"CONTRIBUTIONS TO THE INVESTIGATION OF THYROID ENLARGEMENT IN THE HORSE"

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by

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"A MI CLAN"

SUMMARY

Twelve horses with unilateral thyroid enlargement, were investigated during a one year period.

This study was set up to try to relate thyroid enlargement to thyroid function in the horse.

General clinical examination, ultrasound scanning, TSH stimulation test, serum T4 determination, scintigraphy and histopathological study of the gland was carried out.

Two follicular adenocarcinomas, 1 suspected adenoma of the parafollicular (C) cells, accompanied by thyroid follicular hyperplasia and 4 follicular adenomas and one hyperplastic goitre were diagnosed.

All of them were euthyroid (normal thyroid hormone base levels), and primary dysfunction of the gland could not be detected, indicating that, in our cases unilateral thyroid enlargement did not alter thyroid function.

INTRODUCTION

Cervical swellings in the horse may result from a range of conditions such as lymphadenopathies, abscesses, haematoma, auditory tube empyema and tympany, developmental cysts, goitre and neoplasia. Benign enlargement of the thyroid gland (goitre) is presented generally as a mass in the cranial ventral cervical region just caudal to the mandible. This mass is non painful, with no palpable heat , unattached to the surrounding skin and easily movable in all directions. It is firm and can be smooth and rounded or nodular in nature depending on the pathological changes occurring in the gland.

Thyroid enlargement is often referred to as benign thyroid adenoma and is a cause of aesthetic concern rather than clinical problems. There are few reports on thyroid abnormalities in the horse, especially thyroid function, but it is recognised in other species, that alterations in thyroid function may be associated with a complete group of disorders involving almost every system in the body. This study was set up to determine if there is a relationship between physical abnormalities and function of the gland.

Diseases of the thyroid gland have been known for many thousands of years, having been recorded by the ancient Chinese and epidemic goitre still affects an estimated 200 million people. Most of the notorious endemic goitre areas are located in high mountain regions and certain low lying areas that were subjected to flooding and glaciation during the last ice age. In many of these areas, where endemic goitre occurs in man, it has also been reported in domestic animals (Mason and Wilkinson 1985).

Enlargement of the gland can be due to several causes:

1-Ingestion of goitrogens

There are two basic types of goitrogens: anionic goitrogens such as thiocyanate and organic goitrogens which include thiouracil.

The organic goitrogens represented by thiouracil, sulphonamides, anions of the Hofmeister series and a number of plants from the family Brassicaceae, inhibit the

formation of iodotyrosine and their coupling to form iodothyronines (Mayberry and Astwood 1961). The anionic goitrogens prevent the accumulation of iodide by the thyroid and cause the release of any iodine in the thyroid that is not organically bound (Catt 1970). Certain chemical radicals, thiocyanate, perchlorate, or nitrate, selectively inhibit the operation of the thyroid iodide trap. Thiocyanate is about 25 times as potent as nitrate in inhibiting thyroid function (Greer, Matsuda and Stott 1966). Since this inhibition can be reversed by large doses of iodide, it is thought that these radicals compete with iodide for some component of the trapping mechanism.

Goitre may also be induced by substances such as haemoglobin, cottonseed meal and walnuts which prevent the reabsorption from the alimentary system of thyroxine excreted in the bile. High blood iodide interferes with one or more steps of thyroxigenesis, leading to low blood T4 levels, which results in excessive stimulation of the glands by TSH (Ruegemor, Wagner and Barstow 1967; Linazasoro, Sanchez-Martin and Jimenez-Diaz 1970), and therefore in enlargement of the gland.

2- Dyshormogenesis

When dyshormogenesis occurs the thyroid fails to produce normal quantities of thyroid hormone because of an inborn defect in synthesis.

It has not been yet recorded in the horse, but it has been in Merino sheep and in that species it is associated with a genetic defect (Rac, Hill and Pain 1968).

3- Thyroiditis

Inflammatory lesions of the thyroid are very uncommon in domestic animals and when they do occur they are often associated with septicemia or follow extension from an adjacent abscess (Jubb and Kennedy 1970)

Autoimmune and lymphocytic thyroiditis have been recorded in dogs (Beierwaltes and Nishiyama 1968) but not in the horse.

4- Neoplasia

Tumours of the thyroid gland may involve the follicular, parafollicular (C-cell) cells and the remnants of the thyroglossal duct.

a) Tumours of the follicular cells are classified as follows:

- Adenomas: -Follicular
  -Papillar

- Carcinomas: -Follicular
  -Papillar
  -Compact Cellular:
  -Undifferentiated:
    -Small cells
    - Giant cells

b) Nodular hyperplasia of follicular cells

c) Tumours of the thyroglossal duct remnants:
  - Well differentiated papillary carcinomas

d) Tumours of the parafollicular cells or ultimobranchial arch.

(Capen 1990)

a) Tumours of the thyroid follicular cells

Tumours of the thyroid follicular cells are encountered most often in dogs, cats and horses, with other species affected infrequently (Granbmann 1965; Hayes 1975). They are classified as adenomas or carcinomas. Earlier reports have suggested that the incidence of thyroid tumours is highest in iodine deficient areas where many animals have long-standing diffuse hyperplastic goitre (Marine and Lenhart 1909; Davis 1938). Most animals with thyroid tumours are adult or aged.

The majority of tumours encountered in the thyroid glands of horses have not produced obvious clinical disturbances and have been encountered as incidental findings at post-mortem examination. A palpable firm swelling in the cranioventral region may be detected with certain thyroid carcinomas and larger adenomas, being multinodular in nature. Carcinomas are fixed in position by extensive local invasion of adjacent structures, whereas adenomas are freely movable under the skin. In the few reports of equine thyroid carcinomas, they have been related to a hypothyroid status (Held, Patton, Toal and Geiser 1985) or to an euthyroid status (Joyce, Thompson and Kyzar 1976; Hillidge, Sanecki and Theodorakis 1982). Euthyroid status refers to a normal level of T3 and T4.

The adenomas can be classified into follicular and papillar type. Follicular can be subdivided into microfollicular and macrofollicular in accordance with their histological features. Follicular adenoma can also be classified as oxyphilic, if the cells present a dense eosinophilic granular cytoplasm with little or no colloid formation, and trabecular if they are poorly differentiated (Capen 1990).

Papillary adenomas are very rare in animals and are more frequent in man (Capen 1990).

Thyroid follicular carcinomas have a greater degree of cellular pleomorphism than adenomas. They also can be classified in accordance with their histologic features as follicular, papillar, or compact cellular (solid). Compact cellular (solid) carcinomas are derived from follicular cells of the thyroid not from C-cells as suggested by Williams, Brown and Doniach (1966) (Capen 1990). Invasion of thin walled veins can result in pulmonary metastases, prior to the development of secondary foci of growth

in lymph nodes draining the affected thyroid lobe.

b) Nodular hyperplasia of follicular cells

Non-neoplastic and non-inflammatory enlargement of the thyroid can develop in horses. Iodine deficiency causing diffuse thyroid hyperplasia is recognised in enzootic goitrogenic areas due to the lack of iodine in the diet. Young foals born to mares on iodine deficient diets are more likely to develop severe thyroid hyperplasia and have clinical evidence of hypothyroidism. Foals from mares fed dry seaweed containing excessive iodide may develop thyroid hyperplasia and clinically evident goitre (Baker and Lindsey 1968). The foal's thyroid gland is exposed to higher blood iodide levels than the mare due to the concentration of iodide first by the placenta and then, after parturition, by the mammary gland.

Nodular hyperplasia in thyroid glands of horses appears as multiple white to tan nodules of varying size. The affected lobes are moderately enlarged and irregular in contour. These areas of nodular hyperplasia are not encapsulated and result in minimal compression of the adjacent parenchyma, opposite to the features of thyroid adenomas.

c) Tumours of the thyroglossal duct remnants.

This type of neoplasia has never been recorded in the horse, however they have been encountered in the dog (Harkema, King and Hahu 1984).

A portion of the thyroglossal duct may persist postnatally and form a cyst due to the accumulation of proteinic material secreted by the lining epithelium.

They appear in the ventral midline in the cranial cervical region.

d) Tumours of the parafollicular (C) cells or ultimobranchial arch.

These type of tumours include medullary thyroid carcinoma (MTC) and adenoma. Histological features of MTC are a fairly uniform granular eosinophilic cell type arranged in sheets and lobules of varying sizes and shapes separated by irregular

and sometimes very thin fibrovascular stroma in which amyloid can be demonstrated.

These have been widely reported in cattle, but in the horse there are only two reports (Lucke and Lane 1984; Van der Velden and Meulenaar 1988).

Benign hyperplasia (adenoma) in the horse has also been reported (Turk, Nakata, Leathers and Gallina 1983; Yoshikawa, Yoshikawa, Oyamada and Suzuka 1984, Tateyama *et al* 1988).

Lucke and Lane did not clarify whether the tumour was a hyperplasia or a medullary thyroid carcinoma.

The report of Van der Velden and Meulenaar (1986) represents the only proven case of MTC in the horse. Due to the lack of serum calcitonin levels determination, it is not known if calcitonin had been released from the tumour. The calcium levels were normal and therefore this tumour may have been accompanied by secondary hyperplasia of the parathyroid glands which may indicate a compensatory response to maintain calcium homeostasis.

Amyloid that has been reported to be a common finding in such a tumour in man and frequently in animals has not been reported in the horse.

Bulls with C-cell thyroid tumours frequently show lameness as the result of degenerative osteoarthropathy and ankylosing spondylosis with vertebral osteosclerosis (Capen 1990). These signs have not been seen in the equine reports, but due to the lack of thyroid hormone and calcitonin measurements in these reports nothing can be concluded of the influence of this type of tumour on the calcium metabolism in the horse.

LITERATURE REVIEW

Schlotthauer (1933) reported an incidence of 37 % of thyroid adenomas, 20 % of hyperplastic goitre and 11 % of colloid goitre in an abattoir survey involving 100 horses, establishing that not all pathologic glands were associated with enlargement. He stated that any gland having a weight greater than 0.66 gm. for each kilogram of body weight is abnormal.

However, Dimock, Westerfield and Doll (1944) suggested smaller glands may also show pathological changes. They tried to relate different conditions in the horse such as periodic ophthalmia, wobbler syndrome, bacterial and viral abortion, with the status of the thyroid gland, in terms of weight and histologic features of the stroma, vascularity, follicular pattern, colloid and epithelium of the gland. They did not study the thyroid function but presented a survey in 142 horses relating thyroid to body weight, showing that this relation decreased with age.

In 1968, Baker and Lindsey showed how excess dietary iodide can be responsible for an enlargement of the gland due to an unexplained mechanism. In humans affected by goitre due to excess iodide, the amount of DIT (Diiodotyrosine) is lower than in normal glands. Probably the excess iodide interferes with the coupling mechanism to form DIT and therefore T4. They investigated the thyroid function of those foals affected, but the plasma protein binding (PPB) assay used, has been shown to be an unreliable technique (Thomas and Adams 1978). Their work established that as little as 50 mg of iodide per day consumed by mares during pregnancy can cause congenital goitre in some foals. The majority of foals in this study, did not show evidence of hypothyroidism although, as mentioned before, the unreliability of the techniques used invalidates their findings.

An important piece of work was the one presented by Lowe *et al* (1974). They established the long term effects of thyroidectomy on metabolism and growth in mares and stallions. As a result of this research, clinical signs associated with hypothyroidism were defined:

- Lethargy
- Obese phenotype

- Oedema of the rear limbs
- Coarse, rough, and dull hair coats
- Cold intolerance
- Decreased appetite
- Hypothermia
- Lowered pack cell volume (PCV)
- Increased serum cholesterol
- Delayed growth, dwarfism

All the horses showed a dramatic improvement after thyroid replacement therapy. One conclusion of this work was that in adult horses, hypothyroidism might go unnoticed because it is not life threatening and appears to be compensated by other body processes.

Congenital suspected hypothyroidism was reported by Shaver, Fretz, Doige and Williams (1979) with skeletal manifestations such as weakness and collapse of tarsal bones. These findings correspond with those seen in man with hypothyroidism and the results of Lowe *et al* (1974). Again, PPB assay was used to determine T4 (Kallfez and Lowe 1970).

In 1978, Thomas and Adams adapted a radioimmunoassay (RIA) to determine reference values of equine serum T4 levels (1.57 +/- 0.62 ug/dl). It demonstrated that the test used previously (Plasma Protein Binding assay) had an standard error of 1.47 +/- 0.07 ug/dl, invalidating it for studying thyroid function. In the same year Reap, Caas and Hightower (1978) reported levels of 1.63 +/- 0.51 ug/dl, finding significant difference between stallions and mares. They also used RIA. Since then, RIA has become the most reliable, easy and quick method to determine serum T4 levels in the horse.

There are just a few reports relating thyroid function with pathologic findings in the thyroid gland (Hillidge *et al* 1982; Held *et al* 1985; Hopper, Kennedy and Taylor 1987; Hovda, Shaftoe, Clemmons and Rose 1990; Murray 1990).

In 1984, Held *et al* presented a sampling protocol for the Thyrotropin Stimulation (TSH) test, which is currently the most reliable test to diagnose primary

hypothyroidism. T3 levels are known to be affected by numerous factors that make interpretation of these test results more difficult (Robbins 1981).

None of the pathology reports of thyroid neoplasia (Turk *et al* 1983; Yoshikawa *et al* 1984; Lucke and Lane 1984; Van der Velden and Meulenaar 1986; Chiba, Okada, Numakunai ans Ohshima 1987; Tateyama *et al* 1988) report thyroid values of the horses affected or further investigation of thyroid function.

Other methods which have rarely been used to diagnose thyroid changes in horses, are scintigraphy (Hillidge *et al* 1982; Held *et al* 1985) or needle biopsy (Held *et al* 1985; Hopper *et al* 1987).

This study used a protocol of ultrasonography, scintigraphy, tru-cut biopsy and TSH stimulation test, that has not been reported before in the horse. However it has been extensively used in dogs and humans (Dige-Petersen *et al* 1978; Ikekubo *et al* 1986; Berghout, Wiersinga, Smits and Touber 1988; Azagra *et al* 1990).

The results indicate that thyroid disease in the horse deserves more research with more accurate and advanced methods to reach a full knowledge of the aetiology and the pathogenesis of such conditions.

THE EQUINE THYROID GLAND

EMBRYOLOGY

The parenchymal cells of the thyroid gland are derived from endodermal epithelial cells. The primordium of the thyroid first appears as an evagination on the floor of the foregut between the first two pharyngeal pouches. This ventral outpocketing is called the thyroid diverticulum. A tube like column of these epithelial cells grows ventrally and caudally into the adjacent mesenchyme. There it divides into two portions, each of which will give rise to a lobe of the thyroid. The tubular connection from the developing gland to the point of origin from the pharyngeal pouch is the thyroglossal duct, which is non patent in the horse. Unlike exocrine glands, the thyroid looses its original connection to the floor of the pharynx, and therefore with the original gut tube. The thyroid splits into two lateral lobes that in the horse usually remain connected, ventral to the trachea, by a thyroid isthmus.

Epithelial clusters bud off the ventral wing of the 4th pouch and are infiltrated by neural crest cells that proliferate around the pouch epithelium. These form the parenchymal cells of the ultimobranchial body, which in all mammals, becomes initially associated with the thyroid parenchyma. The crest cells within the ultimobranchial tissue form the C (parafollicular) cells of the thyroid gland. These produce calcitonin in response to hypercalcaemia.

At the same time, the primordial thyroid cells shift caudally, and they contact with tissues associated with the ventral portion of the 4th pharyngeal pouch, allowing the parafollicular cells to become incorporated into the thyroid gland. The thyroid parenchymal cells organize in solid clusters, each of which subsequently forms a lumen and then becomes follicular. These follicles will be the source of thyroxine (Noden 1985).

ANATOMY

The thyroid gland is located on the most cranial portion of the trachea (Fig. 1), to which it is loosely attached by deep cervical fascia. The gland is dark red brown in colour, firm in texture and highly vascular. Normally it is composed of two lateral lobes connected by a narrow isthmus. The lateral lobes are situated on either side of the trachea immediately caudal to the larynx. Their approximate position is indicated by the angle of junction of the jugular and linguofacial veins, but it is difficult to palpate them due to their great mobility. The lateral surface is convex and is covered by the cervical angle of the parotid gland and the sternocephalicus, and omohyoideus muscles. The deep surface of each lobe is related to the first three or four tracheal rings. The cranial pole of the thyroid is large and rounded, while the caudal pole is smaller and usually tapers into a tail-like process which is continuous with the fibrous isthmus. The isthmus usually extends across the ventral surface of the trachea, thus connecting the two thyroid lobes.

In the adult horse the isthmus consists of a thin strand of fibrous connective tissue. In the foal the isthmus is well developed and entirely glandular. In the ass and mule there is a well developed isthmus, and it is not unusual to observe a complete glandular isthmus in the adult.

Variations exist in regard to the distance each lobe is located caudal to the larynx. They may be in contact with the larynx or be situated 2.5 cm. caudal to the cricoid cartilage. The cranial cervical lymph nodes are located near the gland, under cover of the cervical angle of the parotid gland. These lymph nodes may occur between the thyroid and the mandible or above and partly upon the thyroid.

The thyroid gland is highly vascular, receiving arterial blood from the cranial thyroid or thyrolaryngeal artery, the largest collateral branch of the common carotid artery. The cranial thyroid artery arises 5.0 to 7.5 cm. prior to the division of the carotid and curves over the cranial pole of the thyroid, into which it sends several branches. The caudal thyroid artery is a small inconstant vessel which branches from the common carotid artery a variable distance caudal to the cranial thyroid artery. This artery may also arise from the cranial thyroid or the parotid artery. Branches enter the caudal pole of the gland; some supply the trachea, others go to adjacent muscles. The

thyroid vein joins the jugular near the linguofacial vein. It receives radicles from the cranial thyroid, occasionally the caudal thyroid, the laryngeal and pharyngeal veins. The lymphatic vessels drain into the cervical lymph nodes.

The nerve supply is furnished by the autonomic nervous system (Venzke 1975).

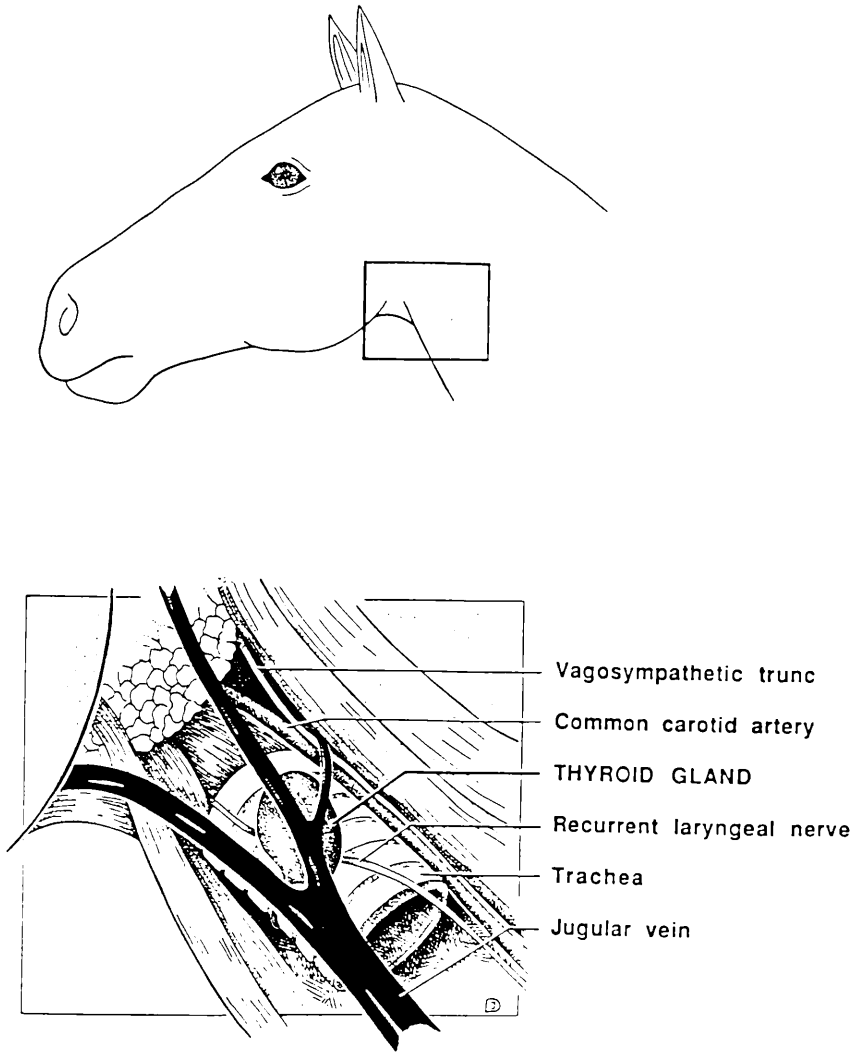


Figure 1 : Anatomy of the cranial cervical area showing the thyroid gland and the adjacent structures.

HISTOLOGY

The thyroid gland is surrounded by a thin capsule of dense irregular connective tissue. Thin trabeculae extend from the capsule into the parenchyma. The collagen fibres of the trabeculae continue into the sparse, loose interstitial connective tissue and into the reticular fibres of the basement membrane which surrounds the thyroid follicles and the capillaries.

Thyroid tissue is composed of follicles consisting of a simple epithelial sphere whose lumen contains colloid, a gelatinous substance. This colloid is amphoteric, staining with both acidic and basic dyes. Because it contains thyroglobulin, a glycoprotein, it is PAS-positive.

The thyroid, as previously stated, is an extremely vascular organ, having an extensive blood and lymphatic capillary network surrounding the follicles. Innervation of the thyroid via the sympathetic and parasympathetic systems, serves an essentially vasomotor function. Small nerve bundles enter the thyroid together with the larger blood vessels. There are primarily post-ganglionic sympathetic fibres which originate in the middle and superior cervical ganglia, although cholinergic fibres are also contributed to by the recurrent laryngeal branch of the vagus. The adrenergic fibres have been shown to terminate near the basal lamina of the follicular cells. These findings, together with evidence that adrenergic and other amines influence thyroid iodine metabolism, indicate that neurogenic stimuli can influence thyroid function (Junqueira 1989).

In normal sections, follicular cells range from squamous to low columnar cells. The morphologic appearance of thyroid follicles varies according to the region of the gland and its functional activity (Fig.2).

In the same gland, larger follicles that are full of colloid and have a cuboidal or squamous epithelium are found alongside follicles that are lined by columnar epithelium. In spite of this variation, the gland is considered hypoactive when the average composition of these follicles is squamous. The ultrastructure of the follicular epithelium exhibits all the characteristics of a cell that simultaneously synthesizes, secretes, absorbs and digests proteins. The basal part of these cells is rich in rough

endoplasmic reticulum. The nucleus is generally round and situated in the centre of the cell. The apical pole has a discrete Golgi complex and small secretory granules with the staining characteristics of follicular colloid. Abundant lysosomes, and some large phagosomes are found in this region. The cell membrane of the apical pole has a moderate number of microvilli. Mitochondria, distended cisternae of rough endoplasmic reticulum, and ribosomes are dispersed throughout the cytoplasm.

Another type of cell, the parafollicular, or C, cell, is found as part of the follicular epithelium or as isolated clusters between thyroid follicles. When parafollicular cells are found inside the follicular basement membrane, as part of the epithelium, their apical surfaces are never in contact with colloid. Thin processes of thyroid follicular cells intervene between parafollicular cells and the colloid. Parafollicular cells are somewhat larger and stain less intensely than thyroid follicular cells. They contain an abundant rough endoplasmic reticulum, long mitochondria, and a large Golgi complex. The most striking feature of these cells is their numerous, small, hormone containing granules. These cells are responsible for the synthesis and secretion of calcitonin. The effect of calcitonin is to lower blood calcium levels by inhibiting bone resorption (Tice 1977).

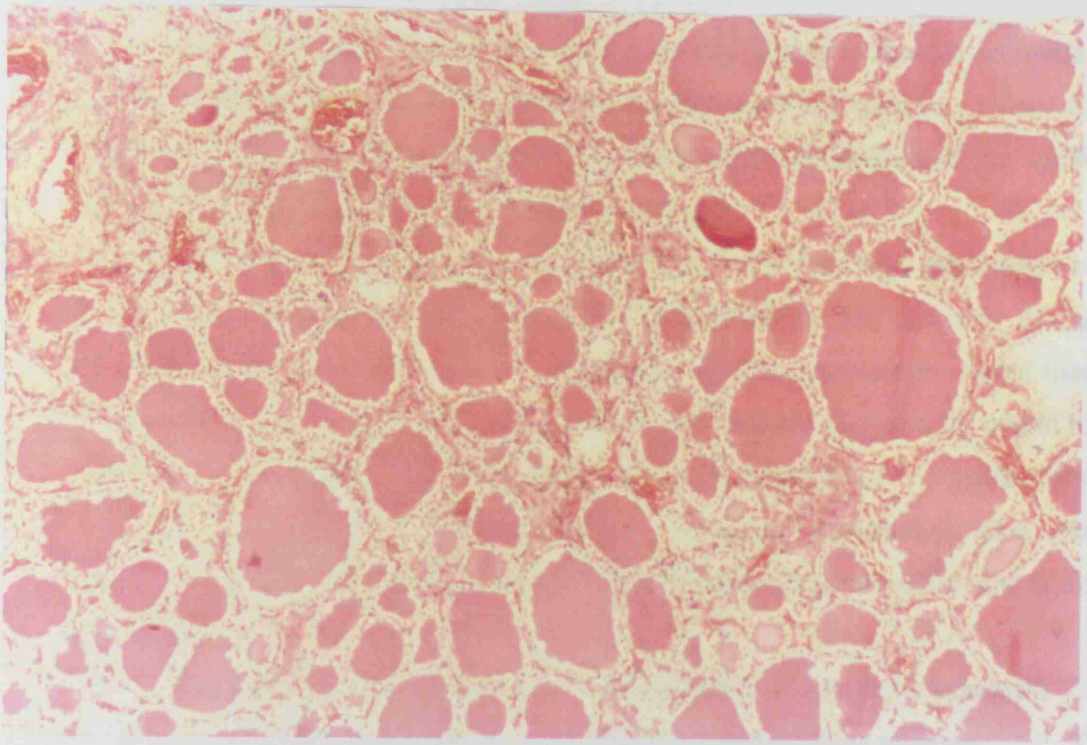


Figure 2 : Microscopic appearance of a normal thyroid gland (H-E; x160). Different size of follicles and follicular cells indicate a normal active gland.

PHYSIOLOGY

The thyroid gland is unique among endocrine glands in that its secretion, the thyroid hormone, includes in its structure a specific chemical element, iodine. The function of the thyroid gland involves the concentration of iodide and the synthesis, storage, and secretion of the thyroid hormone (Fig.3).

Iodine occurs throughout the animal body, but a very high percentage of the total amount is concentrated in the thyroid gland despite the fact that this gland constitutes only 0.2 per cent of the body weight. Iodine is present in animal tissue in two forms, inorganic iodide and organically bound iodine. Organically bound iodine occurs in extremely low concentration and the levels vary greatly. Various forms of organic-bound thyroid iodine are found, including monoiodotirosine (MIT), diiodotirosine (DIT), triiodothyronine (T3), reverse triiodothyronine (Rt3) and thyroxine (T4) (Dickson 1984).

After the iodide is trapped in the thyroid gland ("trapping mechanism"), it is oxidized to iodine in a reaction that is mediated by a peroxidase enzyme. The aminoacid tyrosine is then iodinated to form MIT and DIT. The coupling mechanism of the iodotyrosines to form iodothyronines has two possible routes, the combination of two DIT molecules to form T4, or the combination of one DIT with one MIT to form T3. The thyronines join a molecule of thyroglobulin in a ratio 5:1 (T4:T3). A molecule of thyroglobulin contains up to 6 molecules of thyroxine (T4). Finally the iodinated thyroglobulin is stored in the thyroid colloid.

Thyroglobulin is a glycoprotein, which has a high molecular weight. It is produced in the ribosomes from circulating aminoacids in the blood.

Secretion of thyroid hormone is initiated by endocytosis of colloid near the inner surface of the thyroid cell. The ingested droplets of colloid then are degraded by lysosomal enzymes releasing the iodinated aminoacids. Two of the iodinated aminoacids, T3 and T4, are secreted into the bloodstream, after hydrolysis, to liberate them from the thyroglobulin. The two iodotyrosines are deiodinated within the gland by a deiodinase enzyme. This cycle which is intrathyroidal, reclaims the iodide from tyrosine for use in the manufacture of the more active iodinated thyronine compounds.

Of the two iodinated thyronines, thyroxine (T4), is predominant in all animals; approximately 30% of the total iodine in the thyroid is in the form of T4 and usually less than 10 percent is in the form of T3. About 80 percent of the T3 appearing in the body is the result of extrathyroidal monodeiodination of the thyroxine by the enzyme 5'deiodinase and half of the thyroxine secretion by the gland is utilized for T3 formation. The inhibition of 5'deiodinase by fasting, illness or the administration of glucocorticoids, propylthiouracil or the radiographic contrast media sodium iopodate, will increase the T4:T3 ratio. In these cases rT3 (reverse triiodothyronine) levels are generally either normal or increased and TSH production is usually normal.

Furthermore, the patients are clinically euthyroid despite serum T3 levels which are frequently in the hypothyroid range. Collectively, these conditions have been called the "low T3 syndrome". From the stand-point of clinical medicine, they are perhaps more important for their perturbation of the tests of the thyroid function than as an indicator of abnormal thyroid function requiring therapy. Since this form of T3 formed by target cells is biologically inactive, monodeiodination to form reverse T3 provides a mechanism to attenuate the metabolic effect of thyroid hormones.

Corticosteroid administration results in a prompt fall in circulating T3, occurring within hours. At the same time there is an increase in plasma rT3. It is quite possible that some of the alterations observed in acute illness or stress may result from an increase in corticosteroid secretion (Robbins 1981).

Hood *et al* (1987) demonstrated that horses before onset of acute laminitis (Obel grade I and II) had a decrease serum T3 and T4 levels which could be explained, by the fact that TSH is inhibited in stressful situations. On the other hand, horses with chronic laminitis, show an increase in T3 in accordance with the increase in TSH secretion showed by animals exposed to chronic stress. However, this inhibition of TSH secretion in acute stress is a phenomena not fully understood.

Low circulating T3 is found in fetal or umbilical cord plasma. In such cases, the deiodination of T4 by 5 deiodinase increases resulting in higher levels of rT3, which is an inactive triiodothyronine, and may have antihormone properties (Pittman 1973).

Essentially none of the reverse triiodothyronine is derived from secretion *per se* by the thyroid gland.

In iodine deficiency there is a decrease T4:T3 ratio, and in this instance the increased T3 arises from preferential thyroid gland secretion of T3 as opposed to T4.

In hyperthyroidism, T3 secretion may increase to a greater extent than T4. The *in vivo* potency of T3 is about three times that of T4. This and the lesser binding affinity of T3 by plasma proteins, has led to the suggestion that T4 is a prohormone. However, there is strong evidence that T4 has intrinsic biological activity that is not dependent upon conversion to T3.

T4 is metabolized in the peripheral tissues by phenolic conjugation, deamination, decarboxilation and a cascade of monodeiodinations. Not all of these transformations occur in all tissues; for example, skeletal muscle can only deiodinate, whereas all the conversions listed, take place in the liver.

The T3 distribution through the tissues is quite different than T4, may be due to its lower affinity to bind the serum proteins. The liver contains approximately 5 percent and the plasma 18 percent of the total extrathyroidal T3. The major part, 75 percent, is in equilibrium in the tissues, being mainly stored in muscle, brain and skin.

Negative feedback control of thyroid hormone secretion is accomplished by the coordinated response of the adenohypophysis and certain hypothalamic nuclei to circulating levels of T4 and T3. A decrease in thyroid hormone concentration in plasma stimulate the hypothalamus which synthesise and secrete TRH into the hypophyseal portal circulation. As a result of this the adenohypophysis is activated and secretes TSH which stimulates the thyroid gland, resulting in release of T4 and T3 into the bloodstream.

One of the initial responses by follicular cells to TSH is the formation of numerous cytoplasmic pseudopodia, resulting in increased endocytosis of colloid and release of preformed hormone stored within the follicular lumen.

If the secretion of TSH is sustained, thyroid follicular cells become more columnar and follicular lumens become smaller due to increased endocytosis of colloid. Numerous PAS-positive droplets are present in the luminal aspect of the hypertrophied follicular cells.

The converse of what has been just described occurs in response to an increase in circulating thyroid hormone (T4 and T3) and a corresponding decrease in circulating

pituitary TSH. Thyroid follicles become enlarged and distended with colloid due to decreased TSH-mediated endocytosis of colloid. Follicular cells lining the involuted follicles revert to low cuboidal and there are few endocytic vacuoles at the interface between the colloid and follicular cells.

Thyroxine and triiodothyronine once released into the circulation act on many different target cells in the body. The overall functions of thyroxine and triiodothyronine are similar, though much of the biological activity is the result of monodeiodination to 3, 5, 3' triiodothyronine (T3) prior to interacting with target cells.

The best known function of thyroid hormones in the mammal is their ability to increase the rate of oxygen consumption. It causes increased utilization of carbohydrates, increased protein catabolism as indicated by greater excretion of nitrogen, and greater oxidation of fats as indicated by loss in body weight. The administration of thyroxine will increase the heart rate by a direct effect on heart muscle cells. Nervous function at all levels is influenced by the thyroid. Injection of thyroxine causes increased spontaneous electrical activity in the brain, a decreased threshold of sensitivity to a variety of stimuli, decreased reflex time, and increased neuromuscular irritability.

The overall effects of thyroid hormone are to:

- Increase the basal metabolic rate (BMR).

- Make more glucose available to meet the elevated metabolic demands by increasing glycolysis, glyconeogenesis, and glucose absorption from the intestine.

- Stimulate new protein synthesis.

- Increase lipid metabolism and conversion of cholesterol into bile acids and other substances, activation of lipoprotein lipase and increase the sensitivity of adipose tissue lipolysis by other hormones.

- Stimulate the heart rate, cardiac output, and blood flow.

- Increase neuronal transmission, cerebration, and neuronal development in young animals (Dickson 1984).

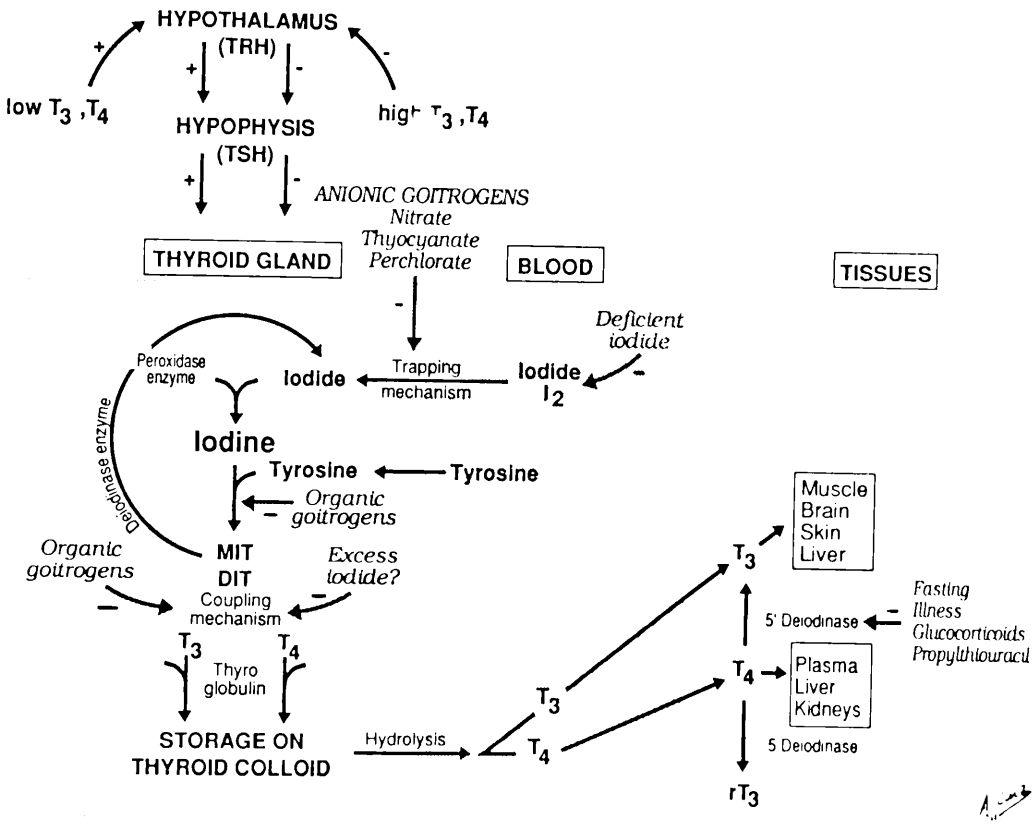


Figure 3 : Brief summary of the main pathways of the thyroid physiology.

FUNCTIONAL DISORDERS

HYPOTHYROIDISM

This condition is not a well recognised clinical entity in the equine. Despite the fact that equine hypothyroidism has been characterised by surgical thyroidectomized animals (Lowe *et al* 1974) the diagnosis still remains difficult due to the poor availability of reliable diagnostic tests. Hypothyroidism has been associated with laminitis (Britton 1959), infertility, alopecia (Stanley and Hillidge 1982), musculoskeletal problems, and respiratory insufficiency (Murray 1990). Some of these reports are based on the fact that treatment with thyroid hormone helped to resolve the clinical condition.

Hypothyroidism is the clinical manifestation of a deficiency of circulating thyroid hormone, therefore, understanding the biological action of the hormone can help establish the expected range of clinical signs that might be seen in the deficient animal. The deficiency of thyroid hormone may be due to primary disease of the gland, either hereditary or acquired, secondary to disease of the pituitary or hypothalamus resulting in a decrease of TSH level, to unavailability of precursors needed for elaboration of thyroid hormone, specifically iodine, or to antithyroid or goitrogenic substances that inhibit synthesis or release of thyroid hormone.

Failure to convert T4 into T3 has been indicated as a possible cause of hypothyroidism in one horse (Stanley and Hillidge 1982), although it could not be confirmed. Also inflammatory lesions, thyroiditis, of the thyroid gland may lead to hypothyroidism but to my knowledge it has not been reported in the horse. Some of these causes are associated with thyroid enlargement, such as ingestion of goitrogens, dyshormogenesis or thyroid neoplasia.

At present there are no reports in the literature which describe a reliable method to investigate the whole complex of thyroid metabolism in the horse and there is a great lack of information about the mechanisms involved in the production of T3, rT3 and subsequent degradation of T4. Since T3 is biologically much more active than T4, specific diagnosis of hypothyroidism is difficult. In the report of Stanley and Hillidge (1982), the horse presented bilaterally symmetrical alopecia, dullness, lethargy,

myxoedema, and a considerable reduction in testicular activity (Plasma testosterone 65 pg/ml) with testicular hypoplasia.

Musculoskeletal problems related to hypothyroidism include severe weakness, defective ossification leading to collapse of the central and third tarsal bones (Shaver *et al* 1979), osteochondrosis (Jeffcott 1991), laminitis (Britton 1959) and "tying up" syndrome (Mc Laughlin and Doige 1981).

A recent report of hypothyroidism associated with respiratory insufficiency in a neonatal foal, showed a similarity with a human syndrome. Thyroid hormones have a central role in the morphologic and physiological development of the fetal and neonatal pulmonary system, and administration of exogenous thyroid hormone enhance synthesis of lung surfactant in clinical conditions. In this report, the histologic changes in the foal's lungs were consistent with impaired or incomplete surfactant development (Murray 1990).

Histologically, the hypothyroid animal may have several changes in the thyroid gland. Hypothyroidism, secondary to long-standing pituitary or hypothalamic lesions that prevent the release of either thyrotrophic hormone TSH or thyrotropin releasing hormone (TRH), shows a gland moderately reduced in size and composed of colloid distended follicles lined by flattened follicular cells. This is due to a lack of TSH induced endocytosis of colloid and secretion of thyroid hormones.

Iodine deficiency produces a colloid goitre (Doidge and McLaughlin 1981). Since iodine is the limitating factor to produce T4, lack of such element results in a decrease of thyroxine which stimulates the feed-back mechanism leading to an increase in TSH which stimulates production of thyroglobulin. Due to the lack of iodine there is no T4 production and the thyroglobulin is stored on its own in the colloid leading to an enlarged gland with a flat follicular epithelium and big follicles.

On the other hand, hypothyroidism due to excess dietary iodide is believed to be produced because the release of T4 is prevented by an unexplained mechanism (Baker and Lindsey 1968), probably through an interference with the "coupling" mechanism.

HYPERTHYROIDISM

There are a few reports of hyperthyroidism in horses. One report associates nervousness, hyperexcitability and sweating with laboratory evidence of increase thyroid function (De Martin 1973). The syndrome has been suspected in other highly-strung, unmanageable horses. In at least one case, thyroidectomy resulted in a change in demeanour of a high-strongly horse; the owner was able to train and ride the animal after surgery, whereas this was impossible preoperatively (Lowe *et al* 1974).

CLINICAL INVESTIGATION

MATERIAL AND METHODS

Ten cases with a history of thyroid enlargement were referred to the Glasgow University Veterinary Hospital (GUVH), and two to the Western College of Veterinary Medicine (WCVM), Saskatchewan, during a one year period.

All of them belonged to different ages, breeds, sex, and areas.

The protocol of investigation consisted of:

a) HISTORY

General signalment of the animal were recorded and a careful note of any reproductive, behaviourial, or locomotor problems (Fig.4).

Special attention was given to the area where the animal came from and the diet, because of the importance of the amount of iodide ingested by the animal. Levels above 35 mg/day have been reported as goitrogenic despite National Research Council (NRC) advice , which is about 0.1 mg/Kg/day (Baker and Lindsey 1968).

b) CLINICAL EXAMINATION

None of the horses presented obvious clinical signs at presentation which could be related to the enlarged thyroid, except case No.11 which was lethargic and always retained a winter coat.

Horses were carefully examined and blood samples were taken for routine haematology and biochemistry profiles (Normal reference values are given in appendix I). In those horses were a TSH stimulation test was carried out, haematology and biochemistry profiles were also investigated after stimulation. Palpation of the thyroid gland was used as a primary method to determine the enlargement.

Thyroid weight was not recorded because thyroidectomy was only carried out in some of the horses, and none of the horses were destroyed. Therefore, a gland was considered enlarged based on visual appreciation and palpation.

c) ULTRASONOGRAPHY

B-mode ultrasonography was undertaken in some of the cases as a method to establish the architecture of the gland, and the quality of the thyroid tissue, through interpretation of the echogenic pattern.

The retropharyngeal area was clipped and shaved around the area caudal to the mandible and above the bifurcation of the jugular vein.

A 5 and a 7.5 MHz probe was used in parasagital and transverse planes to determine the value of the three dimensions, which were measured at their maximum values, obtaining very accurate results. Parallel to this clinical study, morphometric study of the gland was undertaken in three normal horses (Table 2). The volume of the gland was obtained with the following formula:

$$V = 1.6/pi \times Height \times Diameter \times Width$$

(Azagra *et al* 1990).

d) TSH STIMULATION TEST

After ultrasonography a TSH stimulation test was carried out. The reason for proceeding with this after scanning was to avoid the possibility of iatrogenically altering the structure of the gland.

10 mls. of blood were withdrawn from the jugular vein and collected in a plain sterile tube to measure serum T4 levels. There is no difference between serum and plasma T4 levels (Hightower, Miller and Kyzar 1971). Immediately afterwards an intravenous injection of 5 i.U of Bovine Thyrotropin (TSH, Sigma Chemical Co., St.Louis, U.S.A) was made and 4 hours later another 10 mls. blood sample was withdrawn in accordance with the protocol of the test developed by Held and Oliver (1984) (Fig.5).

Serum T4 levels were measured by R.I.A. (Thomas and Adams 1978). The time of the day at which the test was performed, was taken into account, due to the diurnal variations in thyroid hormone secretion (Duckett and Manning 1989). Normal base line values of serum T4 are 1.57+/- 0.62 ug/dl in mature horses (Thomas and Adams 1978) and 4.02 ug/dl in foals (Chen and Riley 1981). The TSH stimulation test

remains the most reliable parameter to differentiate between primary and secondary hypothyroidism.

The R.I.A used in cases No.1, 5, 9 and 10 was not validated, but calibrated for use in the Equine. Since none of the horses were within the limit of hypothyroid values we considered them as reliable. The percentage of increase was calculated to compare the results with the normal ones established by Held and Oliver (1984).

e) SCINTIGRAPHY

The main and most useful application of radioisotope imaging is the detection, localization and quantitative assessment of dynamic tissue change or turnover, although it is used to a limited extent to monitor whole organ function. Radioisotope imaging therefore is useful to indicate the degree of activity of established anatomical change and to predict the initiation of structural abnormalities resulting from continuing pathological activity (Attenburrow and Vennart 1988). Combined use of this technique and ultrasonography has given excellent results in human medicine as a method to diagnose malignant neoplastic processes (Ikekubo *et al* 1896). The principle of radioisotope imaging and scanning is based on the use of pharmaceuticals, which when introduced into the blood stream concentrate in a particular tissue or organ. The pharmaceutical is first labelled with a suitable gamma-ray emitting radioisotope so that its distribution and concentration within a particular organ can be imaged and quantified (Attenburrow and Vennart 1988).

Free ^{99m} TcO_4 was used because any carrier can interact with the normal absorption of the radioisotope (Attenburrow, personal communication). Pertechnetate was chosen because it was readily available and relatively inexpensive. Likewise it has a short half-life, emits a low energy (140 keV) photon well suited to scintigraphy, does not undergo beta decay, and delivers the lowest radiation absorbed dose to thyroid of the available radionuclides.

The equipment used, in cases No.5, 9 and 10 was a photo-multiplier or gamma-counter with a collimator of 8mm of diameter and 0.5 inches length (Fig.6). 3 MBq/Kg of such isotope was injected intravenously and counts were taken every three minutes during the following hour. Counts were taken separately from each thyroid

lobe, with values from the pectoral muscles taken as a reference. These were processed by a computer, to allow easier count manipulation.

In cases number 6, 11 and 12 a gamma camera was used by courtesy of the Dept. of Surgery of Liverpool School of Veterinary Science. The same dose of radioisotope was used and a ventro dorsal view was obtained 30 minutes later. Radioisotope uptake was compared between both lobes as well as any difference in size or density of the scintigraphic image.

f) THYROID BIOPSY

Finally a tru-cut thyroid biopsy was taken from the enlarged lobe and submitted for histopathology. The sample was fixed in 4 % formalin and a routine Haematoxylin-Eosin staining method was used.

CASE No AGE SEX BREED

DATE MAIN DIAGNOSIS

Where has it come from ?

Description of the diet ?

Any other veterinary problems ?

Any kind of therapy at the moment ?

Another owner complaint at the moment?

Type of work?
General history ?

Reasons for admittance in the Veterinary School

GENERAL EXAMINATION

General health : Body condition :

Description of palpation of the thyroid area :

Duration and progression of the process :

Any reproductive problems ?

Any change in behaviour ?

General exam (Ta, H.R, R.R, Haematology, biochemistry, etc...)

ANCILLIARY AIDS

Figure 4 : Signalment form used for this investigation.

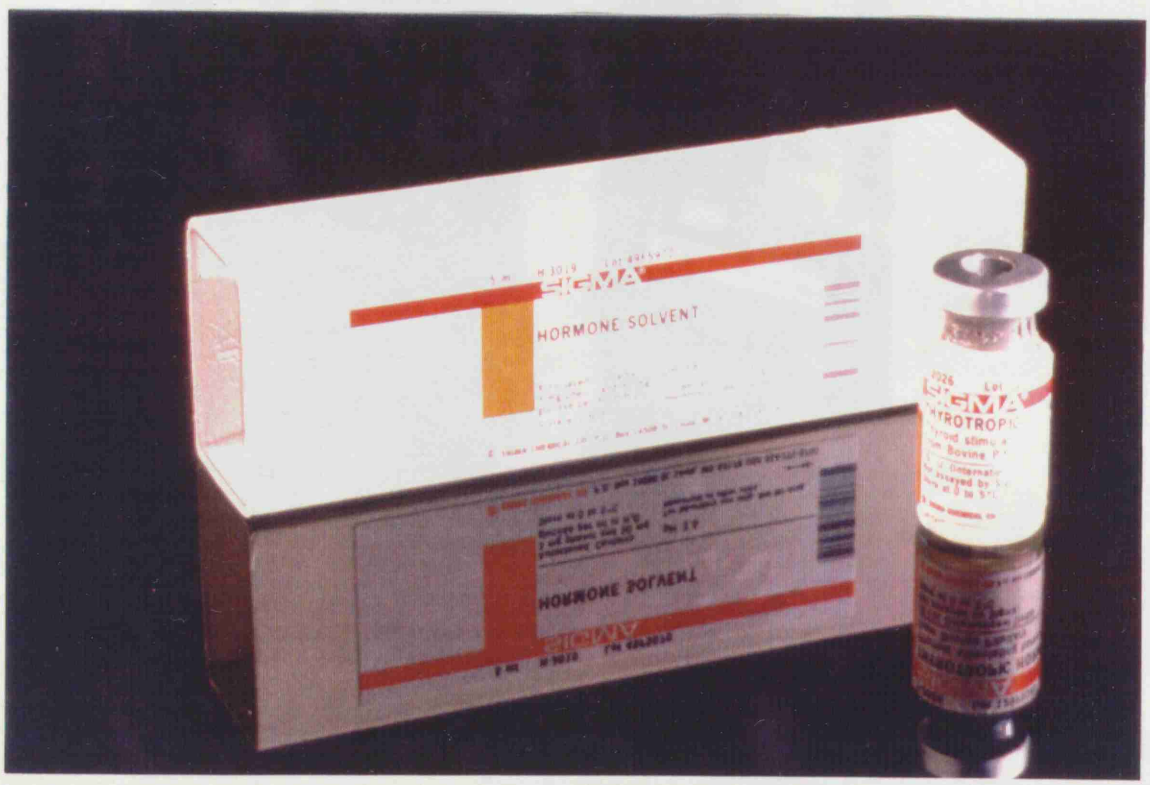


Figure 5 : Bovine thyrotropin used in the TSH stimulation test (Sigma Co. St.Louis, U.S.A).

Figure 6 : Gamma-Counter used for scintigraphic evaluation of the thyroid gland.

CASE RECORDS

CASE 1 (Fig.7)

A 23 yrs.old riding pony mare was presented with a history of right thyroid enlargement for the past three years. Previous history of the mare, in accordance with owner's information, revealed a permanent oestrus behaviour during the last breeding season, which was treated with synthetic progestagens (*Regumate*, Intervet Ltd.) without success.

The mare came from Lanarkshire (Central Scotland) and the diet was composed of bran, nuts, oats, molychop and a vitamin supplement (*Vitahorse*).

The owner reported that the mare was in a very good condition and able to work well.

On palpation the mass was firm, very loose within the deep fascia and situated under the jugular furrow caudal to the larynx in the right part of the neck. It was 9 cms.high, 7 cms.long and 5 cms.wide.

Besides a chronic navicular syndrome the mare remained normal.

Further investigation involved routine haematology and biochemistry profile resulting in normal parameters.

TSH stimulation test was carried out, and a needle biopsy of the gland.

The horse was not clinically affected and after discussion with the owner, he decided not to undertake a thyroidectomy.

CASE 2

A 15 yrs. old hunter gelding was referred to the GUVH, with a history of a mass on the right side of the cranial cervical region.

Within the last three months the lump was observed to increase in size, and at presentation it was approximately 6 x 4 cm. in size.

The horse was from Dumfries (South West Scotland) and the diet was composed of mixed bran and grass. The owner reported the occurrence of a sebaceous cyst removed from the ventral mandible when the horse was two years old and a previous diagnosis of COPD, treated with bronchodilators (*Ventipulmin*, Clenbuterol;

Boheringher Co.) and mucolytics (*Sputulosin).* Otherwise the horse was in good condition and all the clinical parameters were within normal levels.

At the owner's request the mass was surgically resected and the horse remains is in good health.

CASE 3

A 18 yrs. old arab mare presented with a thyroid enlargement for the last year. The lump was ovoid in shape and approximately 15 cms. long and 10 cms. wide.

There was an obvious respiratory problem with nasal discharge and abnormal respiratory noise, but the rest of clinical parameters were normal at presentation.

The mare came from Blackpool (North West England) and the diet was composed of hay, molichop, sugar beet pulp and oats with a mineral supplement. The mass was on the right hand side and at palpation it was firm and loose within the fascia.

One of the main concerns of the owner was that the mare was covered twice, without success, during the last year.

A needle biopsy was taken showing a low grade thyroid adenoma and the gland was surgically removed. After the surgery the mare was successfully covered and the respiratory noises and nasal discharge disappeared.

CASE 4

A 6 yrs. old Pony mare from Liphook (Hampshire), presented an episode of acute laminitis, without displacement of P3 (Obel grade 1).

At presentation the biochemistry and haematological profiles were all normal. The pony presented as an obese phenotype and the left thyroid lobe was noticed to be enlarged. It was firm to touch, loose in the deep fascia and not nodular. Accurate measures were not taken but it was approximately large egg sized.

The pony was treated for the laminitic condition with phenylbutazone (2 g/day) for one week, acepromazine (0.03 mg/Kg) and frog supports. The pony was believed to be hypothyroid and thyroxine tablets were administered by the referring veterinarian at the dose of 1 mg./s.i.d.

After one month the thyroid was reduced in size, and the gait had improved.

Nine months later a TSH stimulation test was performed. The horse remains in good condition and sound.

CASE 5 (Fig.8)

A 5 yrs. old riding pony gelding from Dumfries (South West Scotland) was admitted to the GUVH with a history of headshaking for the last ten months. The owner also reported the growth of a mass in the cranial cervical region for the last ten weeks.

At presentation all the clinical parameters were normal.

The horse was on diet of pony nuts, grass, molichop, hay, sugar beet pulp and seaweed supplement.

At examination a mass was noted to correspond to the left thyroid lobe. It was smooth and not attached to the deep tissues. The dimensions were approximately 5.5 cms. high, 4.8 cms. wide and 2 cms. thick.

Further examination did not reveal any abnormality in the eyes, ears, sinuses, mouth, or external nares. No facial distortion was seen.

At exercise the pony showed an abrupt vertical headshaking more acute if outdoors. Riding with different bits did not make any difference.

After sedation a standing lateral and 25° oblique radiographic views of the head were taken without any abnormality being detected.

Videoendoscopy was carried out and no abnormality detected in the head or neck. Further investigation of the headshaking syndrome was carried out without success.

TSH stimulation test, ultrasonography and scintigraphic study were carried out with no abnormality being detected. A tru-cut biopsy was taken without finding any pathological abnormality.

Removal of seaweed from the diet was advised and six weeks later the horse still headshaked, but the thyroid lobe was notably reduced in size.

CASE 6

A 1 yr. old filly was presented to the Liverpool School of Veterinary Science with a history of thyroid enlargement since birth.

The right one was well circumscribed with a dimensions of 10 cms. x 5 cms. x 3cms. and spherically shaped. It was firm and smooth on palpation.

The general health of the filly was excellent, and all clinical parameters were normal including haematology and biochemistry profiles. The filly's dam received a seaweed supplementation from October through to foaling. This was a new product which the owner had fed to no other mares before or since. The diet of the filly included oats, barley, sugar beet pulp and grass. The mare was clinically normal and had no previous history of foals with thyroid enlargement.

Serum T3 and T4 levels were determined, as well as TSH stimulation test, ultrasound and scintigraphic images and histopathology of the gland.

As a result of this examination we concluded that the lobe was normal, but the owner decided to remove it for aesthetic reasons.

Histological study after thyroidectomy showed macroscopically a large thyroid of 93.5 grams. of weight with dimensions of 8 cms. x 5 cms. x 3.5 cms.

Based on all our findings a diagnosis of hyperplastic goitre was made.

The filly was in perfect general health at the three month follow up check.

CASE 7 (Fig.9)

A 13 years old arab gelding was presented to the WCVM, with a history of thyroid enlargement for the last year. The horse seemed to be in good condition and at clinical examination no abnormality was detected. Serum T4 levels were determined as well as TSH stimulation test, ultrasound examination and biopsy of the gland. Currently the horse is in good clinical condition and awaiting thyroidectomy.

Histopathology showed a malignant process difficult to identify in a single biopsy.

CASE 8 (Fig.10)

A 27 years old TBx gelding was presented to the WCVM with a history of long standing respiratory distress concurrent with an enlarged thyroid. The horse was diagnosed as having a thyroid adenoma 4 years previously.

At presentation the horse was in very poor condition and dyspnoeic. Clinical examination revealed widespread abnormal emphysematous sounds over the entire the lung field.

An obvious enlarged left thyroid lobe was palpated. It was smooth, and loose within the deep tissues. The dimensions were approximately 13 cm x 9 cm x 7 cm.

Because the horse was presented for euthanasia and the strong sentimental attachment to the owners, further investigation could not be carried out, except serum T4 level determination.

CASE 9

A 4 yrs. old TBx gelding with a history of thyroid enlargement for the last 8 months was presented to the Glasgow University Veterinary Hospital.

Initial examination did not reveal any abnormality except a mass on the right cranial cervical area that on palpation was identified as the right thyroid lobe.

The owner reported a change in behaviour since the mass was first noticed, with the horse becoming more excitable.

Serum T4 level, TSH stimulation test, ultrasound exam, scintigraphy and biopsy were undertaken.

Currently the horse is in excellent health.

CASE 10

A 3 yrs. old TBx mare was presented to the GUVH with a history of a mass in the neck for the last month.

The mare was kept outside and received a herbs supplement. It came from the south of Scotland.

At clinical examination a mass on the right cranial cervical area was detected. On careful palpation the mass was smooth, rounded and easily movable, identifying

it as an enlarged thyroid lobe. The left lobe presented the same characteristics on palpation, although it was obviously smaller.

Serum T4, TSH stimulation test, ultrasound scan and biopsy were carried out.

The mare otherwise was in excellent condition, although the owner reported a change in behaviour coincident with the development of the mass.

CASE 11

A 13 yrs. old Welsh pony gelding was presented to the Liverpool School of Veterinary Science with a history of an approximately golf ball sized swelling for the last twelve months, gradually increasing in size during this period. The owner reported that the pony seemed to be much quieter and that he always retained a winter coat during summer months.

At examination the pony was normal except it retained a winter coat. On palpation of the mass, it was located on the left ventral cervical region, freely movable and firm. It was identified as the right thyroid lobe.

A serum T4 determination, TSH stimulation test, scintigraphy and biopsy were performed.

Based on results and owner decision, a right partial thyroidectomy was performed. Recovery was uneventful.

CASE 12

A 14 yrs. old pony gelding was referred to the Liverpool School of Veterinary Science with a left unilateral thyroid enlargement. The pony came from North-West of England.

At clinical examination no abnormality could be detected.

Palpation of the mass revealed a distinct left lobe, larger than the right one, freely movable and non nodular.

Serum T4 level determination, TSH stimulation test, thyroid scintigraphy and biopsy were undertaken.

The pony is in good general health.

Figure 7 : Gross appearance of Case No.1

Figure 8 : Gross appearance of Case No.5

Figure 9 : Gross appearance of Case No.7

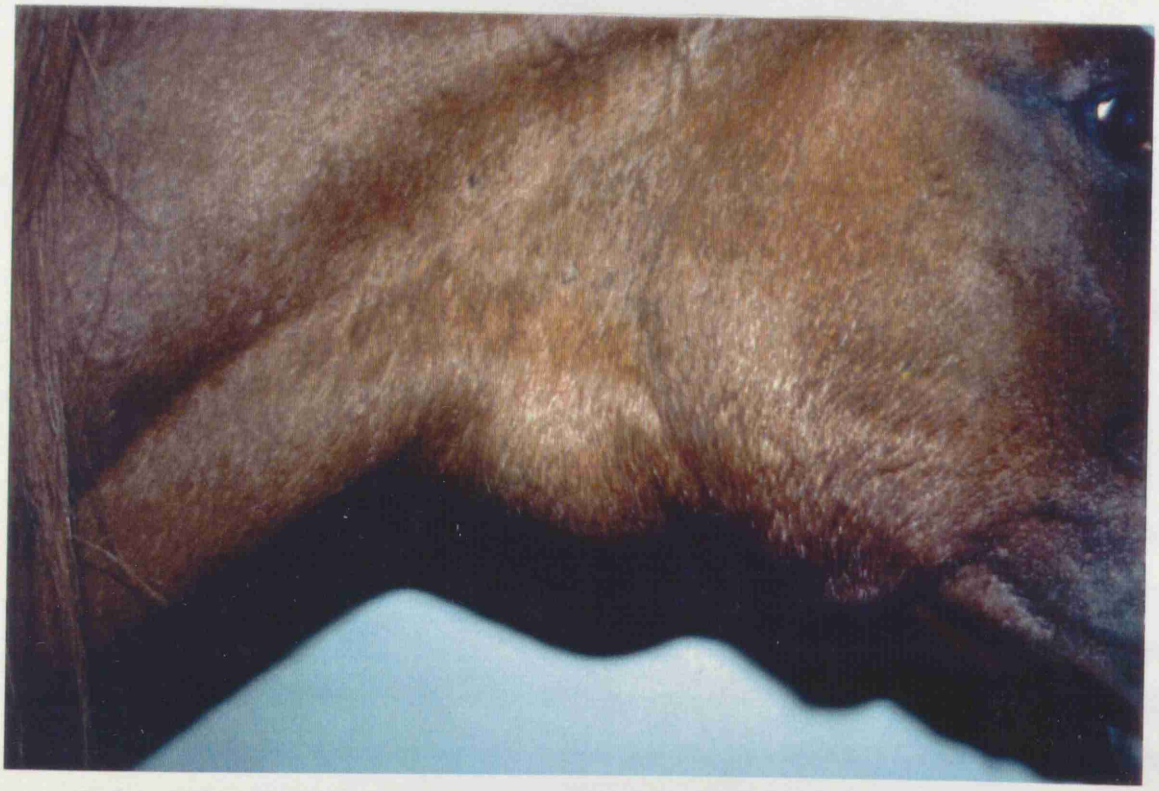


Figure 10 : Gross appearance of Case No.8

RESULTS

Clinical Features

All the horses investigated belonged to different ages,breeds, sex, and areas (Table 1). Except case No.11, none of the horses investigated showed signs of hypothyroidism. However, it is interesting that in the history of most of them there were signs that probably were related to changes in the thyroid function (Table 1). Case No.1 had a history of permanent oestrus during the last season which was refractory to treatment with synthetic progestagens. Case No.3 presented an obvious respiratory problem with dyspnoea and serous nasal discharge which resolved after thyroidectomy. Case No.4 had an obese phenotype and was presented in acute stage (Obel grade 1) of laminitis. In case No.5 the horse was referred because of a history of headshaking, and was a quite excitable horse, although the owners did not report any change in behaviour since the growth of the mass. Cases 9 and 10 were reported with a change in behaviour, and case No.11 was lethargic and had retained a winter coat.

The filly presented in case No.6 was delivered from a mare receiving dietary seaweed supplementation during pregnancy. Case No.8 was diagnosed as a thyroid adenoma four years ago and was presented for euthanasia, due to a chronic respiratory problem and poor body condition accompanied by general weakness.

Biochemistry or haematology panels did not show any abnormality.

On palpation the masses were smooth, firm, and not attached to the deep tissues except cases No.7 and No.8 where the masses were relatively fixed to the cervical fascia. Nodules could not be detected in any of the palpated thyroids.

Ultrasound Examination

Ultrasonography of cases No.6, 7, 9 and 10 was consistent with the histologic findings. Different patterns of echogenicity were recorded, and the affected gland in cases 6 (Fig.11) and 7 were more echogenic than the normal one, revealing a decrease in colloid, and increase in soft tissue density. In case No.6 this was consistent with a hyperplastic goitre and in case No.7 with a thyroid carcinoma. Cases 9 and 10 were normal and the ultrasonography did not show any difference between both lobes (Fig

determined as well as TSH stimulation test in some of the horses (Table 4). Serum T4 levels were within normal levels. Response to TSH was very variable, and in accordance with the results of Held and Oliver (1984) abnormal in cases 1, 5, 7, 9, 10, 11 and 12 (See Table 4). In cases No.2, 3, 4 and 8 TSH stimulation test was not carried out. TSH stimulation test evaluates the Hypophyseal-Thyroid axis and the functionality of the gland, but the thyroid function can be inhibited through several pathways such as the conversion of T4 into T3 (deiodination mechanisms), or the binding of thyroid hormones to serum proteins. More research is needed to identify such pathways and the possible ways that they can be inhibited.

CASE	AGE	SEX	BREED	CONCURRENT CONDITIONS	AREA
1	23	F	Riding pony	Reproductive problems	West Scotland
2	15	G	Hunter	None	South Scotland
3	18	F	Arab	Respiratory and reproducutive	North England
4	6	F	Riding pony	Laminitis	South England
5	5	G	Riding pony	Headshaking	South Scotland
6	1	F	TB	None	North England
7	17	G	Arab	None	West Canada
8	27	G	TB-X	Respiratory distress	West Canada
9	4	G	TB-X	Behaviour change	South Scotland
10	3	F	TB-X	Behaviour change	South Scotland
11	14	G	Riding pony	Lethargy, retained winter coat.	North England
12	13	G	Riding pony	None	North England

Table 1 : General signalment of the horses investigated.

	LENGTH (cm)		THICKNESS (cm)		WIDTH (cm)		VOLUME (ml)		BODY WT (kg)	BODY HT (cm)
	R	L	R	L	R	L	R	L		
A	1.4	1.8	3.4	4.2	1.5	1.7	3.7	6.7	410.8	132
B	2.6	2.5	4.3	4.5	2.5	2.2	15	13	519.8	135
C	2.7	2.5	4.2	4.2	2.2	2.3	13	13	544.5	145

L: Left thyroid lobe; R: Right thyroid lobe

WT: Weight; HT: Height

Table 2 : Shows the results of the dimensions of the thyroid lobes of three normal horses.

CASE	THYROID LOBE	LENGTH (cm)	THICKNESS (cm)	WIDTH (cm)	VOLUME (ml)	BODY WT (kg)
1	R	9	5	7	127.4	378
2	L	NM	NM	NM	NM	NM
3	R	10	8	15	624	457
4	L	NM	NM	NM	NM	NM
5	L	5.5	2	4.8	27.45	443
6	R	10	3	5	78	420
7	R	6	5.1	4.5	70.11	471
8	R	15	8.2	10	626.31	454
9	R	3.9	3.5	3.2	22.24	612
10	R	5.1	3.2	3.8	41.44	563
11	R	NM	NM	NM	NM	?
12	L	NM	NM	NM	NM	?

R: Right thyroid lobe; L: Left thyroid lobe

NM: Not measured

Table 3 : Dimensions of the enlarged lobes taken by ultrasonography.

CASE	AGE	SEX	PRE-TSH T4 (µg/dl)	POST-TSH T4 (µg/dl)	% INCREASE
1	23	F	2.04	2.96	45.5
2	15	G	1.25	?	?
3	18	F	?	?	?
4	6	F	2.22	4.66	109.9
5	5	G	1.4	6.86	390
6	1	F	2.86	5.68	98.5
7	17	G	1.86	2.17	16.6
8	27	G	?	?	?
9	4	G	2.89	3.21	11.07
10	3	F	1.62	1.72	6.17
11	13	G	1.13	1.55	37
12	14	G	1.86	1.86	0

?: Not undertaken; F: Female; G: Gelding

Table 4 : Results of TSH stimulation test

Scintigraphy

Gamma-counter scintigraphy was performed in cases No.5, 9 and 10. As a very experimental method, the results were processed in such a way to see the percentage of increase on absorption of one lobe in comparison with the other one. The results of case No.5 are shown in figure 14. They show that there is no variation in the uptake of the radioisotope in any of the lobes, showing similar counts each time. Out of 21 counts, 13 represent increase in uptake by the right lobe and 8 represent increase by the left lobe which was the one enlarged. In the case of the right lobe, such increase is higher than 50 % on three occasions, between 20 % and 50 % on three occasions and between 0 and 20 % on six occasions, the maximum increase being 87 %. Regarding the affected lobe, the percentage of increase was higher than 50 % on three occasions, between 20 % and 50 % once and lower than 20 % on four occasions, the maximum increase being 68 %. Similar results were seen in cases 9 and 10.

The distribution of these values in the time shows how in both lobes, the uptake is increasing with time, reaching their maximum values at 48 min.(left lobe) and at 54 min.(right lobe), taking into account that only the first hour was recorded. The graph shows how the radioisotope uptake is very uneven in both lobes, while in the reference points (pectoral ms.), the uptake is very regular, showing the maximum values just after injection of the radioisotope, and decreasing uniformly with the time. The absolute value of the uptake of the reference points was much lower than the one of each thyroid lobe, and the distribution of values with time was also different. While the uptake in the thyroid gland increases with time showing a peak around 45 - 55 min., the reference point shows a peak just after injection of the radioisotope, decreasing uniformly with time.

It is very interesting to see that in both thyroid lobes there is an almost parallel distribution of values within the time, although on some occasions they are well apart.

In cases No.6, 11 and 12 a scintigraphic image was obtained. Figure 15 shows the images obtained in case No.6. The right lobe is clearly larger than the left, although there is not difference in the radioisotope uptake intensity.

Histopathology

The histopathology results are very different. Only cases No.5, 9 and 10 showed a normal appearance of the gland with regular follicles and a low cuboidal epithelium, the appearance of a mildly active gland (Figs 22 and 23). In case No.4 a biopsy was not taken.

Case No.1 there was histological evidence of a malignant process composed of follicles, solid cords and small groups of thyroid epithelial cells in fibrous tissue stroma, with a low number of mitotic figures. A diagnosis of thyroid follicular adenocarcinoma was established (Fig.16).

Case No.2 was diagnosed as thyroid follicular adenocarcinoma but neither the pathology report or the slides were available to the author.

Case No.3 showed a marked proliferation of follicular cells with very few follicles, and little colloid. These findings suggest a diagnosis of follicular adenoma (Fig.17).

Case No.6 showed an enlarged gland of 93.5 gms. of weight which dimensions were 8 cm.x 5 cm. x 3.5 cm. Microscopically there was a compressed fibrous capsule covering variable sized but plentiful well-stained follicles with marked peripheral vacuolation. Most follicles were lined by a single layer of flat epithelium. In some follicles there was focal regular thickening of the epithelium. As a conclusion a diagnosis of hyperplastic goitre was made (Fig. 18).

Case No.7 showed an outer fibrous capsule and an inner fibrous trabeculae separating compressed thyroid tissue and what seemed to be an area of neoplastic C-cells. There was some degree of pleomorphism in cell and nuclear size, but there was no increase in mitotic figures. The cytoplasm of these cells was intensely eosinophilic and granular, typical of C-cells. One large nerve fibre was completely surrounded by C-cells. This tumour also contained scattered thyroid follicle-like structures containing eosinophilic colloid-like material. The adjacent thyroid gland was markedly compressed with very few normal thyroid follicles present. As a result of this biopsy we concluded that there was a C-cell tumour which was most likely an adenoma, with an involvement of thyroid tissue, difficult to identify in a single biopsy (Fig.19).

The histological appearance in Case No.8 was that of a follicular adenoma.

There was a small nodule within the other lobe, which appeared normal on gross examination. Sections from the large thyroid mass consisted of fairly uniform sheets of cuboidal cells separated by irregular and variable trabeculae. There were follicles containing colloid scattered through the tissue. The cytoplasm was eosinophilic and no mitotic figures or evidence of invasiveness was found, although the mass was compressing normal thyroid tissue. These findings were consistent with those of follicular adenoma (Fig.20).

The nodule within the other gland showed similar histological appearance, but existence of fibrous capsule could not be detected concluding in a diagnosis of thyroid follicular hyperplasia (Fig.21).

In case No.11 a section of the gland was taken after thyroidectomy. Abnormal proliferation of follicular cells with no apparent fibrous capsule and evidence of capillary invasiveness was seen. Very little colloid was also seen, but no abnormal mitotic figures. Thyroid follicular carcinoma was diagnosed (Fig.22).

Biopsy in case No.12 represented an abnormal proliferation of follicular cells, with some follicles scattered through the biopsy field. No evidence of invasiveness or abnormal mitotic figures could be detected. These findings lead us to diagnose thyroid follicular adenoma (Fig.23).

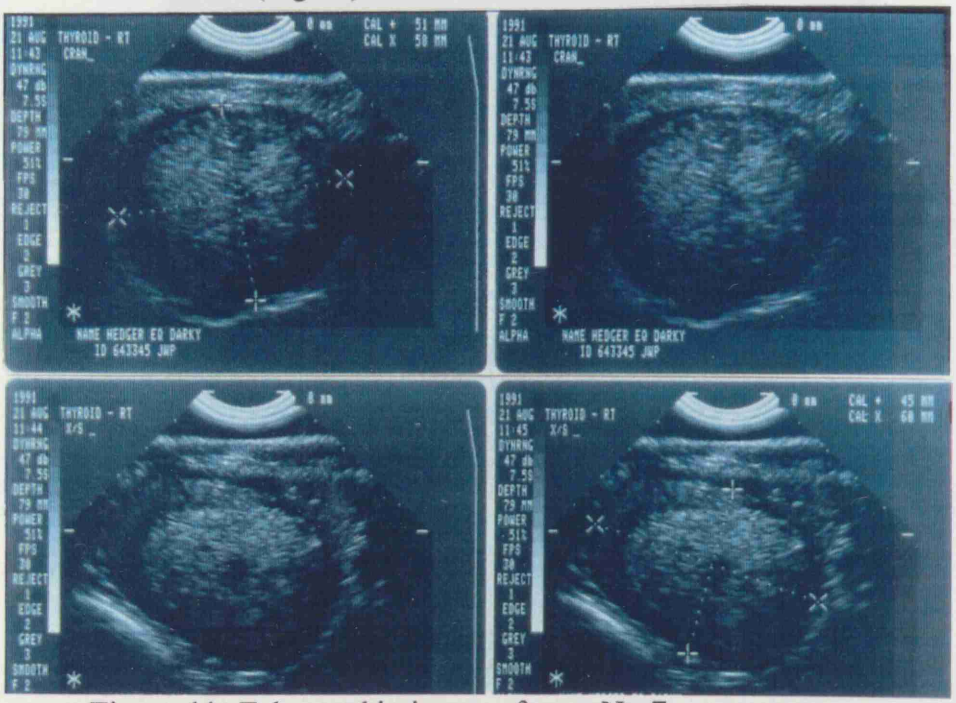
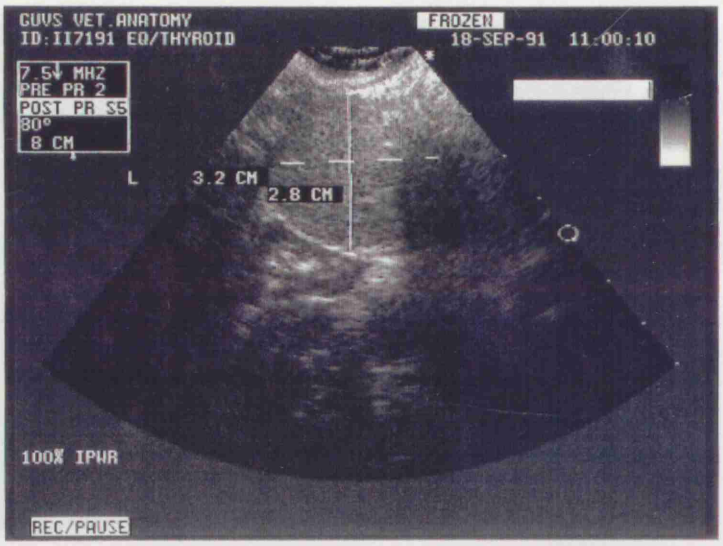


Figure 11: Echographic image of case No.7

A



B

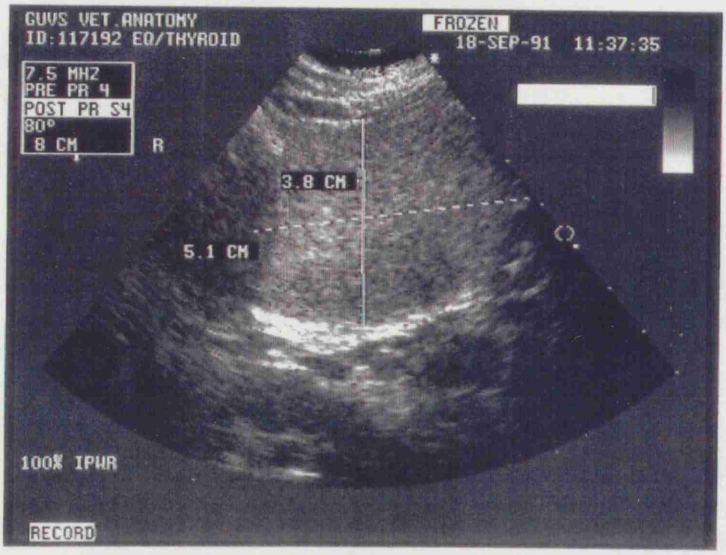


Figure 12: Echographic images of cases No.9 (A) and 10 (B).

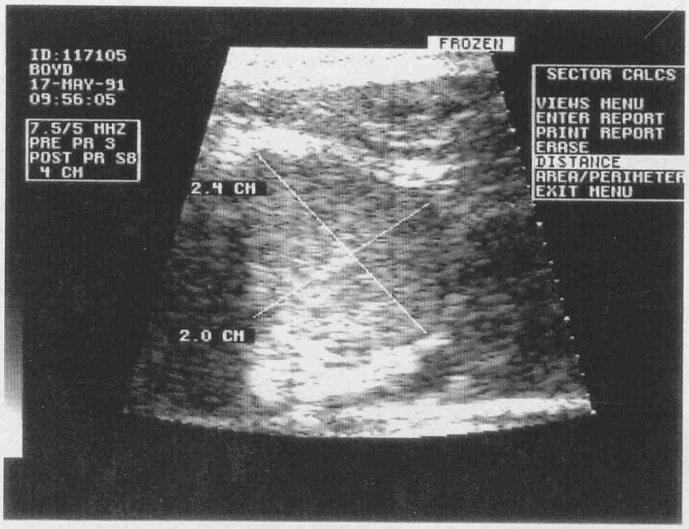


Figure 13: Echographic imaged obtained in Case No.5.

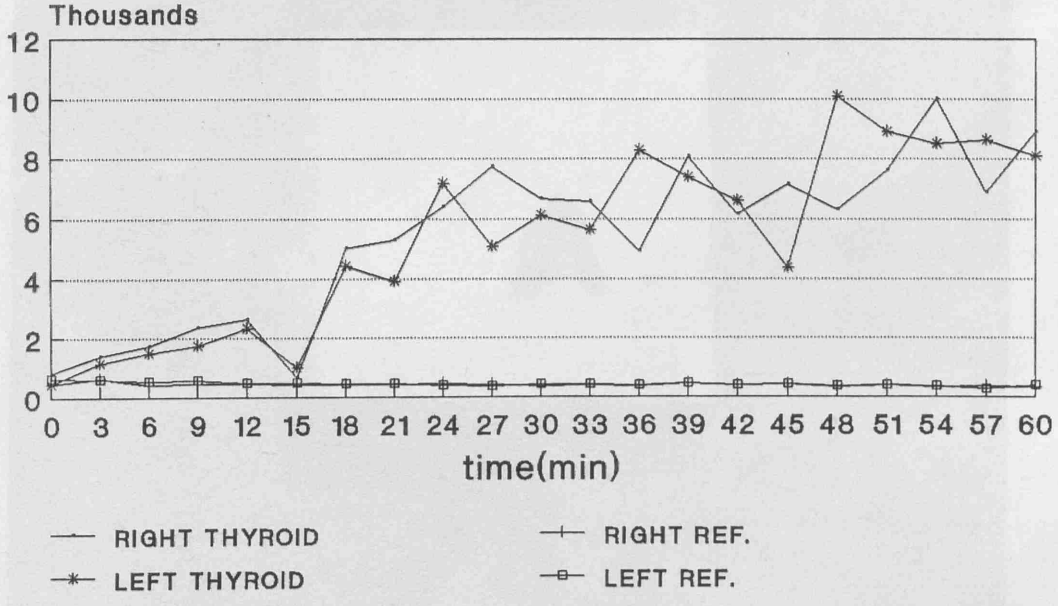


Figure 14: Graphic display of the distribution of values obtained by gamma-counter in Case No.5.

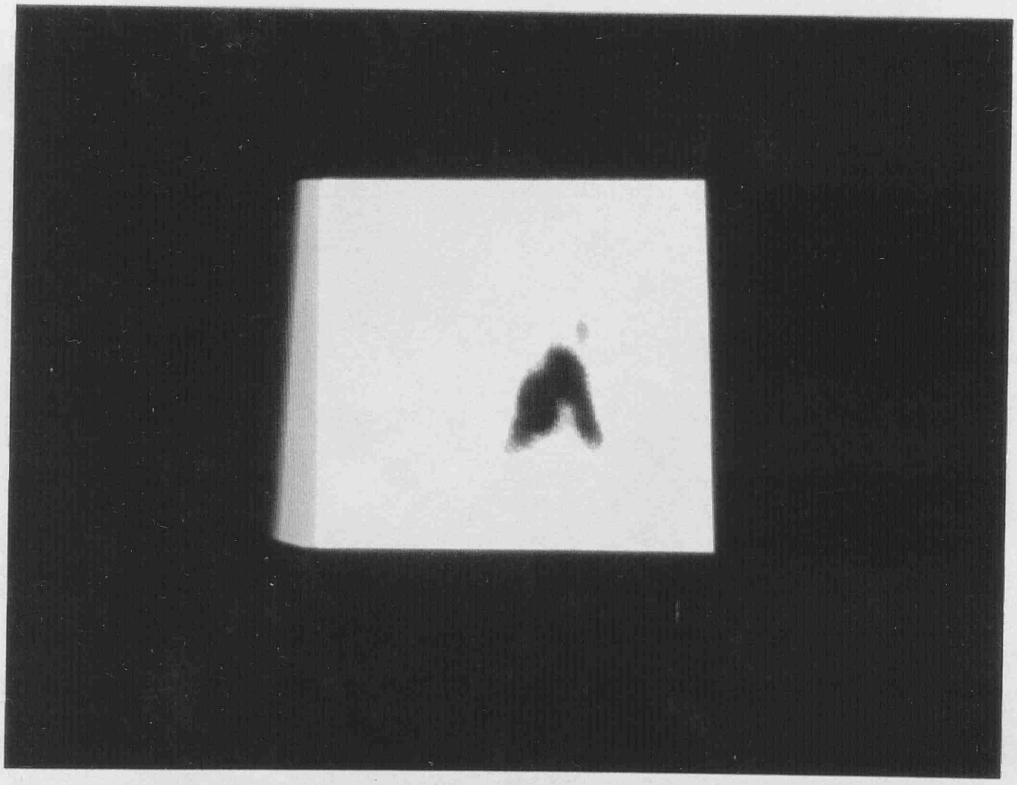


Figure 15 : Scintigraphic image of Case No.6, obtained 1hr. post-injection of $^{99m}TcO_4$, showing the difference in size between both thyroid lobes.

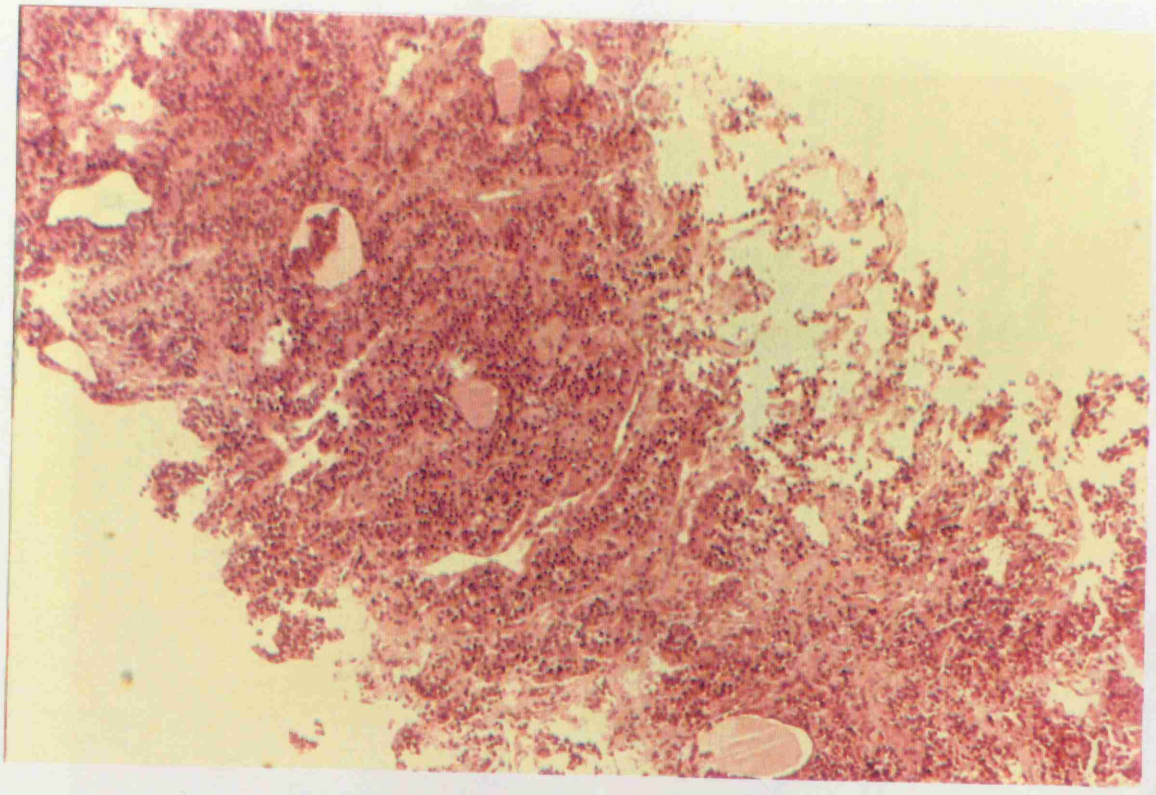


Figure 16 : Microscopic appearance of Case No.1.

(H-E. x63)

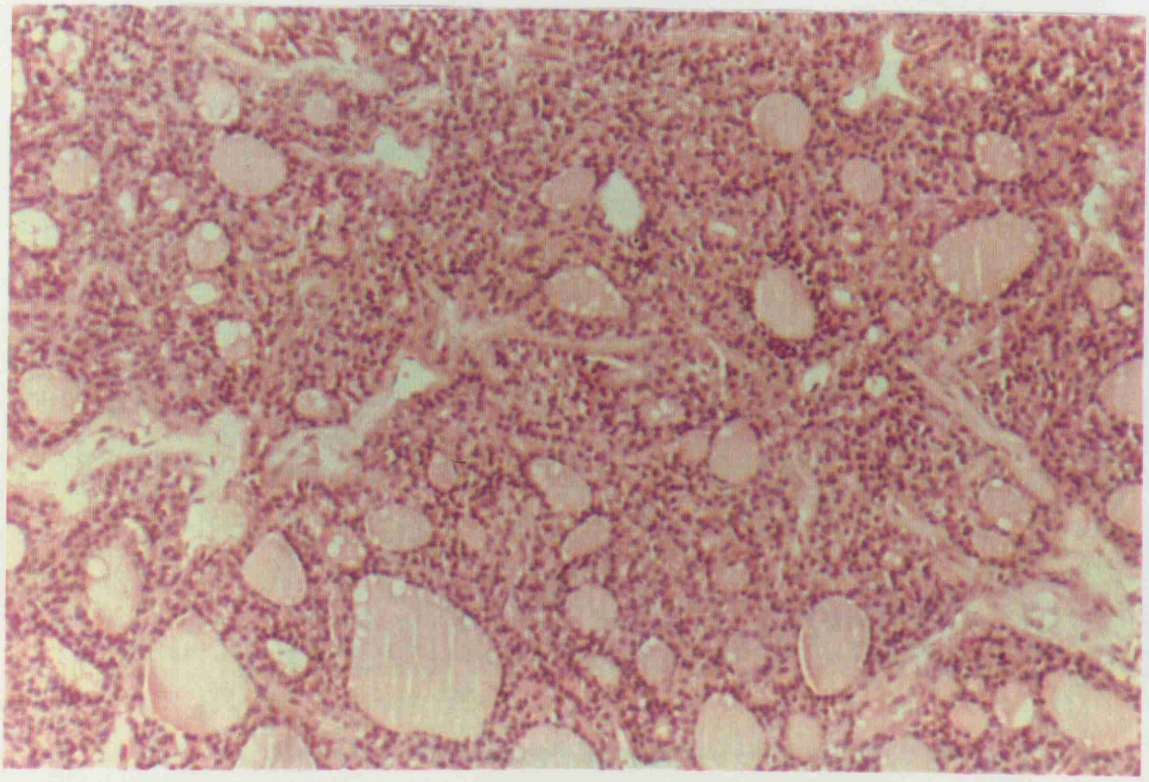


Figure 17 : Histological appearance of Case No.3, (H-E, x160).

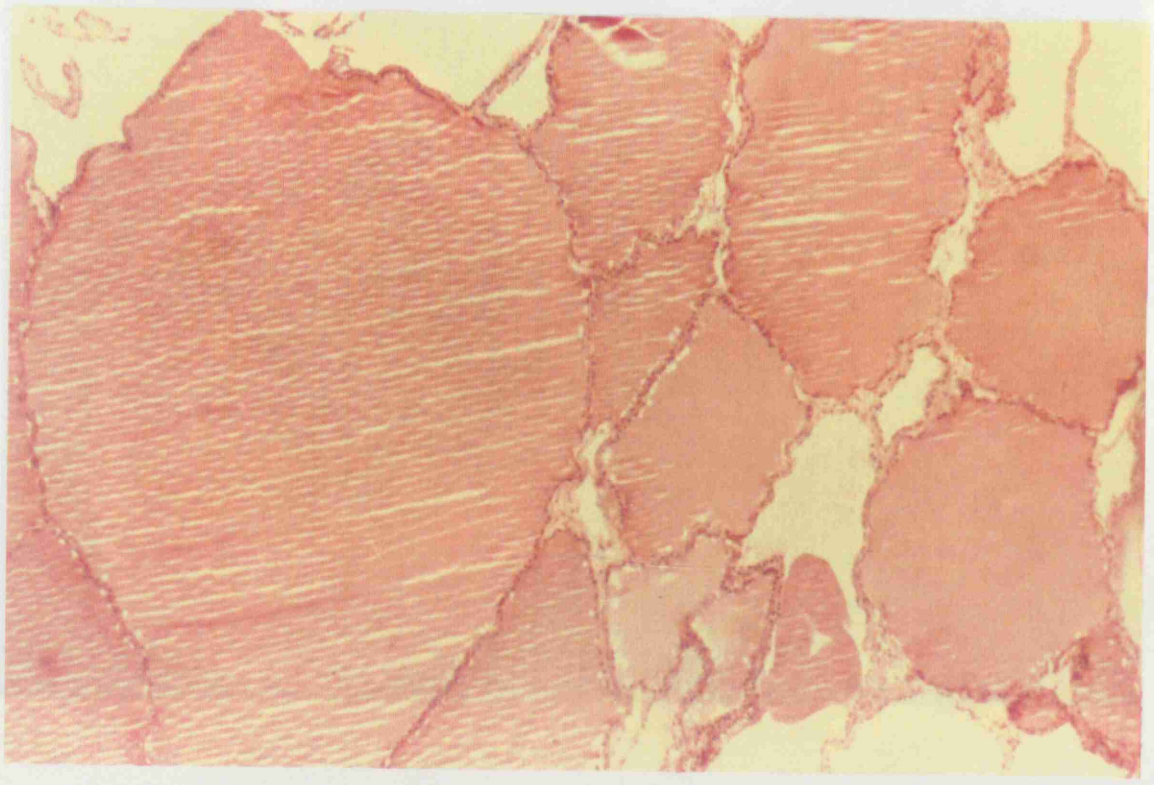


Figure 18 : Microscopic appearance of the affected thyroid lobe in Case No.6. (H-E. x250)

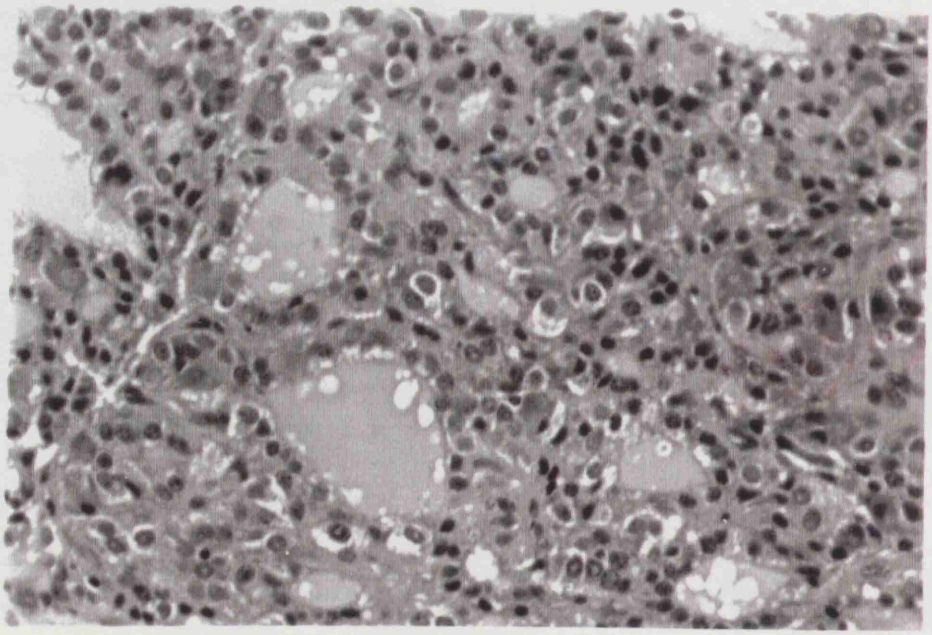


Figure 19: Microscopic appearance of Case No.7 (H-E. x250)

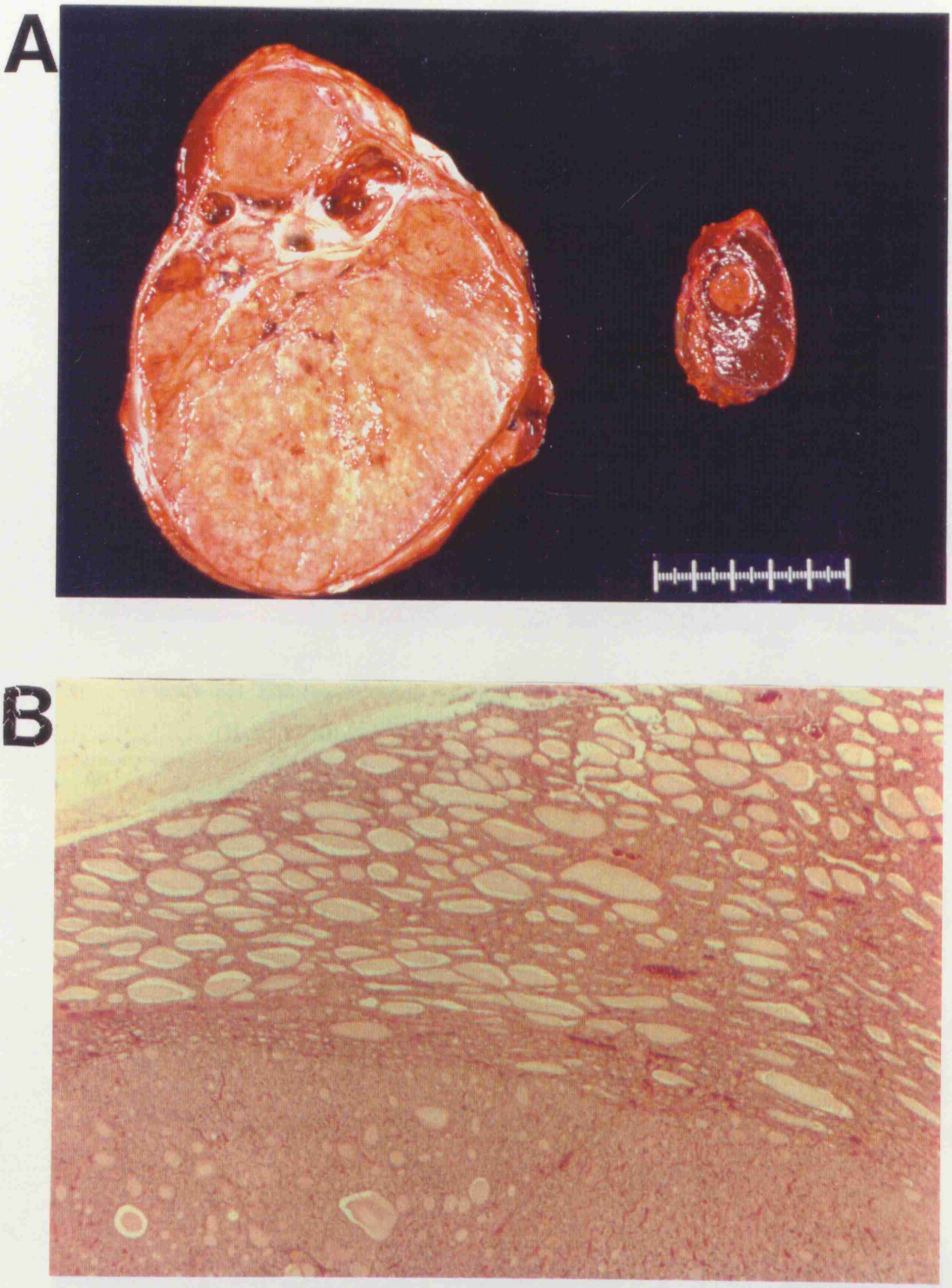


Figure 20: The macroscopic (A) and microscopic (B) (H-E. x 63) appearance of the thyroid lobe in Case No.8.

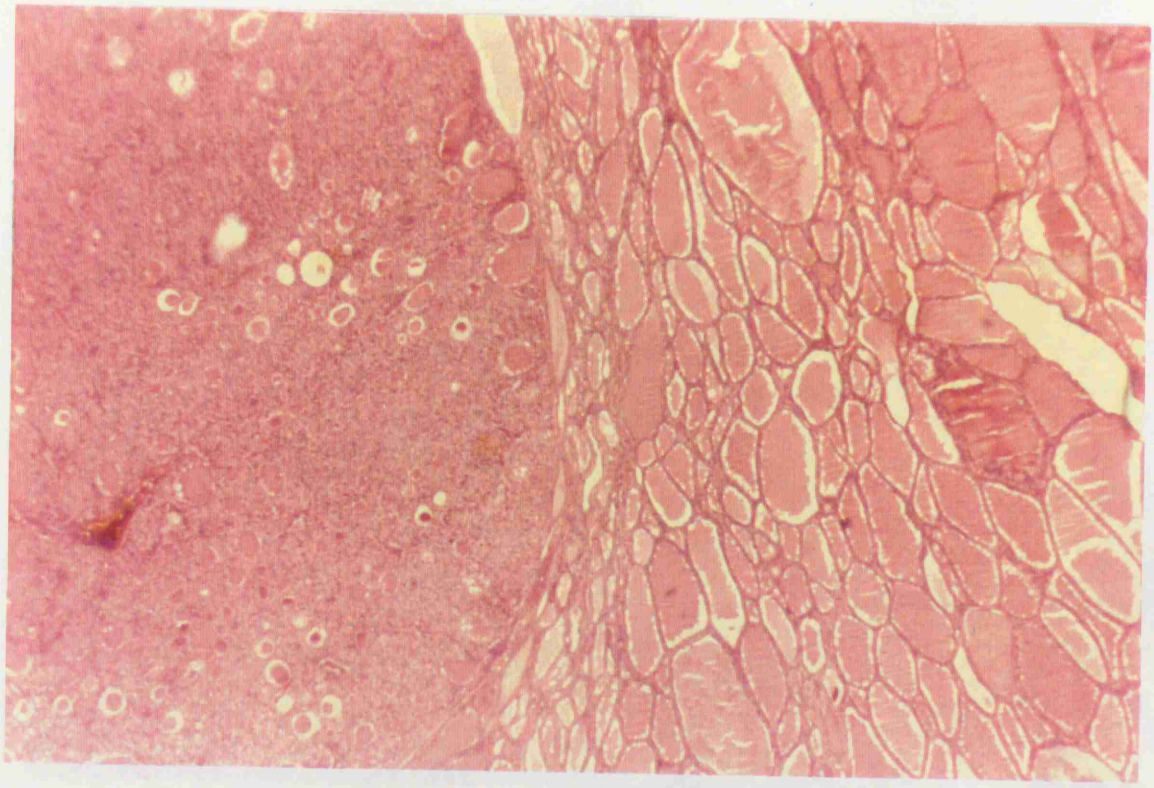


Figure 21: The microscopic appearance of the left thyroid lobe in Case No.8. (H-E. x160).

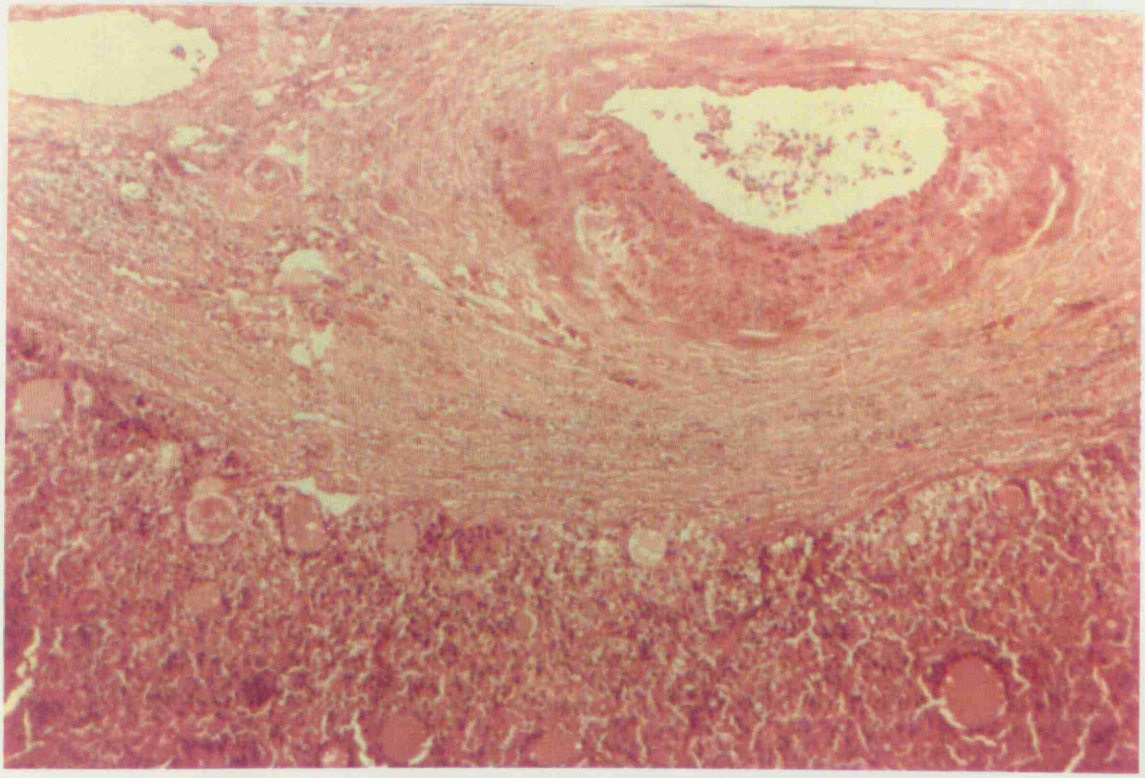


Figure 22 : Case No.11, thyroid microscopy. (H-E. x63)

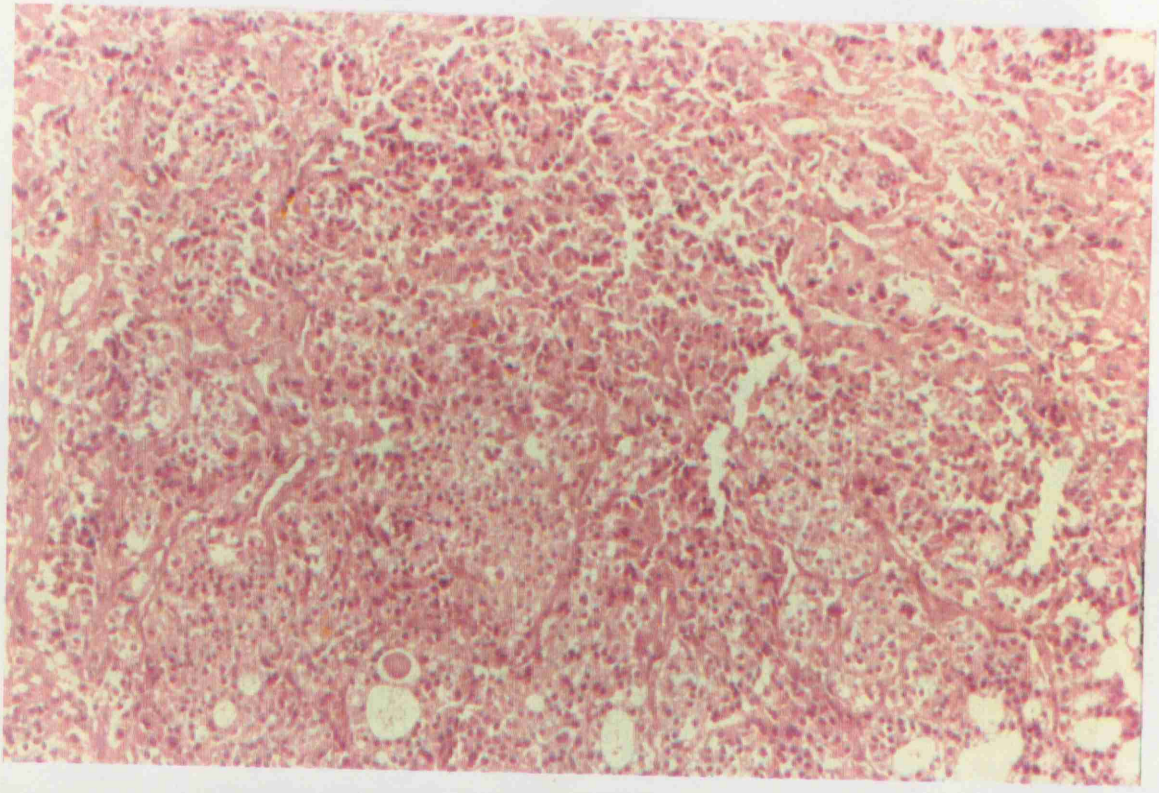


Figure 23: Microscopic image of the affected thyroid lobe in Case No.12. (H-E. x63)

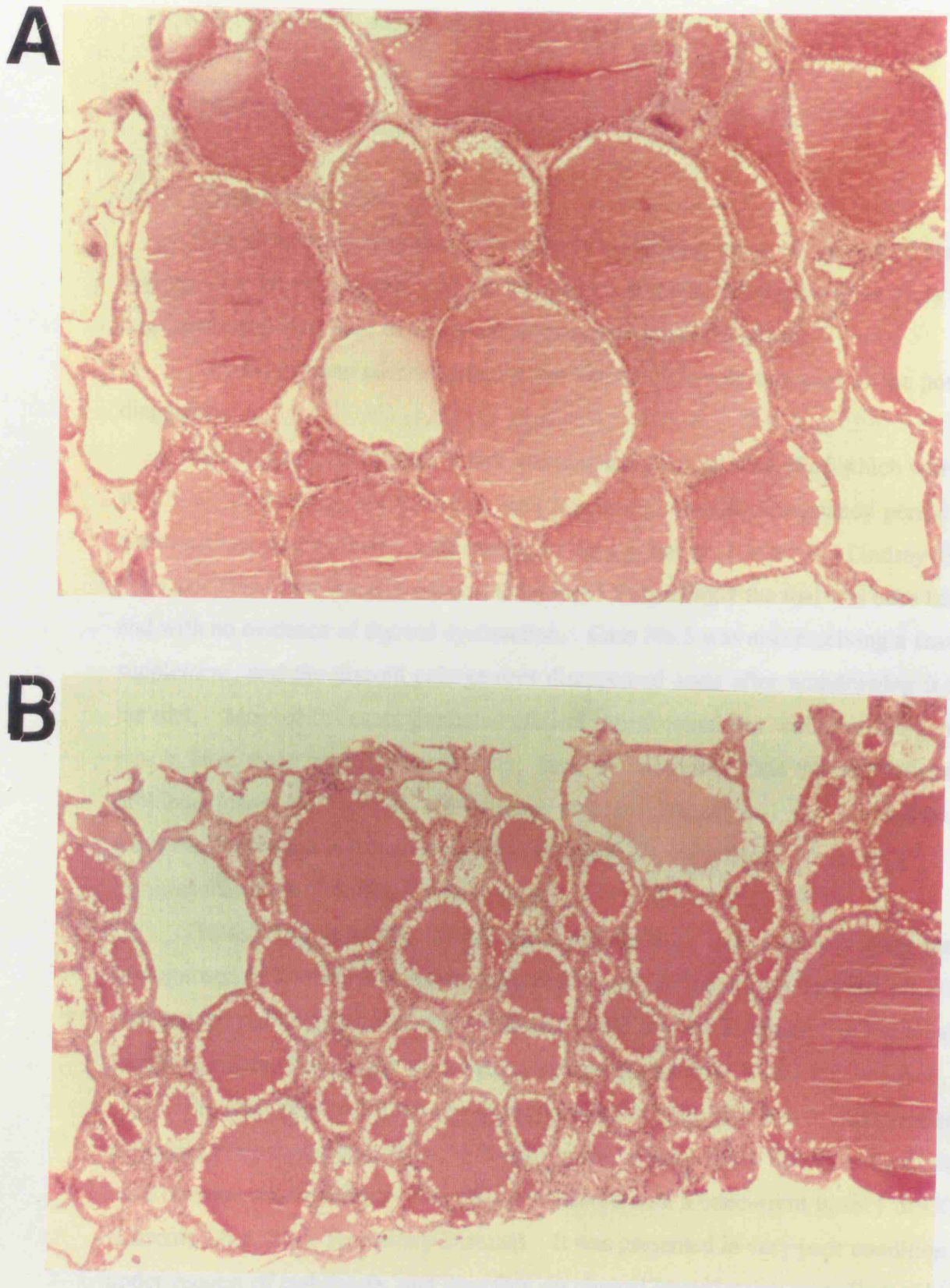


Figure 24 : Microscopic appearance of Cases No.9 (A) (H-E. x160) and 10(B) (H-E. x160)

DISCUSSION

Clinical Features

The differential diagnosis for any space-occupying lesion in the cranial cervical region of a horse includes lymphadenopathy, abscess, haematoma, auditory tube empyaema and tympany, developmental cyst, goitre and neoplasia.

The history and clinical presentation helped us to rule out most of the possible diagnoses.

All of them presented as a slow growing mass except case No.6 which was born with it . In this case the mare had been fed during the entire pregnancy period with a seaweed supplement that is well recognised as a goitrogen (Baker and Lindsey 1968). The mechanism for this still remains unknown. Surprisingly the foal was born healthy and with no evidence of thyroid dysfunction. Case No.5 was also receiving a seaweed supplement, and the thyroid enlargement disappeared soon after withdrawing it from the diet. Most of the cases presented clinical signs, coincident with the enlargement, which have been reported previously, such as laminitis (case No.4), respiratory problems (cases No.3 and 8), reproductive problems (cases No.1 and 3), headshaking (case No.5), change in behaviour (cases No.9 and 10) and lethargy (case No.11). The significance of these findings is not known.

Headshaking is a very difficult syndrome to diagnose and most of them remain undiagnosed or idiopathic. Space-occupying lesions in the cranial cervical region are well recognized as a cause of this syndrome. We can not conclude that the enlarged lobe was responsible for the headshaking syndrome, because it was not removed.

In cases No.3 and 8 the respiratory problems originated due to compression of the upper respiratory tract (U.R.T) by the mass. In case No.3 the signs disappeared once the lobe was removed. Case No.8 presented with a concurrent history of COPD (Chronic Obstructive Pulmonary Disease). It was presented in very poor condition and under request of euthanasia, and therefore no clinical investigation was allowed by the owners. Post-mortem examination was only carried out on the thyroid gland.

We can not explain the reproductive problems of cases No.1 and 3. Case No.1 was euthyroid with a normal TSH response and thyroxine levels in case No.3 were not

determined. Experiments carried out by Lowe *et al* (1987) showed how thyroidectomized mares, do not have altered the cycling pattern, although one of the mares in the study showed oestrus behaviour for an abnormal long period of time. In this study, serum TSH levels were not determined and therefore the influence of it in the reproductive function can not be assessed. In case No.3 we do not know about the management conditions of the mare. However, in the work done by Lowe *et al* (1987), anoestrus was not present in hypothyroid mares.

Cases No.2, 6, 7, 9 and 10 did not present any obvious problem coincident with the thyroid enlargement, and the main owner's concern was the aesthetic problem and the possibility of further complications.

Palpation lead us to identify the thyroid lobes as two masses either side of the trachea and just caudal or in the area of the cricoid cartilage. The author gained experience on differentiating between normal and abnormal thyroid glands by thoroughly palpating the thyroid glands of a large number of horses. All horses except Nos.7 and 8 had a smooth, firm, non painful and very loose gland within the deep fascia. In cases No.7 and 8 the gland felt attached to the deep fascia. We believe that palpation is a reliable method to differentiate between normal and abnormal glands. For instance, malignant neoplasia shows a gland rather nodular and attached to the surrounding fascia due to the infiltration of the tumour in adjacent tissues.

Ultrasonography

Ultrasonography examination was carried out in five horses. A parallel ultrasonographic exam in three normal horses was used to assess the appearance of a normal gland. Ultrasound scan has been reported in humans as a very reliable method to diagnose and differentiate between benign and malignant neoplastic process of the gland (Ikekubo *et al* 1986). Our results indicate that it could be a very useful technique in horses, because in most of our cases, there is an obvious change on the echogenic pattern in the affected lobes with respect to the normal ones. Further study is first necessary to establish the normal appearance and then to relate the abnormal ultrasound scan with pathological processes in the gland. We believe that it is an open field for investigation with a very promising future. In addition, the dimensions

of the gland were measured by ultrasound scan giving us very accurate results.

TSH Stimulation Test and Thyroxine Levels

Validated radioimmunoassay for T4 determination was only available for cases No.6, 7, 8, 11 and 12. A calibrated radiommunoassay was used in the rest of the cases.

The results of serum T4 base levels are very variable all of them being within the normal values. In case No.4 serum T4 base levels were not determined on presentation, but it was decided that the pony was hypothyroid based on clinical findings, lethargy and obese phenotype. Thyroxine supplementation, helped to resolve the enlargement of the gland, leading us to think that probably low T4 levels were responsible for the enlargement. This can be explained in different ways:

- Deficient/Excess iodine in the diet
- Intrathyroidal prevention of T4 production
- Prevention of T4 release into the blood stream
- Defect in the conversion of T4 in T3
- Production of antithyroid substances

Further investigation of thyroid function such as serum T3, TSH base level determination, TSH stimulation test, thyroid biopsy, ultrasonography and scintigraphy would have helped us to clarify the etiology. We could not establish a relationship between laminitis, thyroid enlargement and the subsequent resolution after thyroxine treatment.

Case No.6 was a yearling and the values of T4 for yearlings are higher than for adult horses. The age should be considered when evaluating the serum T4 base line values. Responses to TSH stimulation were also very variable. In accordance with Held and Oliver (1984) the normal response varies between 2 and 2.5 times the value of the basal line. Nine cases were investigated through this method. Cases No.4 and 6 yielded a normal response, but the rest of the cases were abnormal in accordance with the results of Held and Oliver (1984). The low response showed by case No.1 can be explained because the T4 serum level was on the top range of the normal values and

this has been associated with a low response to TSH stimulation (Held and Oliver 1984). We can not explain the high response to TSH in case No.5, although Held and Oliver (1984) got similar results in some of the horses surveyed. Case No.7 showed a extremely low response (13.5 % increase) and one possibility was that the malignant neoplasia was producing an antithyroid substance. However, because the horse was euthyroid and without signs of hypothyroidism we think that the proliferation of C-cells did not allow space enough for the follicular cells to proliferate, but the contralateral lobe was assuming the whole role of thyroid hormone secretion. Cases No.9 and 10 showed a decrease in response, although the rest of the investigation was normal. Case No.11 showed an increase of 37 % after stimulation. Although the serum T4 base levels were in the lower range, the weak response could be due to the old age of the animal, and the time of the year. It has been reported that in summer months thyroid secretion is reduced due to a reduction of the basal metabolic rate (Britton 1959). Case No.12 did not respond to the stimulation and on microscopy the gland was devoid of colloid. The absence of colloid was due to the overwhelming proliferation of hyperplastic cells. Because we only measured T4, we do not know whether in this case was a preferential secretion of T3 as it is showed in early cases of hypothyroidism or it was truly hypothyroid. These results lead us to think that none of the horses, despite having histological abnormalities of the gland, suffered a primary thyroid disease, involving a dysfunction of the gland.

T4 determination has been reported as the best single procedure for evaluation of thyroid function in domestic animals (Held and Oliver 1985). As mentioned earlier T3 has been reported to be affected by several situations and factors such as disease, starvation, environmental influences such as cold exposure and drugs such as phenobarbital or phenylbutazone (Robbins 1981). Phenylbutazone is highly protein bound in the blood and able to compete for transport sites, usually occupied by other drugs or hormones and although total T3 and T4 concentrations are decreased, concentrations of free hormones may remain unchanged (Morris and Garcia 1983). Therefore in horses presented with a non-especific lack of performance, which has been reported as a consequence of hypothyroidism (Waldron-Mease 1979), investigation of thyroid status has to be done considering that many of the horses in training or

competition are being medicated with phenylbutazone.

The amount of factors implicated in the evaluation of T3 levels make it not very worthwhile as a method to investigate the thyroid status of the animal, although it may be considered as a complementary test to further determine the thyroid status of the animal.

We have obtained very variable results to the TSH stimulation test, believing that they were not abnormal. We think that the test should be subject of revision and further research regarding influence of age, sex, time of the year, time of the day and assay used.

Histopathology

Biopsy of Case No.7 was inconclusive, and the horse is currently awaiting for thyroidectomy. After the surgery, we will be able to determine the histopathological changes affecting the gland. Amyloid was not detected in the tumour and calcium levels were within the normal limits.

The lobes were not investigated separately and none of the thyroids were bilaterally involved. It is possible that the normal lobe assumed the major role in the production of thyroid hormones.

Scintigraphy

Non-invasive methods, ultrasound scanning and scintigraphy, were used together with the aim of detecting abnormal structure of the gland and its relation to the activity of the gland. Nuclear imaging has been used to differentiate between normal and neoplastic thyroid tissue in the dog (Harari, Patterson and Rosenthal 1986). We think that the alternate peaks observed in the absorption of the radioisotope may be explained by the fact that pertechnetate is metabolised by the gland in a very similar way to iodine producing a regular "trapping" cycle giving us a wavy values corresponding with the absorption of the radioisotope. Also same disparate results obtained during the procedure may be a consequence of the difficulty in performing the procedure because it was difficult to maintain the probe in the correct position for the time necessary to read the count (10 sec.). It is very risky to speculate about the results obtained from

the scintigraphic procedures, because only six horses were investigated with this technique. However based on human and small animal results, this area deserves to be investigated regarding its use in the equine. Combination of ultrasound scan and scintigraphy have been used extensively in human medicine (Berghout *et al* . 1989; Ikekubo *et al* . 1986; Azagra *et al* . 1990) with a very good results, as a method to differentiate between benign and malignant processes and they are currently used in small animals.

Unfortunately only five horses were fully investigated and we think that the results are not representative of a standardised method of investigation, but they are good enough to consider these techniques as a matter of further research with a very optimistic future. Further investigation need to be undertaken to adjust these methods to their use in the equine. They have the advantage of being non-invasive, quick and very reliable in giving the clinician an idea of what is happening in the gland.

APPENDIX I

Normal haematological parameters (Glasgow University Veterinary School)

WBC	5.40 - 14.30	$x10^9/L$	Neu.	5.5 - 12.5	$x10^9/L$
RBC	8.80 - 12.90	$x10^{12}/L$	Lym.	1.50 - 5.50	$x10^9/L$
Hb	11 - 19	g/dl	Mon.	0 - 0.80	$x10^9/L$
Hct	32 - 53	%	Eos.	0 - 0.93	$x10^9/L$
MCV	37.00 - 58.50	fl.	Bas.	0	$x10^9/L$
MCH	12.30 - 19.70	pg.			
MCHC	31 - 37	g/dl			
PLTS	100 - 600	$x10^9/L$			

Normal serum chemistry values (Glasgow University Veterinary School)

Sodium	130 - 151	mmol/L
Potassium	2.6 - 5.2	mmol/L
Chloride	94 - 113	mmol/L
Calcium	2.78 - 3.38	mmol/L
Magnesium	0.69 - 1.27	mmol/L
I. PO_4	0.9 - 1.93	mmol/L
Bilirubin	0 - 25.6	umol/L
Glucose	2.7 - 5.55	mmol/L
Urea	0 - 6.64	mmol/L
Cholesterol	2.06 - 4.51	mmol/L
Creatinine	62 - 133	umol/L
T. Protein	60 - 83	g/L
Albumin	30	g/L
Globulin	38	g/L
Alk.Phos.	18 - 280	IU/L
Asp.Trans.	90 - 240	IU/L
Ala.Trans.	0 - 23	IU/L

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