990) and include, the risk of anaesthesia, and potential complications such as haemorrhage, laryngeal oedema, laryngeal paralysis, hypoparathyroidism, Horner's syndrome, voice change, persistent hyperthyroidism and recurrence due to regrowth of tissue left *in situ* at the time of surgery. Chronic antithyroid drug therapy (using propylthiouracil, methimazole or carbimazole) requires daily pill administration. In addition, propylthiouracil has been associated with serious haematological abnormalities and is now considered too toxic for use in cats (Peterson, Hurvitz, Leib, Cavanagh & Dutton, 1984b). Methimazole appears to be safer than propylthiouracil with fewer and less serious adverse effects (Peterson, Kintzer & Hurvitz, 1988). It is not available in this country. The only alternative is carbimazole and although serious adverse reactions have not been associated with its use, mild side effects of vomiting and depression can persist requiring withdrawal of the drug (Mooney, Thoday & Doxey, 1992a).

Mode of action of radioactive iodine

Radioactive iodine, like stable iodine, is actively concentrated by the thyroid gland. It emits both beta particles and gamma radiation. The beta particles, which cause over 80

% of the tissue damage, travel a maximum of 2 mm in tissue and have an average pathlength of 400 um. It is therefore locally destructive and spares adjacent atrophic thyroid tissue, parathyroid glands and other cervical structures. Radioiodine produces damage by two mechanism, acute radiation thyroiditis and chronic gradual thyroid atrophy (Solomon, 1986). Thyroid follicles vary in their uptake of radioactive iodine. Thus, some cells will be subjected to high doses and intense radiation and die immediately. This acute radiation response generally occurs from three to 10 days following administration of radioactive iodine. Later, a progressive atrophy is noted with an obliterative endarteritis and interstitial fibrosis. The progressive decline in function of surviving cells may be a result of the development of genetic abnormalities that lead to a shorter survival time and impaired replication.

Estimation of a therapeutic dose and efficacy of radioactive iodine administration

The aim of radioactive iodine therapy is to restore euthyroidism with the smallest possible single dose of radiation, whilst avoiding the development of hypothyroidism or the persistence of hyperthyroidism. Considerable controversy surrounds the optimal method of dose selection in man. Each method, with or without some modification, has been attempted in the cat and this review will concentrate primarily on this species.

Tracer kinetic studies

Theoretically the most valid method of estimation of the optimum dose of ^{131}I, and the most complicated, is to calculate a therapeutic dose aiming to deliver approximately 15,000 to 20,000 rad (as with external radiation) per g of thyroid tissue. This method involves the administration of a small tracer dose of ^{131}I (3.7 to 11.1 Mega Becquerel (MBq)) to determine various parameters of thyroid kinetics including the effective half life (which accounts for both the physical half life and duration of radioiodine retention by the thyroid gland) and the peak thyroidal uptake. These parameters, together with an estimated weight of thyroid tissue determine the required therapeutic dose. This method obviously requires two separate isotope injections (the tracer and therapeutic doses) and although manual techniques of data retrieval and calculation are applicable (Broome, Turrel & Hays, 1988) they are time consuming, so that sophisticated computerised nuclear medicine equipment is preferred.

Peterson *et al.* (1983a) were the first workers to evaluate this method in a series of 11 hyperthyroid cats. They attempted to deliver 15,000 rad to thyroid tissue using the above method of dose formulation. The administered therapeutic dose ranged from 37 to 185 MBq with a mean $\pm$sem of 99.9 $\pm$11.1 MBq. The serum total T4 concentration decreased to within the reference range in one to nine days with a mean $\pm$sem of 4.1 $\pm$0.7 days. Signs consistent with hypothyroidism developed in one cat together with a subreference range serum total T4 concentration, necessitating T4 supplementation. Relapse of hyperthyroidism occurred in one cat 18 months after therapy, but was successfully retreated with a second injection. Nine cats remained euthyroid for one to

26 months. The mean ±sem survival time in the 11 cats was 13.5 ±2.9 months, and of these five were still alive five to 25 months after treatment.

Turrel *et al.* (1984) attempted to assess the efficacy of treatment using the above method of dose estimation compared to cats given a dose based on an "educated guess" and to identify some of the factors that affected eventual outcome. Eleven cats were treated with ^{131}I. Previous unsuccessful treatments included unilateral thyroidectomy (two cats) and medical management with propylthiouracil (seven cats) for one to 14 months, while two cats had had no prior treatment. Tracer studies were performed in seven cats. Thyroid weight was estimated by digital palpation and the appearance of a sodium pertechnetate ($^{99m}TcO_4$) scan. The calculated therapeutic dose of ^{131}I aimed to deliver 20,000 rad to the thyroid gland and ranged from 37 to 218.3 MBq. Seven cats became clinically and serologically euthyroid after therapy, although one of these required a second injection at 6 months. Clinical improvement was noted within 1 month of therapy and clinical signs were absent within 3 months, while serum total T4 concentrations were usually within the reference range within 1 month of therapy. $^{99m}TcO_4$ scans performed within 2 to 10 months of therapy depicted decreased activity and size of the thyroid gland. Two cats had a partial response to therapy with serum total T4 concentrations decreasing to less than half of their pretreatment concentrations. These cats were not retreated with ^{131}I. Hypothyroidism developed in the remaining two cases within one and 11 months of therapy, respectively. One of these cats died without being treated while in the other, the lethargy, obesity and subreference range serum total T4 concentration were reversed with thyroid hormone supplementation. Treatment with a single dose of ^{131}I was successful in those cats that had had no prior antithyroid medication, had undergone unilateral thyroidectomy or where propylthiouracil treatment had been withdrawn at least one month prior to radiation therapy. Of four cats that had received propylthiouracil within one month of ^{131}I therapy, two remained hyperthyroid despite multiple treatments, the third required a second injection and the fourth became acutely hypothyroid. It was suggested that, as in humans, antithyroid drug therapy may increase the radioresistance of the thyroid tissue. Retrospective calculations based on the therapeutic dose showed that the radiation dose delivered to the thyroid gland actually ranged from 7,100 to 64,900 rad. The variation between the prescribed and actual therapeutic doses resulted in part because of the four cats which did not have tracer scans. This resulted in marked underdosing of two and overdosing of two animals. Of those having tracer scans, peak thyroidal uptake and/or the biological half life of the therapeutic dose differed significantly from the tracer kinetic studies. In addition, the thyroid gland weight was difficult to estimate and thus introduced a source of error in the calculation. Markedly elevated pretreatment serum total T4 concentrations also adversely affected ultimate outcome, particularly if associated with large thyroid lobes. Thyroid tissue was subsequently available for histopathological examination in two treated cats at necropsy and areas of necrosis were observed. The authors concluded that quantitation of thyroid activity to determine

radiation dose, using sophisticated computerised nuclear medicine equipment, may not improve the proportion of cats that respond to treatment, when compared with dosages empirically selected on the basis of pretreatment factors.

Despite these reservations, calculation of the therapeutic dose of ^{131}I using the tracer kinetic formula have been used in the two largest studies on the efficacy of radioactive iodine therapy in hyperthyroid cats. Peterson & Turrel (1986) aiming to deliver 15,000 to 20,000 rad/g of thyroid tissue, treated more than 300 cats using a dose ranging from 37 to 370 MBq ^{131}I. Over 90 % of the cats became euthyroid within 3 months after a single dose, while the remaining cats were successfully cured with a second injection, and very few cats developed hypothyroidism. Feldman & Nelson (1987) aimed to deliver 20,000 rad to the thyroid gland and this resulted in a success rate of over 90 % of 350 cats with 10 % remaining hyperthyroid.

The time to return of euthyroidism in 31 of the cats described by Feldman & Nelson (1987) was reported in detail by Meric, Hawkins, Washabau, Turrel & Feldman (1986). The therapeutic dose, based on ^{131}I tracer kinetic studies, ranged from 55.5 to 226.81 MBq. Serum total T4 concentrations decreased rapidly in all cats during the first two weeks of therapy, with the most rapid decrease being during the first three to six days. Of the 31 cats, 16 (55 %) had serum total T4 concentrations within the reference range by day four, while 23 (74 %) had reference range values by day eight. Two weeks after treatment, one cat died from undetermined causes, while one was not available for reevaluation. One month after treatment, 24 (83 %) of the remaining 29 cats had serum total T4 concentrations within the reference range. The serum total T4 concentrations of three cats remained elevated, albeit decreased from pretreatment values, and they had gained weight, had normal appetites and had resumed normal activity. Further evaluation of these cats was not reported. Two cats had subreference range serum total T4 concentrations but did not have clinical signs of hypothyroidism.

Broome, Turrel & Hays (1988) subsequently evaluated the predictive value of tracer studies for ^{131}I in hyperthyroid cats. Peak thyroidal ^{131}I uptakes and effective half lives were determined after administration of tracer and therapeutic doses of ^{131}I in 76 cats. In six additional animals, only peak thyroidal uptakes after administration of tracer and therapeutic doses were determined. A good correlation was found between peak thyroidal uptakes of tracer and therapeutic doses, but only a fair correlation between effective half lives. In 79 % of the cats, the effective half life for the therapeutic dose was longer than that for the tracer dose. These data suggested that the effective half life determined from a tracer dose was of limited value in calculating a therapeutic dose of ^{131}I. However, the estimation of thyroid gland weight proved to be the largest potential source of error in the calculation since it was recognised that there is no reproducible method for accurately determining thyroid gland weight in cats. Despite this, the estimation of a tracer compensated therapeutic dose came significantly closer to the therapeutic goal than the hypothetical administration of a uniform dose of 111 MBq although this latter was a relatively low dose.

Fixed low dose

A second method of dose determination is to select a relatively low dose of ^{131}I without determining thyroid gland kinetics. Peterson & Turrel (1986) reported that administration of 74 to 148 MBq of ^{131}I would produce euthyroidism in the majority of cats but would also result in the under or overtreatment of a significant number of cases. The obvious advantages of this method are that the need for sophisticated nuclear medicine equipment is obviated, two isotope injections are not required and the time required to assess thyroid kinetics using a tracer dose is avoided. Several workers have reported on the success or otherwise of this method of therapy.

Meric, Rubin & Shaw (1987) treated 25 hyperthyroid cats with a fixed dose of 148 MBq ^{131}I. Further details were not reported. Subsequently, Meric & Rubin (1990) reviewed the medical records of 62 hyperthyroid cats treated with a fixed dose of 148 MBq ^{131}I. Fifty cats (81 %) had not been treated for hyperthyroidism prior to presentation. One cat had undergone a unilateral thyroidectomy, and another cat had undergone a bilateral thyroidectomy at least 2 years previously. Nine cats had been treated orally with either propylthiouracil or methimazole. In these cats, treatment was discontinued at least 3 weeks before therapy. The history of the remaining cat was not reported.

Two cats died within 3 weeks of therapy (one from a cerebral cortical haemorrhage and ischaemia and the other of unidentified causes). The remaining 60 cats were reevaluated within a mean of 204 days (range; 30 - 850 days) Fifty cats (81 %) were biochemically euthyroid at this stage. Five cats (8 %) remained persistently hyperthyroid, although the serum total T4 concentrations had decreased from pretreatment values. Two of these animals had been given antithyroid drugs. Three of the cats had pretreatment serum total T4 concentrations > 263.84 nmol/l. However, six other cats with comparable values were successfully treated with a single dose of ^{131}I. Three of the five persistently hyperthyroid cats were subsequently retreated with 148 MBq ^{131}I. Two responded completely, while the remaining animal died from undetermined causes after a third dose of 370 MBq. The owners of the remaining two cats declined further treatment.

Serum total T4 concentrations in five cats (8 %) were below the reference range when evaluated within 60 days of treatment, although none had clinical signs of hypothyroidism. Three of these cats were reevaluated six months after therapy, when the serum total T4 concentrations had increased into the reference range. The remaining two cats were not reevaluated.

Zuber & Allan (1990) also used a fixed dose of 148 MBq ^{131}I in a series of 21 hyperthyroid cats. At the time of publication, 18 cats were alive and 16 of these were euthyroid while two had not yet been evaluated. Three cats were euthanased for reasons unrelated to hyperthyroidism or its treatment. Further details were not reported.

Fixed high dose

A third method of dose determination is to select a fixed high dose of ^{131}I, ranging from 370 to 1110 MBq (Peterson & Turrel, 1986). However, such doses will destroy both hyperplastic and atrophic thyroid tissue and although effective in treating hyperthyroidism would undoubtedly induce hypothyroidism in most cases. Such large doses are therefore only recommended for treatment of cats with hyperfunctioning thyroid carcinomas (Peterson & Turrel, 1986). Turrel, Feldman, Nelson & Cain (1988) treated 12 hyperthyroid cats with thyroid carcinoma. Four cats were known to have thyroid carcinoma at the time of treatment, and were given doses of between 740 and 1110 MBq, resulting in thyroid ablation in three of the cats. Eight cats were treated with relatively low doses of ^{131}I (37 to 259 MBq), determined on the basis of tracer kinetic studies. Three of these cats required further treatment with ^{131}I or surgical treatment before euthyroidism was achieved. The treatment was unsuccessful in the remaining five cats which eventually died from complications of the disease process itself.

Miscellaneous methods of dose estimation

Several miscellaneous methods of dose determination have been recommended, some of which would be difficult to reproduce. Chambers, Hightower & Tveter (1987) treated 43 cats with a dose of ^{131}I ranging from 103.6 to 329.3 MBq. Each individual dose was estimated on the basis of "previous experience and the appearance of the $^{99m}TcO_4$ thyroid scan". All of the cats showed clinical improvement after treatment as demonstrated by improvement in weight gain and behaviour. When measured, most cats had serum total T4 concentrations below the reference range but in only "a few" of the cases were signs consistent with hypothyroidism observed, necessitating thyroid hormone supplementation. However, serum total T4 or T3 concentrations after therapy were not available from 33 cats and a definitive success rate is difficult to interpret.

Zuber, Allan & Church (1990) treated 15 hyperthyroid cats with ^{131}I using an empirical dose in 12, and based on $^{99m}TcO_4$ uptake by the thyroid gland in three cats. Ten cats received 148 MBq, three cats 111 MBq and two cats 74 MBq. Two of the cats remained hyperthyroid after treatment and required a second dose of ^{131}I within 3 months of therapy.

From all of these studies it was recognised that markedly elevated serum total T4 concentrations prior to therapy, large goitre and previous antithyroid drug therapy potentially adversely affected the response to ^{131}I therapy. Jones *et al.* (1991) therefore suggested using a dose of ^{131}I individually based on the severity of the clinical signs, the size of the thyroid gland, and the circulating free or total T4 concentration. The clinical signs were graded as mild, moderate or severe (score 1, 2, 3, respectively), the serum free T4 concentration as 30 to 60 pmol/l, 60 to 100 pmol/l and >100 pmol/l (score 1, 2, 3, respectively) and thyroid lobe size as not palpable, < 1.5 x 0.5 cm and > 1.5 x 0.5 cm or bilateral enlargement (score 1, 2, 3, respectively). Total scores of 3 to 5 received 39 to 60 MBq ^{131}I, 5 to 7, 60 to 75 MBq and 7 to 9, 75 to 100 MBq. Thirty-

two cats were treated in this manner. However, in three cases serum free T4 concentrations were not available. The serum total T4 concentration ranged from 84 to 260 nmol/l in these cats, but a scoring system for this parameter was not provided. Three cats (9 %) remained hyperthyroid after treatment with no reduction in their serum free T4 concentrations. One of these cats had received antithyroid drug therapy, although this had been discontinued at least 21 days prior to treatment. In all three cases, the pretreatment serum T4 concentration was very high, the clinical signs severe and the thyroid gland large and easily palpable, but there were other cats with equivalent parameters which apparently responded completely to a single dose of ^{131}I. Retreatment was successful in two of these cats, but was not yet evaluated in the remaining animal. One cat had a serum free T4 concentration below the reference range three months after therapy, but this had increased into the reference range by six months. One cat which had previously undergone a bilateral thyroidectomy required thyroid hormone supplementation after ^{131}I therapy.

Route of administration of radioactive iodine

In all of the previous reports, ^{131}I was administered as a single intravenous bolus. Two separate groups of workers recognised the potential dangers to the handler of injecting a radioisotope intravenously to a hyperthyroid cat, together with the problem of removing a contaminated catheter and the difficulties of obtaining ^{131}I for intravenous use in certain countries, and therefore studied the efficacy of treatment using the oral route. Klausner, Johnston, Feeney & Walter (1987) treated 23 hyperthyroid cats using a fixed dose of 185 MBq ^{131}I administered orally in a gelatin capsule. One cat died within 3 days of therapy exhibiting grand mal seizures, but the exact cause of death was not identified. In the remaining 22 cats, serum total T4 concentrations were within the reference range in 19 (86 %) at the time of evaluation 12 to 40 days later. Two cats (9 %) remained hyperthyroid after therapy one of which had previously received methimazole therapy. Three other cats which were successfully treated had also received antithyroid drug therapy. Serum total T4 concentrations were below the reference range in two cats (9%) 11 and 8 months after therapy, respectively. One of these cats was severely uraemic while in the other, the clinical features of lethargy and obesity were reversed with thyroid hormone supplementation. Side effects of, or potential dangers associated with, oral administration were not mentioned.

Malik, Lamb & Church (1993) also administered ^{131}I orally in a gelatin capsule to a series of 40 hyperthyroid cats. The cats received capsules containing between 200 and 300 MBq ^{131}I, and each cat typically received 250 MBq. This intermediate dose was obtained by administering the capsule earlier or later than the date of calibration. Cats with marked thyroid enlargement received the highest doses. Disposable latex gloves were worn while the cats were being dosed. Vomiting within a few hours of dosing was noted in some cats during the early part of the study but was subsequently avoided by introducing a 12 hour fast. Thirty-six (90 %) cats were successfully treated on the basis

of a resolution of clinical signs and a serum total T4 concentration either within or below the reference range. One cat subsequently required thyroid hormone supplementation. Four cats remained hyperthyroid after treatment. One cat was given only 200 MBq ^{131}I, another had an extremely large thyroid gland, while a third had received carbimazole before treatment. The drawbacks of oral administration of ^{131}I were reported and included the need for a higher dose which may reflect incomplete absorption of iodine from the gastrointestinal tract in hyperthyroid cats, greater risk of radioisotope spillage during dosing and vomiting after dosing. Despite this, potential advantages were considered to be the less stressful administration procedure and minimal delay in initiating treatment. The discrepancy between the relatively low dose used by Klausner *et al.* (1987) and the higher dose used by Malik, Lamb & Church (1993) was not addressed.

Care of cats treated with radioactive iodine

Regardless of the amount or route of ^{131}I administered, all treated cats should be confined to restricted areas that have minimal traffic and should be housed in cages where urine and faeces can be collected easily and safely. Personnel handling the cats should wear laboratory coats, disposable plastic gloves and appropriate dosimeter badges. All material removed from the cages should be classified as radioactive waste and disposed of accordingly. Cats can be discharged when the radiation dose level has decreased to a predetermined level defined by local radiation regulations. Definite levels have been reported by several workers; cats were not discharged to their owners until the thyroid surface-dose rate was less than 4.5 milliRoentgens/hour (mR/hr) (Turrel *et al.*, 1984; Meric *et al.*, 1986), 2.5 mR/hr (Chambers, Hightower & Tveter, 1987; Klausner *et al.*, 1987) or 1.5 mR/hr (Meric & Rubin, 1990). This required hospitalisation from five up to 40 days in rare cases and also required frequent monitoring of the thyroid area of treated cats. In other studies, where mentioned, hospitalisation periods varied between one to three weeks (Peterson & Turrel, 1986; Feldman & Nelson, 1987; Jones *et al.*, 1991; Malik, Lamb & Church, 1993). When reported, all cats appeared to tolerate the hospitalisation period well.

Adverse reactions to radioactive iodine therapy

In humans, potential immediate complications of ^{131}I therapy include an inflammatory response in the thyroid tissue resulting in swelling and tenderness around the thyroid gland or exacerbation of the thyrotoxicosis, owing to the release of large amounts of colloid from damaged follicles. The latter can be prevented by prior antithyroid drug therapy (Solomon, 1986). Other complications are extremely rare but include laryngeal nerve paralysis and overt hypoparathyroidism (Bauer & Blahd, 1957; Orme & Conolly, 1971). Adverse reactions appear to be extremely uncommon in cats. In one study, a transient change of voice was noted in one cat (Turrel *et al.*, 1984). The development of hypothyroidism months to years after radioactive iodine therapy is the most significant

complication in humans (Solomon, 1986). Although overt hypothyroidism has been reported, this complication does not appear to be prominent in cats. The difference may be related to the large number of human patients with Graves' disease, rather than toxic nodular goitre, who present for radioactive iodine therapy.

8.2 INTRODUCTION

Since the first description of feline hyperthyroidism in 1979, radioactive iodine (usually as ^{131}I) has consistently been recommended as a simple, effective and safe treatment for affected cats. Methods of estimating a dose of ^{131}I for each individual cat have previously involved administration of a small tracer dose of ^{131}I to determine iodine kinetics and subsequent calculation of a therapeutic dose using computerized nuclear medicine equipment (Peterson *et al.*, 1983a; Turrel *et al.*, 1984; Meric *et al.*, 1986; Peterson & Turrel, 1986; Feldman & Nelson, 1987). Alternatively a fixed dose of ^{131}I can be administered to each cat (Klausner *et al.*, 1987; Meric & Rubin, 1990; Zuber & Allan, 1990) but this may involve overtreatment of a number of animals. Other workers have recommended a dose based on previous experience and the appearance of a technetium scan, although such recommendations are difficult to reproduce (Chambers, Hightower & Tveter, 1987; Zuber, Allan & Church, 1990). The severity of the clinical thyrotoxicosis, high circulating thyroid hormone concentrations and large goitres have all been implicated as possible reasons for the failure of ^{131}I therapy (Jones *et al.*, 1991). In the study described here, a simple method of dose estimation was devised based on these three factors but using the more frequently measured serum total rather than free T4 concentrations as suggested by Jones *et al.* (1991). In addition, although ^{131}I has usually been administered intravenously, a comparison of efficacy using the simpler subcutaneous route was made. This is the first large study on the efficacy of radioactive iodine therapy in hyperthyroid cats in the United Kingdom and suggests a protocol that can easily be adopted at other referral institutions.

8.3 MATERIALS AND METHODS

Clinical material

The case material comprised a series of 50 hyperthyroid cats. Two of these cats had each had a bilateral thyroidectomy eight and 36 months previously. One further cat had had two surgical thyroidectomies, the last eight months previously. Thirty four of the cats were female (all ovariohysterectomised) and 16 were male (all castrated). They ranged in age from 9.0 to 19.0 years. There was only one pedigree cat (a Persian), the remainder being either domestic short (n = 46) or long haired (n = 3). In each case, a tentative diagnosis of hyperthyroidism was made on the basis of historical and clinical features and the diagnosis was confirmed by demonstrating an elevated serum concentration of total T4 as described in Chapter 4. In 26 of the cases, corresponding serum free T4 concentrations were measured as described in Chapter 4 and these results are discussed in Chapter 7. Due to the constraints of handling radioactive material, any animals with evidence of severe concurrent non-thyroidal illness (as assessed by clinical examination, full biochemical and haematological profiles and radiography if necessary) were refused this mode of treatment.

Estimation of dose

The dose selected for each individual cat was based on the severity of the clinical signs, the circulating total T4 concentration and the size (length and width) of goitre estimated by palpation, each being graded out of a possible score of 5, respectively (Table 13). Low scores (3 to 9) obtained a dose of less than or equal to 120 MBq, medium scores (9 to 12) 120 to 150 MBq and high scores (> 12), 160 MBq or more with greater weighting being given in each group to the size of the goitre.

Score	Severity of clinical signs	Serum total T4 concentration	Size of goitre
1	Mild	< 80	Palpable with difficulty
2	Mild - moderate	< 100	1.0 x 0.5 cm
3	Moderate	100 - 150	1.5 x 0.5 cm
4	Moderate - severe	150 - 400	> 1.5 x 0.5 cm
5	Severe	> 400	Visible to naked eye

Table 13. The scoring system for estimation of a dose of radioactive iodine (^{131}I) for treatment of hyperthyroid cats. The serum total thyroxine (T4) concentrations are expressed in nmol/l.

Treatment protocol

Each animal was sedated with a ketamine (Vetalar, Parke-Davis) and midazolam (Hypnovel, Roche) mixture (10 mg/kg and 0.2 mg/kg, respectively) administered intramuscularly. A 22 gauge catheter was placed in a cephalic vein if the isotope was to be injected intravenously. The ^{131}I was injected intravenously as sodium iodide in an approximate 2 ml volume. The catheter was subsequently flushed with sterile saline and removed. The solution was diluted with an equal volume of sterile water if injected subcutaneously. After injection of isotope, the animals were housed in an isolation unit for 30 days and all excreta were disposed of as radioactive waste. At 30 days the thyroid gland and injection site were monitored for detectable radiation. A full clinical examination was carried out and a blood sample obtained for a total T4 determination prior to being discharged to the owners. Each animal was rechecked, as far as was possible, three months following therapy and every six months thereafter.

If applicable, at the time of euthanasia or immediately following death, the thyroid lobes were removed and fixed in 10 % formol saline. After fixation, the thyroid tissue was processed to paraffin and 4 to 5 um sections were stained with haematoxylin and eosin and sirius red.

Statistical analyses

Student's paired t test was used to analyse the serum total and free T4 concentrations before and after treatment. All other data were analysed using Fisher's exact probability test or where appropriate, Chi-square analysis.

8.4 RESULTS

The pretreatment serum total, and where applicable, free T4 concentrations, dose of ^{131}I administered, route of administration and immediate post treatment free and/or total T4 concentrations for each individual cat are detailed in Appendix 13.

Efficacy of ^{131}I treatment

The mean dose of ^{131}I administered was 143 $\pm$24 MBq. All of the cats improved over the course of the isolation period as noted by a decrease in the severity of observable clinical signs and an obvious gain in weight. When initially palpable, there was a notable decrease in the size of the goitre. There was a significant ($P < 0.001$) decrease in the serum total T4 concentration from a mean $\pm$sd of 181.29 $\pm$111.40 nmol/l (range; 43.83 - 575.57 nmol/l) to a mean $\pm$sd of 19.03 $\pm$29.57 nmol/l (range; 2.00 - 175.73 nmol/l) 30 days following the injection of radioisotope (Figure 11).

Five cats remained hyperthyroid at the 30 day check, although the serum total T4 concentration had decreased from a mean $\pm$sd of 273.01 $\pm$210.53 nmol/l (range; 86.67 - 575.57 nmol/l) to a mean $\pm$sd of 93.32 $\pm$46.83 nmol/l (range; 61.46 - 175.73 nmol/l). Two of these cats became euthyroid within three and five months of therapy, respectively. Two cats were not re-examined, (one dying from an acute neurological illness four months after treatment, whilst the other was reportedly well but thin 12 months later). The remaining cat was successfully retreated four months later using a low dose (80 MBq) of ^{131}I.

The serum total T4 concentration was below the reference range in 29 (58 %) cats 30 days following treatment. In 19 of these cases, the serum total T4 concentration increased into the reference range 1.5 to 20 months later. In seven cases, the serum total T4 concentration has remained low in follow up periods of one to 15 months. Two cats died apparently from unrelated causes 12 and 13 months following treatment, respectively and repeat samples were not available. Repeat samples were not available from the remaining cat. Clinical signs suggestive of hypothyroidism were not apparent in any of the cats examined.

The treatment was deemed effective in controlling the hyperthyroidism in 47 (94.0 %) cats. Recurrent hyperthyroidism did not develop in any cat in follow-up periods of one to 32 months (mean $\pm$sd; 10.9 $\pm$9.0 months).

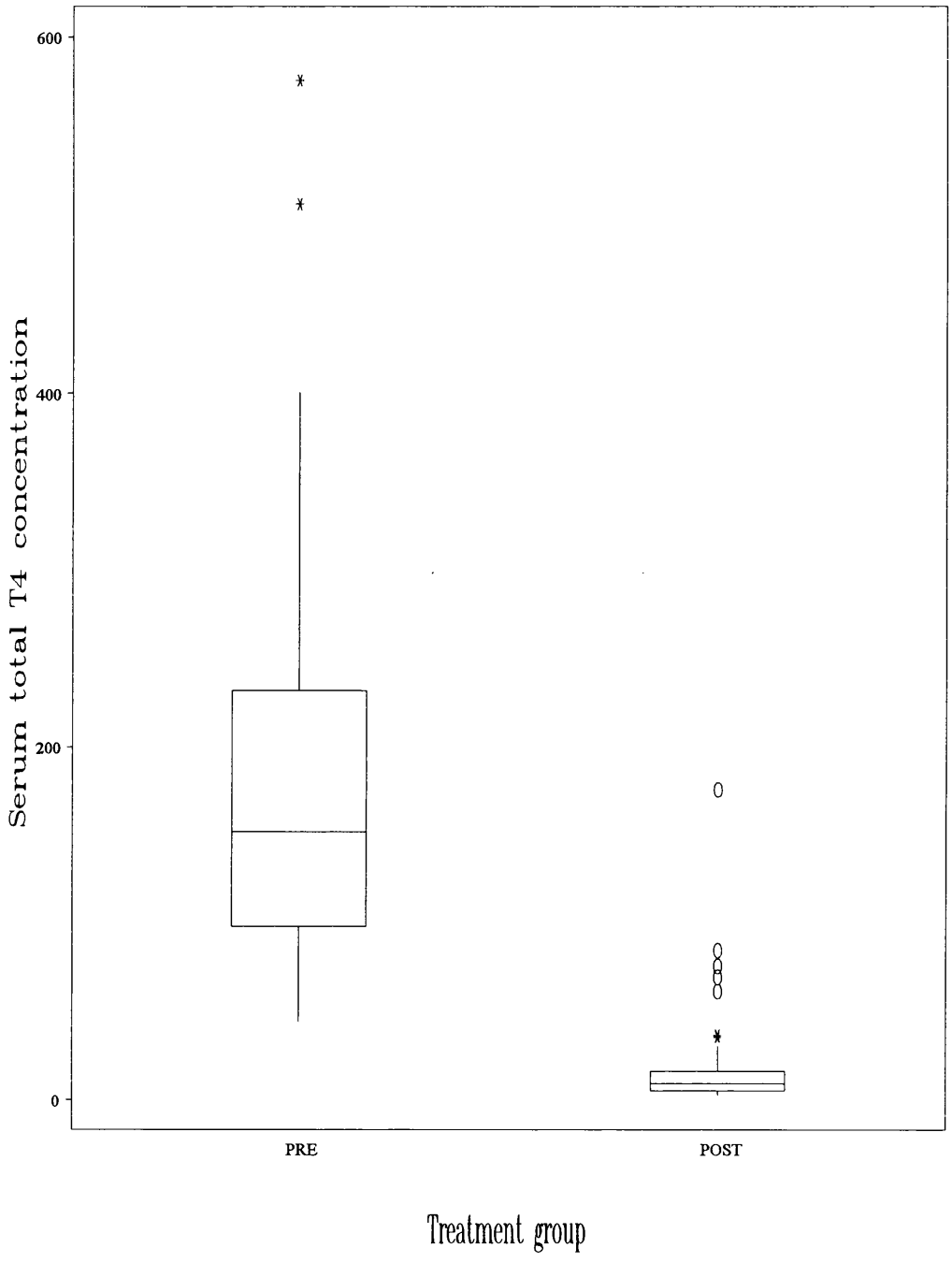


Figure 11. Box and whisker plot of serum total thyroxine (T4) concentrations in 50 cats before and 30 days after treatment with ^{131}I. The box encloses the middle half of the data and is bisected by a line representing the median. The whiskers represent the range of typical values. Possible outliers are represented by * and probable outliers by 0. The probable outliers post treatment correspond to the five cats that remained persistently hyperthyroid.

Efficacy of dose

The results of the three dose categories are presented in Table 14. All 12 cats treated with the low dose were effectively cured. One cat treated with the medium dose remained hyperthyroid at 30 days, but subsequently became euthyroid four months later. Goitre was not palpable in this cat when originally computing the dose. Four cats treated with the high dose remained hyperthyroid at thirty days, although one subsequently became euthyroid within three months. There was a significant difference ($P < 0.05$) in the number of cats that remained hyperthyroid after treatment with the high dose than those that remained hyperthyroid after treatment with low and medium doses taken together. There was no significant difference ($P > 0.05$) in the number of cats with serum total T4 concentrations below the reference range after treatment with the three different doses.

		Pretreatment total T4		Post treatment total T4			
Dose	n	mean ±sd	range	mean ±sd	range	n with subnormal T4	n with elevated T4
80-120	12	145.8 ±93.5	43.8-382.1	12.9 ±9.3	3.2-35.5	8	0
130-150	21	162.9 ±89.6	70.6-400.8	14.1 ±14.0	2.0-61.5	12	1
160-200	17	227.3 ±130.1	86.7-575.6	29.4 ±46.9	2.0-84.44	9	4

n = number of cats

Table 14. The effect of the three different dose categories of ^{131}I (MBq) on serum total thyroxine (T4) (nmol/l) concentrations in hyperthyroid cats. Thirty days elapsed between the pre- and post treatment results. The number of cats with subreference range and elevated serum total T4 concentrations after therapy are included.

Efficacy of injection route

The radioactive iodine was injected intravenously in the first 27 cats presented, and subcutaneously in the remaining 23 animals. There was no significant difference ($P > 0.05$) in the number of cats treated with the low, medium and high dose between these two groups. There was no significant difference ($P > 0.05$) in the outcome between the group injected intravenously and the group injected subcutaneously. None of the cats exhibited any obvious side effects when injected using either route.

Effect of therapy on serum free thyroxine concentrations

Serum free T4 concentrations were measured before and 30 days after therapy in 26 cats. There was a significant ($P < 0.001$) decrease in the serum free T4 concentrations from a

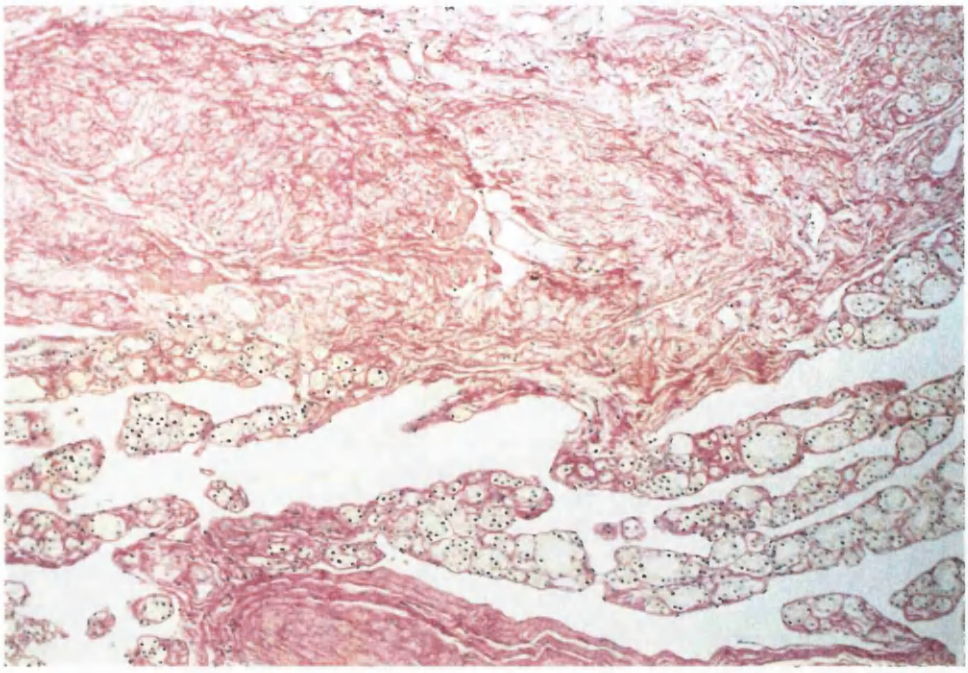
mean ±sd of 233.32 ±177.08 pmol/l (range; 46.88 - 687.61 pmol/l) to a mean ±sd of 24.89 ±40.49 pmol/l (range; 1.00 - 186.13 pmol/l). In four cats, the serum free T4 concentration was above the reference range after therapy, as were the corresponding serum total T4 concentrations. In 10 cases, both the corresponding serum free and total T4 concentrations were below the reference range. In three cases, the total T4 concentration was below the reference range after therapy while the corresponding free T4 concentrations were just within the reference range at 12.66, 12.73 and 9.94 pmol/l, respectively. In one case, the serum free T4 concentration was below the reference range, while the corresponding serum total T4 concentration was just within the reference range at 11.00 nmol/l. In all the remaining cats both corresponding serum free and total T4 concentrations were within the reference range.

Histopathological appearance of radioablated thyroid tissue

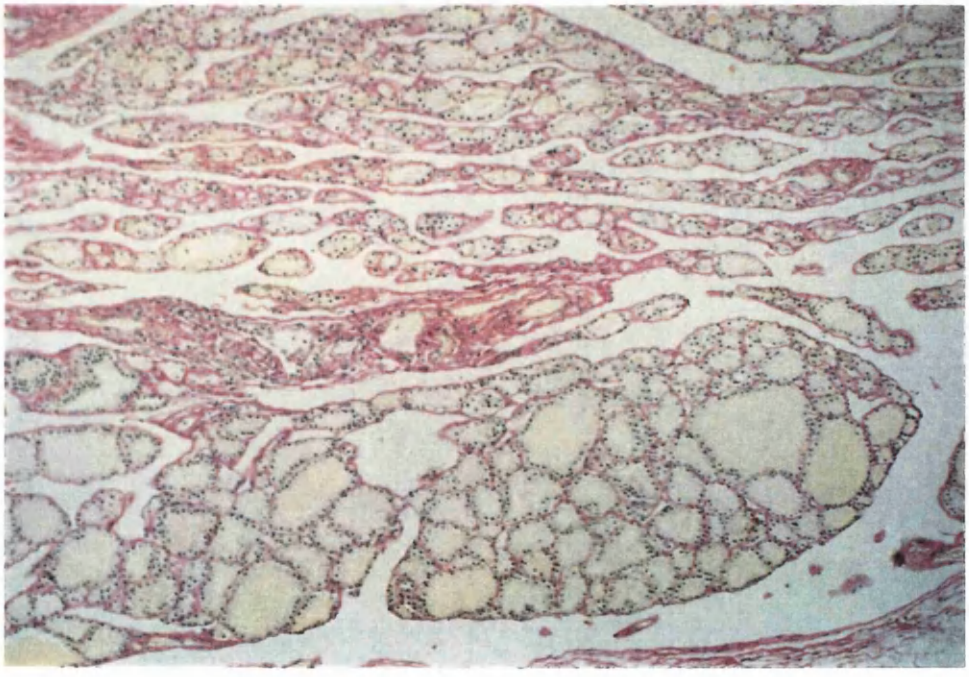
Thyroid tissue was available from two cases following ^{131}I therapy. Case number 117880 was euthanased 18 months after therapy because of persistent vomiting resulting from an alimentary lymphosarcoma. Case number 119218 died suddenly 11 months after therapy from hypertrophic cardiac disease resulting in acute congestive cardiac failure. In both cases, the thyroid lobes were grossly small with no visible nodules. The histopathological appearance of the thyroid tissue from case number 117880 is presented in Figure 12. There was marked fibrosis of the tissue with the acini containing poorly stained colloid and lined by cuboidal epithelium. Normal thyroid tissue and a few areas of hyperplasia were interspersed throughout.

8.5 DISCUSSION

This study emphasises the advantages of radioactive iodine therapy in hyperthyroid cats and exemplifies the locally destructive action of ^{131}I. As exhibited by these cats, adjacent parathyroid tissue and other important cervical structures were spared and there were no untoward systemic effects. ^{131}I therapy also appeared to spare atrophic thyroid tissue since a return to euthyroidism was presumably a result of reactivation of this tissue. Serum total T4 concentrations below the reference range developed in over 50 % of the cats immediately after treatment and there was often a lag period before biochemical euthyroidism was restored. During this period clinical signs suggestive of hypothyroidism (Thoday, 1990b; Rand, Levine, Best & Parker, 1993) were not apparent. When measured, corresponding serum free T4 concentrations also tended to be low, and thus an increased free T4 fraction could not account for the maintenance of clinical euthyroidism. In the face of a low circulating T4 concentration there may be increased peripheral conversion of T4 to the more metabolically active T3 thereby maintaining euthyroidism (Mooney, Thoday & Doxey, 1992a). Serum total T3 concentrations were not measured in this study.



(a)



(b)

Figure 12 (a) and (b). The histopathological appearance of radioablated thyroid tissue (case number 117880) stained with sirius red. The thyroid tissue is largely replaced by fibrous tissue (a). More normal thyroid tissue is apparent in (b).

In both humans and cats controversy has surrounded the most accurate method of estimating the lowest possible dose that will restore euthyroidism without producing hypothyroidism. Using the most sophisticated method, various parameters of thyroid kinetics are determined using a small tracer dose of ^{131}I and a therapeutic dose is calculated aiming to deliver between 15,000 to 20,000 rad/g of thyroid tissue. Doses of between 37 and 370 MBq have been used (Peterson *et al.*, 1983a; Turrel *et al.*, 1984; Meric *et al.*, 1986; Peterson & Turrel, 1986; Feldman & Nelson, 1987) resulting in a success rate of from 64 % (Turrel *et al.*, 1984) to 90 % in the largest study of over 350 cats (Feldman & Nelson, 1987). Subsequently, the use of higher fixed doses of 185 MBq (Klausner *et al.*, 1987) and 148 MBq (Meric & Rubin, 1990) were recommended and these effectively controlled hyperthyroidism in 91 % of 22 and 60 cats, respectively. Zuber & Allan (1990) also used a fixed dose of 148 MBq with a known success in 16 (88.9 %) of 18 cats. Such a method may however under or overdose cats unnecessarily.

The method of dose estimation used in the present study was easy to compute and effectively controlled the thyrotoxicosis in 94 % of the cats, a success rate comparable to or higher than either the tracer-compensated dose or fixed dose regimen. Computerised facilities were not required, and by comparison with the fixed dose regimen, over 60 % of the cats were effectively treated with a dose of less than or equal to 150 MBq. Few required a dose approaching or in excess of 185 MBq. Using a low dose was desirable both with a view to radiation safety and the constraints imposed on the premises of a maximum allowable dose of 250 MBq and may explain why clinical hypothyroidism has not been seen to date in any of the cats. The method of dose estimation described is similar to that recommended by Jones *et al.* (1991). However, in their study a scoring system was only reported for serum free T4 concentrations which ranged from 42 to 238 pmol/l in 29 cats, by comparison to a range of 46.88 to 687.61 pmol/l as reported in 26 cats in this study. The lower serum free T4 concentrations may explain why a similar success rate was achieved in their study when using lower doses of 39 to 100 MBq.

Five of the cats in this study remained biochemically hyperthyroid 30 days after ^{131}I therapy. Two cats subsequently became euthyroid without further treatment. The effects of ^{131}I are variable in any one gland since there is a difference in the uptake of iodine between individual follicles (Solomon, 1986). Some cells are subjected to intense radiation and die immediately. Others undergo various degrees of damage resulting in progressive atrophy and fibrosis and possible genetic damage in surviving cells ultimately impairing survival, explaining the lag period seen in some cases before euthyroidism is achieved. In humans, persistent hyperthyroidism is rarely retreated until four months have elapsed from the original treatment (Toft, Campbell & Seth, 1981; Solomon, 1986). Based on the results of this study, a similar time period is recommended but with monthly checks to monitor serum total T4 concentrations as an indicator of a possible late return to euthyroidism. If rapid control of hyperthyroidism is required, another form of therapy should be sought because of this possible lag period before euthyroidism is achieved.

The reasons for an incomplete response to ^{131}I are unclear but suggestions have included errors in dose estimation, previous antithyroid drug therapy, size of the goitre, high circulating thyroid hormone concentration, severity of the thyrotoxicosis and the presence of a thyroid carcinoma (Turrel *et al.*, 1984; Meric *et al.*, 1986; Turrel *et al.*, 1988; Meric & Rubin, 1990; Jones *et al.*, 1991). In the present study, none of the cats had recently been treated with antithyroid drugs and there was no clinical evidence (large, immobile locally invasive mass, distant metastases) to suggest the presence of a carcinoma. In one of the five persistently hyperthyroid cats, goitre was not palpable at the time of initial presentation and a medium dose was administered which may have been lower than that required. In the remaining four cats a high dose was required, based on the severity of the clinical signs, serum total T4 concentration and size of the goitre. If cats requiring such a high dose were not treated, a success rate approaching 100% would have been achieved. It is possible that a combination of surgical excision followed by low dose therapy would be more successful in such cases particularly if the premises are constrained with regard to the maximum dose allowed. In such cases, a potential benefit of radioactive iodine therapy is that unlike surgery (Welches, Scavelli, Matthiesen & Peterson, 1989; Swalec & Birchard, 1990), recurrence of hyperthyroidism appears to be extremely rare, was not seen in any of the treated cats in follow-up periods of up to 32 months and has been previously described in only one cat (Peterson *et al.*, 1983a). Recurrence of hyperthyroidism, once euthyroidism has been achieved, is also extremely rare in humans (Solomon, 1986).

^{131}I is usually administered to cats by intravenous injection (Peterson *et al.*, 1983a; Turrel *et al.*, 1984; Meric *et al.*, 1986; Peterson & Turrel, 1986; Chambers, Hightower & Tveter, 1987; Feldman & Nelson, 1987; Meric & Rubin, 1990; Zuber, Allan & Church, 1990; Jones *et al.*, 1991) although oral administration is equally as effective (Klausner *et al.*, 1987) but may be complicated by vomiting, an increased risk of spillage and a higher dose requirement of 200 to 300 MBq (Malik, Lamb & Church, 1993). In the present study, there were no complications associated with either the intravenous or subcutaneous routes of administration and neither affected the ultimate outcome. The use of the subcutaneous route was simpler and safer to personnel since prior insertion of a catheter was not necessary and potential complications such as removal of the contaminated catheter or the need for haemostasis at a contaminated site were avoided. The subcutaneous route is therefore recommended for routine administration of ^{131}I.

For radiation safety, cats are not usually discharged to their owners until the thyroid surface-dose rate is less than 4.5 mR/hr (Turrel *et al.*, 1984; Meric *et al.*, 1986), 2.5 mR/hr (Chambers, Hightower & Tveter, 1987; Klausner *et al.*, 1987) or 1.5 mR/hr (Meric & Rubin, 1990) and this requires hospitalisation from five up to 40 days in rare cases. In this study, cats were routinely hospitalised for 30 days by which time there was no detectable radiation from the neck region or injection site. All of the cats tolerated this period of isolation and no untoward effects were noted. The fixed hospitalisation

period avoided unnecessary handling of the cat to measure thyroid radioactivity and was well accepted by all the clients.

The histopathological appearance of the thyroid lobes after radioablation was as expected and similar to that described in humans, the major changes being atrophy and fibrosis (Solomon, 1986). Although some areas of hyperplasia were apparent, these were not considered to indicate a recurrence of the problem. Non-functional or "cold" nodules of hyperplasia are known to exist in affected thyroid tissue, normal thyroid tissue and even atrophic thyroid tissue (Carpenter *et al.*, 1987).

Administration of ^{131}I is an effective method of controlling hyperthyroidism in cats. By comparison to humans, where multiple doses are often required to control hyperthyroidism (Solomon, 1986), a single injection, as administered in this study appears to be effective in most cats. The dose for each individual cat can be calculated simply on the basis of the severity of the clinical signs, a serum total T4 concentration and the size of goitre estimated by palpation. In addition, the development of overt hypothyroidism or recurrence of hyperthyroidism appears to be extremely rare. ^{131}I can be injected subcutaneously rather than intravenously. Modification of the technique in this way reduces the health risk to personnel by reducing their handling of, and exposure to ^{131}I.

CHAPTER 9
GENERAL CONCLUSIONS AND FUTURE RESEARCH AREAS

9.1 INTRODUCTION

The objectives of this study were outlined in Chapter 1. The completion of the work has increased our knowledge of the older cat in general and thyroid dysfunction in particular. However, as with most studies of this kind more questions were posed than answered and even greater deficiencies in our knowledge uncovered. In this chapter, the findings are briefly summarised and possible areas of future research highlighted.

9.2 THE GERIATRIC CAT

The medical diseases affecting geriatric cats seen between 1990 and 1992 were compared to those seen between 1970 and 1972. Neoplastic and renal disorders have remained of significance in the older cat population. However, viral infections and hyperthyroidism were common in the latter time period, but were not diagnosed in any cats in the former. For viral infections, this presumably was related to a lack of awareness of their presence rather than a true increase in incidence. For hyperthyroidism, there was no conclusive evidence of its presence between 1970 and 1972. This supports the post-mortem study of Peterson & Ferguson (1989a) who reported that thyroid abnormalities were rare prior to 1977. Thus, it would appear that feline hyperthyroidism has shown a true increase in incidence.

There was a disproportionate increase in the number of aged cats (greater than five years old) seen between the two time periods, and between 1990 and 1992 the cats were significantly older. This supports the premise that the emergence of hyperthyroidism may be partly related to an increased longevity of cats (Peterson, Randolph & Mooney, 1994). This study did not address the possibility that extra- or intra-thyroidal stimuli enhance or sustain the progression of the disease. This therefore, remains an important area for future research.

This study was undertaken in a referred population of cats and provides a starting point for future research. It is clear however, that a more detailed demographic study of the current proportion of aged cats in the population, disease categories and their prevalence should be undertaken.

9.3 THYROID HORMONE CONCENTRATIONS IN HEALTH AND DISEASE

The equilibrium dialysis kit method designed for use with human serum was capable of measuring serum free T4 concentrations in cats and avoided the problems associated with other, in particular analogue, kit methods (Ekins, 1985) Although a relatively expensive test, it was readily adaptable for routine application in a clinical laboratory.

Apart from the necessary validation steps, the assay required no modification and would appear to be applicable to a wider range of species. Through the use of this assay and a total T4 RIA, absolute serum free T4 concentrations and the free T4 fraction were reported for healthy, euthyroid cats. Given that the measurement of free T4 parameters for cats have been rarely reported, this study provided the necessary database for a comparison of possible abnormalities in disease states. The effects of age, sex, breed, heredity and environment on circulating total T4 concentrations have been reported. Due to the experimental limitations of this study the effects of these factors on serum free T4 concentrations were not addressed and require further study.

As expected, and as previously reported by Peterson & Gamble (1990) serum total T4 concentrations were profoundly depressed in cats with non-thyroidal diseases. This was a consistent response to illness similar to the situation in the dog, but occurred more frequently than in humans (Ferguson, 1988; Nicoloff & LoPresti, 1991). As in humans, there was an excellent inverse correlation between mortality and serum total T4 concentrations (Slag *et al.*, 1981a; Kaptein *et al.*, 1982b). Thus, apart from its use as a diagnostic test for hyperthyroidism, measurement of serum total T4 concentrations can now be considered as a useful prognostic indicator.

The measurement of serum free T4 concentrations and calculation of the free T4 fractions in sick cats, previously unreported, provided an important insight into thyroid abnormalities and the possible mechanisms involved in disease states. Despite depression of serum total T4 concentrations in sick cats, euthyroidism was maintained because serum free T4 concentrations tended to remain within the reference range and the free T4 fraction increased. Serum free T4 concentrations were occasionally elevated but tended to occur in association with reference range serum total T4 concentrations. Serum free T4 concentrations were rarely subnormal. The abnormalities found closely resembled the situation in humans when serum free T4 concentrations are measured using similar techniques (Faber *et al.*, 1987; Nelson & Tomei, 1988; Surks *et al.*, 1988). Thus , in the cat as in man, suppression of serum TSH is not an important mechanism in depression of serum total T4 concentrations. Since controversy surrounds the possible mechanisms involved in humans, the cat could now be regarded as an appropriate model for future research. Most importantly, the existence of thyroid hormone binding inhibitors, believed to be involved in humans (Chopra *et al.*, 1979b; Woeber & Maddux, 1981; Oppenheimer *et al.*, 1982; Chopra *et al.*, 1982, 1984, 1986) requires investigation in the cat.

Hypothyroidism is a common disease in the dog, but is difficult to diagnose because serum total T4 concentrations are depressed in a variety of non-thyroidal illnesses. If serum free T4 concentrations are largely unaffected by non-thyroidal disease as they are in cats, its measurement could be useful for the diagnosis of hypothyroidism. Attempts have been made to address this issue, but the controversial analogue kit methods, which are unlikely to reflect the true circulating free T4 concentration, have

been used (Montgomery *et al.*, 1991; Nelson *et al.*, 1991). This issue requires future study using the more appropriate technique of equilibrium dialysis.

As expected and as previously reported, serum free T4 concentrations are elevated in hyperthyroidism (Ferguson, Peterson & Nachreiner, 1989). However, its measurement provided no additional diagnostic information over measurement of circulating total T4 concentrations alone. Importantly, because of the correlation between these two parameters, the degree of hyperthyroidism could be inferred by measurement of serum total T4 concentrations alone. However, from this study measurement of serum free T4 concentrations at least by equilibrium dialysis, is not recommended as a sole diagnostic test for hyperthyroidism since concentrations may be elevated in euthyroid cats with non-thyroidal diseases.

9.4 RADIOACTIVE IODINE THERAPY FOR FELINE HYPERTHYROIDISM

The dose of radioactive iodine was simply chosen on the basis of serum total T4 concentrations, clinical severity of the thyrotoxicosis and size of goitre estimated by palpation. Hyperthyroidism was effectively cured without side effects in over 90 % of cases. This technique obviated the need for sophisticated computerised nuclear medicine equipment and avoided fixed dosing of cats with its attendant problems. In addition, injection of the isotope was equally effective when administered intravenously or subcutaneously but the latter was considered simpler and safer. This method of dose estimation and administration is now recommended for routine use and can easily be adopted at other referral institutions.

Serum total T4 concentrations were frequently below the reference range after radioactive iodine therapy, but clinical signs of hypothyroidism were not noted. Corresponding serum free T4 concentrations were also below the reference range. Thus the maintenance of euthyroidism is presumably related to circulating T3 concentrations, as it is during carbimazole therapy (Mooney, Thoday & Doxey, 1992a).

In a small number of cases there was a lag period after treatment before euthyroidism was achieved. Thus retreatment with ^{131}I is not recommended until four months have elapsed after administration of the first dose.

LIST OF APPENDICES

APPENDIX 1

SUPPLIERS OF REAGENTS AND EQUIPMENT

Analytical Software,

1958 Eldridge Avenue, PO box 130204, St. Paul, MN 55113, USA

Boehringer Mannheim GmbH,

Boehringer Mannheim UK (Diagnostics and Biochemicals) Limited, Bell Lane, Lewes, East Sussex, England.

Corning Limited,

Halstead, Essex, England.

P.R. Edwards,

Department of Molecular Endocrinology, University College & Middlesex School of Medicine, Mortimer Street, London, England.

IDEXX Laboratories,

Milton Court, Churchfield Road, Chalfont St. Peter, Nr Gerrards Cross, Bucks., England.

Immunodiagnostic Systems Limited,

Boldon Business Park, Boldon, Tyne & Wear, England.

Instrumentation Laboratories,

Kelvin Close, Warrington, Cheshire, England.

Nichols Institute Diagnostics,

White House, High Street, Newport, Saffron Walden, Essex, England.

Packard Instrument Company,

Canberra Packard, Brook House, 14 Station Road, Pangbourne, Berks., England

Parke-Davis,

Parke-Davis Research Laboratories, Lambert Court, Chestnut Avenue, Eastleigh, Hants., England

Roche,

Roche Products Limited, PO Box 8, Welwyn Garden City, Herts., England.

Sarstedt,

68 Boston Road, Leicester, England

Scottish Quality Assessment Scheme,

Biochemistry Department, Victoria Infirmary, Glasgow, Scotland.

Sigma Chemical Company Limited,

Fancy Road, Poole, Dorset, England.

Technicon Instruments Corporation,

Hamilton Close, Houndsmills, Basingstoke, England.

UKEQAS,

United Kingdom External Quality Assurance Scheme, Department of Clinical Chemistry, Queen Elizabeth Medical Centre, Edgbaston, Birmingham, England.

APPENDIX 2

Case numbers for the geriatric cats seen at the Small Animal Clinic, University of Glasgow Veterinary School between January 1990 and June 1992

84488	114484	116203	117456	119150
94222	114510	116205	117472	119160
98872	114764	116320	117486	119218
105374	114810	116434	117664	119239
109077	114861	116438	117670	119242
109384	115020	116439	117673	119282
109692	115155	116492	117703	119330
110531	115162	116588	117727	119364
112094	115182	116596	117732	119408
113117	115304	116607	117735	119415
113174	115449	116681	117756	119451
113235	115452	116742	117790	119457
113275	115459	116746	117793	119480
113280	115570	116817	117850	119489
113345	115628	116862	117880	119521
113414	115649	116890	117934	119624
113480	115699	116982	117935	119662
113578	115713	116986	117958	119691
113607	115769	117020	117989	119885
113632	115813	117085	118355	119898
113635	115931	117093	118383	119919
113669	115935	117094	118405	119940
113703	115938	117116	118406	120189
113704	115953	117245	118430	120191
113822	116040	117246	118483	120207
113866	116042	117249	118629	120210
113929	116055	117254	118645	
113961	116057	117263	118681	
113995	116059	117265	118694	
114007	116117	117266	118746	
114166	116128	117396	119072	
114198	116132	117403	119077	
114332	116168	117449	119140	
114450	116201	117454	119145	

APPENDIX 3

Case numbers for the geriatric cats seen at the Small Animal Clinic, University of Glasgow Veterinary School between January 1970 and June 1972

42530	44355	45918	47813	48979
42723	44686	46106	47868	49025
42935	44710	46417	47992	49034
43059	44835	46553	48067	49620
43188	44850	46812	48101	49694
43436	45018	47018	48106	49901
43823	45103	47058	48498	49957
44038	45225	47384	48550	50121
44039	45567	47399	48576	50157
44093	45574	47438	48711	
44291	45676	47697	48790	
44320	45834	47795	48936	

APPENDIX 4

Total thyroxine (T4) concentrations of the medium pool serum assayed repeatedly for calculation of intra-assay precsion.

Number	Concentration (nmol/l)
1	31.33
2	30.09
3	30.07
4	28.99
5	27.97
6	30.59
7	24.75
8	32.52
9	31.39
10	33.55
11	30.29
12	30.47
13	27.45
14	32.16
15	30.10
16	32.15
17	29.86
18	31.77
19	30.39
20	30.56
Mean $\pm$sd	30.33 $\pm$1.96

APPENDIX 5

Free thyroxine (T4) concentrations of the medium pool serum assayed repeatedly for calculation of intra-assay precision.

Number	Concentration (pmol/l)
1	25.73
2	20.68
3	27.04
4	21.65
5	26.65
6	23.48
7	23.89
8	24.00
9	22.90
10	24.18
Mean $\pm$sd	24.02 $\pm$2.04

APPENDIX 6

Total thyroxine (T4) concentrations of the low, medium and high pool sera assayed for calculation of interassay precision. For the calculation of possible drift the low, medium and high pools were placed at the beginning and the end of the first four assays, respectively. All concentrations are expressed in nmol/l.

	Beginning of assay			End of assay		
Assay	Low	Medium	High	Low	Medium	High
1	16.07	32.68	139.57	13.38	25.23	128.81
2	14.64	33.64	155.75	12.34	29.25	134.23
3	16.90	31.98	150.89	16.29	34.96	155.23
4	14.07	33.68	146.38	14.48	29.74	149.26
5	18.13	33.90	160.62			
6	17.29	31.66	163.92			
7	17.27	33.20	155.20			
8	16.59	30.98	149.81			
9	18.33	29.89	166.68			
10	15.56	33.04	137.91			
11	16.39	41.49	162.74			
12	16.54	34.07	157.09			
13	13.49	33.54	154.12			
14	13.99	28.38	148.51			
Mean $\pm$sd	16.09 $\pm$1.54	33.01 $\pm$2.95	153.51 $\pm$8.65			

APPENDIX 7

Free thyroxine (T4) concentrations of the medium and high pool sera assayed for calculation of interassay precision. All concentrations are expressed in pmol/l.

Assay	Medium	High
1	25.52	115.74
2	33.49	129.97
3	27.55	143.73
4	22.04	154.51
5	22.31	115.62
6	26.32	
Mean ±sd	26.20 ±4.19	131.91 ±17.18

APPENDIX 8

The recovery of thyroxine (T4) added to serum samples for assessment of accuracy for the total T4 assay. Each sample was spiked with 24.70 nmol/l T4. All concentrations are expressed in nmol/l.

Sample	Basal concentration	Spiked concentration	Amount recovered	% recovered
Low	6.52	30.66	24.14	97.7
	4.49	28.22	23.73	96.1
	4.76	31.14	26.38	106.8
	1.24	27.81	26.57	107.6
	4.30	25.80	21.50	87.0
Medium	31.72	57.49	25.77	104.3
	30.33	55.35	25.02	101.3
	30.89	52.56	21.37	86.5
	26.23	56.19	29.96	121.3
	29.68	53.66	23.98	97.1
High	155.58	182.16	26.58	107.6
	165.78	186.10	20.32	82.3
	150.97	177.61	26.64	107.9
	149.98	173.21	23.23	94.0
Mean $\pm$sd				99.8 $\pm$10.5

APPENDIX 9

Free thyroxine (T4) concentrations found in the quality control samples supplied by the manufacturer. Sample J had a predicted concentration of between 8.62 - 23.17 pmol/l and Sample K, 25.74 - 50.19 pmol/l. All concentrations are expressed in pmol/l.

Assay	Sample J	Sample K
1	13.23	37.25
2	16.30	43.55
3	9.76	37.19
4	13.19	33.11
5	13.54	36.70
6	13.69	37.39

APPENDIX 10

Serum total and free thyroxine (T4) concentrations and the calculated free T4 fraction (%) in 50 healthy, euthyroid cats.

Animal	Breed	Sex	Age	Total T4 (nmol/l)	Free T4 (pmol/l)	% free T4
1	Burmese	Mn	8.0	31.33	25.36	0.08
2	SHD	Fn	2.0	11.67	20.61	0.18
3	SHD	Mn	2.0	12.50	30.51	0.24
4	SHD	Fn	11.0	21.68	20.44	0.09
5	SHD	Mn	8.0	12.94	47.72	0.37
6	SHD	FN	8.0	21.01	26.61	0.13
7	SHD	Mn	5.5	22.04	19.53	0.09
8	SHD	M	1.1	17.07	6.63	0.04
9	SHD	Fn	5.0	30.78	34.00	0.11
10	SHD	Mn	11.0	20.81		
11	Siamese	F	4.0	16.79	15.62	0.09
12	SHD	Mn	4.0	17.66	26.25	0.15
13	SHD	Mn	3.0	31.37	24.02	0.08
14	SHD	Fn	7.0	33.63		
15	SHD	Mn	5.0	18.01	13.58	0.08
16	SHD	Fn	6.0	42.28	22.98	0.05
17	Chinchilla	M	0.1	33.26	21.78	0.07
18	Persian	F	1.0	25.49	19.49	0.08
19	SHD	Fn	13.0	18.20		
20	SHD	F	0.5	29.00	41.38	0.14
21	SHD	M	0.5	35.21	35.48	0.10
22	BSH	Mn	0.6	26.56	14.89	0.06
23	Birman	M	2.0	34.85	29.51	0.08
24	SHD	M	6.0	30.01		
25	Birman	F	4.0	33.39	18.25	0.05
26	Birman	F	1.0	44.33	20.21	0.05
27	BSH	Mn	9.0	34.47	18.97	0.06
28	BSH	Fn	5.0	34.55	29.18	0.08
29	SHD	Mn	0.8	17.55		

APPENDIX 10 (continued)

Animal	Breed	Sex	Age	Total T4 (nmol/l)	Free T4 (pmol/l)	% free T4
30	Siamese	M	2.5	27.53	21.44	0.08
31	SHD	M	7.0	21.70	24.68	0.11
32	SHD	F	14.0	36.53	18.05	0.05
33	SHD	F	2.0	28.69		
34	SHD	F	1.0	19.79		
35	SHD	F	0.6	21.29		
36	Persian	F	4.0	14.73		
37	Persian	F	4.0	20.45	25.03	0.12
38	SHD	Mn	12.0	28.63		
39	SHD	Fn	5.0	36.68	32.13	0.09
40	Siamese	Mn	1.1	26.86	11.38	0.04
41	SHD	Fn	8.0	31.37	28.64	0.09
42	SHD	Fn	7.0	30.38		
43	SHD	F	1.6	26.88	35.81	0.13
44	SHD	Mn	2.6	21.65	20.58	0.10
45	Burmese	Fn	2.0	29.30	35.26	0.12
46	SHD	M	1.0	23.76		
47	SHD	Mn	3.0	23.55	21.64	0.09
48	SHD	Mn	3.0	23.19	27.40	0.12
49	SHD	Mn	3.9	21.96	23.74	0.11
50	SHD	Fn	3.2	26.64	33.41	0.13

SHD, Short haired domestic
BSH, British Short Hair
M, Male
Mn, Castrated male
F, Female
Fn, Ovariohysterectomised female

APPENDIX 11

Serum total and free thyroxine (T4) concentrations, and the calculated free T4 fraction in 107 sick cats.

Case number	Breed	Sex	Age	Total T4 (nmol/l)	Free T4 (pmol/l)	% free T4
84488	SHD	Mn	14.0	2.16		
94222	LHD	Mn	6.0	8.28		
105374	SHD	Fn	15.0	17.48	31.11	0.18
109212	SHD	Fn	7.1	4.37	21.70	0.50
109384	SHD	Mn	6.3	22.37	22.07	0.10
110531	SHD	Fn	14.5	16.16	70.84	0.45
112094	SHD	Mn	5.2	19.87	27.84	0.14
112570	SHD	Fn	13.7	25.16	43.39	0.17
113048	Persian	Mn	2.5	24.19	30.72	0.13
113117	Siamese	Fn	10.0	20.36	35.20	0.17
113129	SHD	Fn	4.0	23.63	32.72	0.14
113235	SHD	Fn	9.0	15.62	75.31	0.48
113275	SHD	Mn	6.0	14.01	22.80	0.16
113351	SHD	Mn	2.0	20.06	27.31	0.14
113414	SHD	Fn	10.0	14.63	21.44	0.15
113669	SHD	Mn	6.0	10.06	8.89	0.09
113701	SHD	Mn	4.0	2.00	42.06	2.10
113703	SHD	Mn	16.0	26.63	16.95	0.06
113929	SHD	F	7.0	22.12	75.54	0.34
114049	SHD	Mn	1.0	17.82	62.31	0.35
114450	SHD	Fn	5.0	23.52	48.12	0.20
114484	SHD	Mn	18.0	13.33	40.33	0.30
114510	Siamese	Fn	8.0	9.88	26.48	0.27
114579	SHD	Mn	3.0	31.39	29.51	0.09
114584	SHD	Mn	3.0	2.00	1.52	0.08
114640	Persian	Fn	2.0	25.68	24.59	0.10
114764	Siamese	Fn	11.0	17.68	15.57	0.09
114769	SHD	Mn	1.5	2.00	35.47	1.80
114854	SHD	Mn	1.0	6.43	33.57	0.52
114861	SHD	Fn	7.0	45.33		
114894	SHD	Fn	3.0	16.97	23.21	0.14

APPENDIX 11 (continued)

Case number	Breed	Sex	Age	Total T4 (nmol/l)	Free T4 (pmol/l)	% free T4
115128	Burmese	Mn	2.6	16.22	38.33	0.24
115155	SHD	Fn	13.0	14.55	25.78	0.18
115182	SHD	Mn	5.0	17.52	31.10	0.18
115304	SHD	Mn	13.0	12.53	8.45	0.07
115332	Burmese	F	0.5	12.41	48.10	0.39
115378	SHD	Mn	3.4	30.58	23.93	0.08
115449	Siamese	Fn	7.0	5.21	30.94	0.59
115452	Siamese	Fn	7.0	19.48		
115459	SHD	Mn	12.0	11.05	24.27	0.22
115485	SHD	Fn	1.5	10.03	51.34	0.51
115570	SHD	Mn	11.0	25.02	26.59	0.11
115769	SHD	Fn	13.0	11.87	36.30	0.31
115938	Siamese	Fn	13.5	16.41	20.23	0.12
115950	SHD	M	4.5	2.41	23.06	1.00
115953	SHD	Mn	7.1	4.64	38.26	0.82
116040	SHD	Mn	12.0	27.97	25.86	0.09
116117	SHD	Fn	7.0	22.95	37.30	0.16
116128	SHD	Mn	11.0	15.02	55.08	0.37
116132	SHD	Mn	12.0	11.42	14.37	0.13
116168	Persian	F	6.0	20.60	14.62	0.07
116201	SHD	Fn	15.0	15.46	23.11	0.15
116205	SHD	Fn	20.0	10.22	25.41	0.25
116320	SHD	F	12.0	26.87	42.79	0.16
116330	SHD	F	3.5	2.00	6.85	0.34
116392	SHD	M	1.5	19.21	24.44	0.13
116439	SHD	Fn	6.0	17.85	24.81	0.14
116492	SHD	Fn	13.0	20.38	19.08	0.09
116596	SHD	Fn	11.0	10.28	20.48	0.20
116967	SHD	Fn	4.0	13.03	22.05	0.17
117020	Persian	Mn	14.0	11.68	16.58	0.14
117116	SHD	Mn	11.0	4.75	17.92	0.38
117245	BB	M	8.0	10.62	35.64	0.34
117246	BB	M	8.0	19.57	27.78	0.14

APPENDIX 11 (continued)

Case number	Breed	Sex	Age	Total T4 (nmol/l)	Free T4 (pmol/l)	% free T4
117249	SHD	Mn	5.0	19.54	31.83	0.16
117254	Siamese	Mn	13.5	17.81	15.37	0.09
117268	Persian	Mn	4.0	2.89	4.06	0.14
117396	SHD	Fn	5.0	12.43		
117416	SHD	Mn	4.0	26.53	42.20	0.16
117524	Persian	M	3.0	9.65	27.37	0.28
117934	Siamese	Fn	8.0	17.95	23.57	0.13
117935	SHD	Fn	8.0	15.26	19.88	0.13
117989	Siamese	Mn	6.0	43.77	38.29	0.09
118355	Persian	Mn	5.5	20.87	19.04	0.09
118383	SHD	Mn	7.0	15.58	33.29	0.21
118430	SHD	Mn	10.0	29.29	40.82	0.14
118645	LHD	Mn	8.0	13.39	10.33	0.08
118676	LHD	Mn	0.5	28.23	23.51	0.08
118677	LHD	Mn	0.5	24.40	14.87	0.06
119077	SHD	Mn	8.1	21.03		
119145	SHD	Mn	15.0	23.18	30.73	0.13
119163	SHD	Mn	1.8	30.55	24.13	0.08
119165	SHD	Mn	4.0	15.39	21.42	0.14
119282	Siamese	Mn	13.0	2.00	14.86	0.74
119364	Siamese	F	9.6	23.88	32.68	0.14
119415	SHD	Fn	9.0	23.58		
119451	SHD	Mn	8.5	9.99	19.66	0.20
119556	DR	M	0.5	17.96	25.65	0.14
119624	SHD	Mn	14.0	33.51	25.08	0.13
119856	SHD	Mn	2.0	18.52	20.97	0.11
119898	Birman	Fn	12.0	28.73	25.08	0.09
120191	SHD	Mn	13.0	18.19		
120466	SHD	Fn	13.0	23.47	15.40	0.07
120532	SHD	Fn	15.0	20.96	19.24	0.09
121224	SHD	Fn	12.0	23.13	26.25	0.11
121273	Siamese	Mn	0.6	21.78	20.78	0.10
121501	LHD	Mn	5.0	14.80	15.05	0.10

APPENDIX 11 (continued)

Case number	Breed	Sex	Age	Total T4 (nmol/l)	Free T4 (pmol/l)	% free T4
121548	SHD	Mn	7.0	14.59	29.89	0.20
121563	SHD	Fn	5.6	16.11	16.80	0.10
122198	SHD	Mn	14.0	22.95		
122298	Siamese	Mn	1.9	20.30	22.19	0.11
122354	Siamese	Mn	9.0	12.77	23.35	0.18
122377	LHD	Mn	10.0	20.82	19.51	0.09
122548	LHD	Mn	2.0	20.94	29.38	0.14
122578	SHD	Fn	15.6	7.01	16.92	0.24
122648	SHD	Fn	13.0	11.36	12.69	0.11
122677	SHD	Mn	5.0	30.76	34.89	0.11

SHD, Short haired domestic
LHD, Long haired domestic
BB, British Blue
DR, Devon Rex
M, Male
Mn, Castrated male
F, Female
Fn, Ovariohysterectomised female

The case number refers to the hospital number at the Small Animal Clinic, University of Glasgow Veterinary School.

APPENDIX 12

Serum total and free thyroxine (T4) concentrations and the calculated free T4 fraction in 95 hyperthyroid cats.

Case number	Breed	Sex	Age	Total T4 (nmol/l)	Free T4 (pmol/l)	% free T4
98872	SHD	Mn	15.0	51.60		
109077	SHD	Fn	15.2	74.77		
109692	SHD	Mn	9.4	65.02		
113280	SHD	Mn	11.0	161.78		
113578	LHD	Mn	15.0	60.27		
113607	SHD	Fn	12.6	79.76		
113632	Persian	Mn	10.0	46.57		
113635	SHD	Fn	13.0	382.05		
113704	LHD	Mn	16.0	54.84		
114166	SHD	Mn	13.0	97.51		
114332	SHD	Fn	10.0	546.20		
115020	SHD	Fn	11.6	193.30	218.63	0.11
115628	SHD	Fn	15.0	400.77	281.63	0.07
115649	SHD	Mn	12.0	67.74		
115713	SHD	Mn	14.0	82.14		
115931	SHD	Mn	11.5	151.71		
115935	SHD	Fn	14.5	98.73		
116055	SHD	Mn	15.5	79.21		
116057	SHD	Fn	14.0	147.60		
116059	SHD	Mn	14.5	111.11		
116203	SHD	Fn	13.0	117.70		
116438	SHD	Mn	14.0	200.81		
116588	SHD	Mn	11.0	210.60		
116681	SHD	Mn	14.0	76.40		
116742	SHD	Fn	12.0	282.70	347.06	0.12
116746	SHD	Fn	15.7	270.96		
116862	SHD	Mn	13.0	58.65		
116890	LHD	Fn	13.0	220.70		
116982	SHD	Fn	11.6	187.73	242.47	0.13
116986	SHD	Mn	16.1	505.80	649.84	0.13
117085	SHD	Mn	16.0	167.61		

APPENDIX 12 (continued)

Case number	Breed	Sex	Age	Total T4 (nmol/l)	Free T4 (pmol/l)	% free T4
117093	LHD	Fn	15.0	214.49		
117099	SHD	Mn	16.0	181.72		
117265	LHD	Fn	11.1	93.03	105.91	0.11
117449	SHD	Mn	15.0	294.48	208.09	0.07
117454	SHD	Fn	14.0	146.72	174.90	0.12
117456	SHD	Mn	12.0	165.57		
117670	SHD	Fn	14.1	271.37	252.41	0.09
117703	SHD	F	18.0	144.52		
117727	SHD	Mn	12.0	152.98	183.21	0.12
117732	SHD	Fn	17.0	168.43		
117790	SHD	F	10.0	167.69		
117793	SHD	Fn	13.0	165.87		
117850	SHD	Fn	UK	272.50	687.61	0.25
117880	SHD	Fn	10.0	43.83	46.88	0.11
117958	LHD	Mn	14.5	191.56		
118405	SHD	Fn	15.0	167.32	199.39	0.12
118406	SHD	Fn	14.6	287.80		
118483	SHD	Fn	11.0	98.21	101.89	0.10
118629	SHD	Mn	13.0	110.94	121.70	0.11
118681	SHD	Fn	11.0	296.30		
118694	SHD	Fn	14.0	172.80	232.47	0.13
118746	SHD	Fn	14.0	62.88		
119072	SHD	Mn	10.5	62.34	91.83	0.15
119140	LHD	Fn	9.0	95.31		
119150	SHD	Fn	15.0	117.31	115.46	0.10
119160	SHD	Fn	10.0	575.57	635.01	0.11
119218	SHD	Mn	13.0	296.19	240.26	0.08
119239	SHD	Fn	15.0	214.64		
119330	SHD	Mn	12.0	143.69		
119408	SHD	Fn	12.6	167.81		
119480	SHD	Mn	10.0	78.29		
119489	SHD	Fn	9.0	70.60		
119521	LHD	Fn	14.0	309.15	381.14	0.12
119919	SHD	Mn	10.0	78.29		

APPENDIX 12 (continued)

Case number	Breed	Sex	Age	Total T4 (nmol/l)	Free T4 (pmol/l)	% free T4
119940	SHD	Mn	14.6	93.57		
120189	SHD	Mn	12.2	90.73	74.57	0.08
120207	SHD	Fn	14.0	178.43	160.41	0.09
120395	SHD	Mn	16.0	95.92	65.30	0.07
120467	SHD	Fn	19.0	101.26	122.78	0.12
120473	SHD	Fn	12.0	138.56		
120712	SHD	Mn	9.0	232.18		
120713	SHD	Fn	14.0	114.17		
120819	Persian	Fn	14.0	194.56		
121015	SHD	Mn	14.0	105.19		
121027	SHD	Fn	15.6	150.84		
121116	SHD	Mn	10.0	39.69		
121427	SHD	Fn	11.5	156.64		
121534	SHD	Fn	13.3	130.18		
121561	SHD	Fn	13.0	112.74		
121646	SHD	Fn	10.0	128.12		
121778	LHD	Fn	14.0	78.41		
121785	SHD	Mn	16.0	129.28		
121801	SHD	Mn	15.5	176.11		
121853	SHD	Fn	16.0	98.00		
122097	SHD	Fn	14.0	245.17		
122102	SHD	Fn	12.0	264.44		
122175	SHD	Fn	14.0	124.27		
122205	SHD	Fn	11.0	137.10		
122254	SHD	Fn	18.0	97.15		
122256	SHD	Mn	10.5	179.31		
122278	SHD	Fn	14.0	91.98		
122346	SHD	Fn	11.0	122.55		
122381	SHD	Fn	12.0	158.24		
122528	SHD	Mn	12.0	196.43		

SHD, Short haired domestic

LHD, Long haired domestic

Mn, Castrated male

APPENDIX 12 (continued)

F, Female
Fn, Ovariohysterectomised female
UK, unknown

The case number refers to the hospital number at the Small Animal Clinic, University of Glasgow Veterinary School.

APPENDIX 13

Serum total and free thyroxine (T4) concentrations in 50 cats prior to and 30 days after administration of radioactive iodine (^{131}I) for the treatment of hyperthyroidism. The ^{131}I was administered intravenously in the first 27 cases, and subcutaneously in the remainder.

Case number	Dose of ^{131}I (MBq)	Pre total T4 (nmol/l)	Pre free T4 (pmol/l)	Post total T4 (nmol/l)	Post free T4 (pmol/l)
115020	100	193.30	218.63	6.94	5.76
115628	150	400.77	281.63	61.46	54.28
116057	120	147.60		3.24	
116438	160	200.81		84.44	
116588	150	210.60		8.12	
116681	120	76.4		10.32	
116742	160	282.70	347.06	11.00	7.18
116982	160	187.73	242.47	2.00	1.00
116986	160	505.80	649.84	2.71	1.00
117265	150	93.03	105.91	14.04	13.77
117449	150	294.48	208.09	30.49	37.11
117403	180	86.67	125.50	69.34	52.55
117454	170	146.72	174.90	3.60	1.48
117670	160	271.37	252.41	12.13	12.33
117727	180	152.98	183.21	4.03	5.18
117850	160	272.50	687.61	27.36	40.96
117880	100	43.83	46.88	9.31	12.66
118405	140	167.32	199.39	3.36	1.00
118483	110	98.21	101.89	10.63	12.73
118629	150	110.94	121.70	12.99	17.76
118694	160	172.80	232.47	3.47	4.70
119072	110	62.34	91.83	7.64	9.94
119160	160	575.57	635.01	175.73	186.13
119150	150	117.31	115.46	2.00	1.00
119218	130	296.19	240.26	6.40	2.41
119489	140	70.60		8.58	

APPENDIX 13 (continued)

Case number	Dose of ^{131}I (MBq)	Pre total T4 (nmol/l)	Pre free T4 (pmol/l)	Post total T4 (nmol/l)	Post free T4 (pmol/l)
119521	200	309.15	381.14	16.39	34.35
119940	130	93.57		9.09	
120189	120	90.73	74.57	22.98	17.24
120207	160	178.43	160.41	6.09	1.00
120395	150	95.92	65.30	12.41	9.32
120467	170	101.26	122.78	75.61	104.36
120712	140	232.18		9.68	
120713	150	114.17		2.00	
120819	170	194.56		2.00	
121027	120	150.84		5.14	
113607	130	79.76		6.90	
121015	140	105.19		14.67	
121427	160	156.64		2.39	
121561	130	112.74		24.04	
121646	140	128.12		6.73	
121778	130	78.41		13.25	
121801	150	176.11		5.12	
121853	167	98.00		2.00	
122102	150	264.44		9.12	
113635	110	382.05		35.46	
122097	120	245.17		13.29	
122205	100	137.10		8.16	
122256	130	179.31		36.44	
122346	80	122.55		21.10	

The case number refers to the hospital number at the Small Animal Clinic, University of Glasgow Veterinary School.

GLOSSARY

ACTH	adrenocorticotropin hormone
AP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BEI	butanolol-extractable iodine
CBPA	competitive protein-binding displacement assay
c.v.	coefficient of variation
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FCoV	feline coronovirus
FeLV	feline leukaemia virus
FIV	feline immunodeficiency virus
FIP	feline infectious peritonitis
FTI	free thyroxine index
HDL	high density lipoprotein
IgM	immunoglobulin M
^{130}I	^{130}Iodine
^{131}I	^{131}Iodine
kBq	kilo Becquerel
LDH	lactate dehydrogenase
MBq	Mega Becquerel
mR/hour	milliRoentgens/hour
n	number of observations
NSB	non-specific binding
PBI	protein-bound iodine
RIA	radioimmunoassay
rT3	3,3',5'-L-triiodothyronine (reverse T3)
sd	standard deviation
sem	standard error of the mean
T3	3,5,3'-triiodothyronine
T4	3,5,3',5'-L-tetraiodothyronine (thyroxine)
TBG	thyroxine-binding globulin
TBPA	thyroxine-bindingprealbumin
TC	total count
$^{99m}TcO_4$	pertechnetate
THUT	thyroid hormone uptake test
TRH	thyrotropin releasing hormone
TSH	thyroid stimulating hormone (thyrotropin)
VLDL	very low density lipoprotein

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