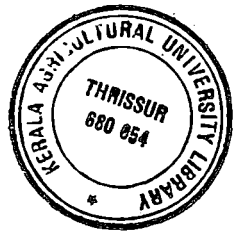
INCIDENCE AND PATHOLOGICAL FEATURES OF HYPOTHYROIDISM IN CATTLE



By

MAMMEN J. ABRAHAM

THESIS

submitted in partial fulfilment of the requirement for the degree

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DECLARATION

I hereby declare that this thesis entitled INCIDENCE AND PATHOLOGICAL FEATURES OF HYPOTHYROIDISM IN CATTLE is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Signature of the candidate

Name of the candidate : Mammen J. Abraham

Mannuthy,
20-6-'86.

CERTIFICATE

Certified that this thesis entitled INCIDENCE AND PATHOLOGICAL FEATURES OF HYPOTHYROIDISM IN CATTLE is a record of research work done independently by Sri. Mammen J. Abraham under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Valsala.

Name of guide : Dr K.V. Valsala
(Chairman, Advisory Board)

Designation : Associate Professor,
Department of Pathology.

Mannuthy,
20-6-'86.

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(Mammen J. Abraham)

DEDICATED TO MY
BELOVED PARENTS

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Introduction

1. INTRODUCTION

The thyroid gland has a significant role in regulating the normal growth and reproduction in animals. This gland is unique in that 70 to 80% of the total body iodine is present in its product, thyroxine. The synthesis of thyroxine is greatly influenced by the supply of iodine and the deficiency of this element could cause different thyroid disorders.

Ever since the demonstration by Daniel Marine that iodine deficiency is an important cause of endemic goitre, the incidence of endemic goitre in man and animals due to absolute or relative deficiency of iodine has been reported from many parts of the world. Kelly and Sndden (1960) while documenting the geographical distribution of endemic goitre in Asia have identified certain regions in India as endemic zones of goitre. This included coastal areas of Kerala. It is known that endemicity of goitre parallel iodine deficient soil zones and it has also been established that the iodine content of the soil varies with the geography of the land and the climatic conditions. In heavy rainfall regions like Kerala, loss of iodine due to leaching of the surface soil is bound to occur. Further the modern practice of adding synthetic nitrogenous fertilizers containing no iodine is likely to cause iodine deficient soil. Besides this, the role of goitrogenic substances widely distributed in nature, cannot be overlooked in precipitating a hypothyroid state in man and animals.

Goitre has been recognized as a clinical syndrome in goats in certain parts of India as early as 1935. Sreekumaran (1976) experimentally induced hypothyroidism in kids and studied the sequence of pathological changes in different stages of hypothyroid state. Reddy (1982) made an in-depth study on the reproductive pathology in experimental hypothyroidism in goats. The results of these studies brought to light the fact that subclinical hypothyroidism is an important factor in inducing subfertility and infertility in livestock.

Although, investigations have been undertaken on the effect of hypothyroidism in goats and useful information gathered, there has not been any investigation on the thyroid pathology in cattle. Recognition of the existence of subclinical hypothyroid state in the animal population and consequent increased susceptibility to infection and subfertility is of paramount importance for adopting suitable preventive measures to reduce reproductive disorders and loss due to lowered production and mortality in animals. The work carried out by Reddi (1983) demonstrated that certain spontaneous cases of subfertility in goats were associated with hypothyroidism. An investigation, was therefore, undertaken to assess the thyroid function in certain cases of non-infectious subfertility in cattle. Besides this, an experimental model of hypothyroidism was induced in male calves using thiourea as the goitrogen and the thyroid pathology was studied with special reference to the reproductive organs.

Review of Literature

2. REVIEW OF LITERATURE

2.1. Historical resume

The name "thyroid" or oblong shield for the gland was first suggested by Wharton (1856) though the diseases of the thyroid was recognized for many centuries back. The relationship between the thyroid and the various body functions was studied by experimental thyroidectomy and the concept of thyroid as an organ of internal secretion was formulated by King (1836). Kocher (1883) observed development of myxoedema like syndrome following thyroidectomy. The association of iodine with the working of the thyroid was made in 1896 by Baumann who discovered a particularly high concentration of this element within the gland. As early as 1900, Gley and Bourcet identified the presence of organic iodine in the plasma in combination with the serum protein. The work of Kendall (1915) lead to the isolation, characterisation and finally synthesis of thyroxine from the thyroid gland. Smith and Smith (1922) first demonstrated that thyroid activity was regulated by the pituitary hormone. Harington and Barger (1927) identified the chemical structure of thyroxine. Kimbal (1937) noted the association of hypothyroidism and endemic goitre. Gross and Pitt-Rivers (1952) identified triiodothyronine (T_3) in the gland and in plasma. This compound proved to be physiologically more potent and more rapid in onset of action than tetraiodothyronine (T_4) with four iodine atoms.

Moreover, these workers speculated that tetraiodothyronine is the form in which thyroid hormone is secreted while T_3 (Triiodothyronine) is the form which is active at the tissue level. Thyroglobulin was classified as a glycoprotein and most of the carbohydrate was found accounted by glucosamine and mannose (Gottschalk and Ada, 1954).

2.2. Thyroid pituitary relationship

Niepce (1851) was the first to observe the relationship between the thyroid and pituitary. The experimental removal of the thyroid resulted in hypertrophy of the pituitary and atrophy of the thyroid after removal of the pituitary. These studies indicated that the pituitary and the thyroid were related (Ragowitsch, 1889). The activity of the thyroid was found to be influenced by the thyroid stimulating hormone (TSH), a glycoprotein formed in the specific basophilic cells in the pituitary called the thyrotroph (Adams, 1946). Normal thyroid function depended on a physical connection of the pituitary to the hypothalamus but isolated anterior pituitary retained some function. Hypothyroidism has been induced in animals and in man by section of the pituitary stalk (Scow and Greer, 1955). The stimulus for TSH secretion is presumably transmitted from the hypothalamic centres to the anterior pituitary in the form of chemical mediators called thyrotropin releasing factor (TRF) by way of hypophyseal portal venous system. Moreover the response of the hypothalamic centres was presumed to be initiated by a metabolic effect of the

iodothyronines (Goldberg et al., 1955). Sufficiently large amounts of thyroxine completely blocked the effect of TRF on TSH discharge. Over and above, the inhibitory effect of moderate doses of thyroxine was overcome by sufficiently large amounts of TRF. Thus the secretion of TSH was governed by two interacting forces - hypothalamic drive on the thyrotrope cell, mediated by TRF and feed-back inhibition exerted by tetraiodothyronine (T_4) directly on the pituitary. In the absence of hypothalamic drive the pituitary was easily inhibited by T_4; on the other hand, the hypothalamus was capable of "breaking through" normal inhibitory effects of T_4 under such conditions as cold exposure (Vale et al. 1967). TSH was found to have a number of effects on the thyroid gland. Due to the action of TSH, the gland was increased in size, the height of the follicular epithelial cells was increased and there was loss of colloid (Kaneko, 1970). TSH hormone stimulated the accumulation of iodine, its organification and release of thyroxine (Jubb and Kennedy, 1970). The response of the thyroid to TSH was also influenced by the level of stable iodine intake. When the level of stable iodine intake was low there was an increase in the number and size of the cells and in the uptake and release of iodine and these changes were attributed to the increased level of TSH in circulation. Iodine was also reported to enhance the hydrolysis of thyroglobulin liberated from the gland (Jubb and Kennedy, 1970).

2.3. Thyroid hormone synthesis

Iodine entering the thyroid gland was rapidly incorporated into the colloid of thyroglobulin which was the initial substrate for iodination. The iodinated thyroglobulin within the colloid of thyroid follicle represent the stored hormone. Release of thyroxine and triiodothyronine from the thyroglobulin is accomplished by a group of peptidases and proteases present in the thyroid gland (De Robertis, 1941). All the tissues though contained iodine, 25 to 35 % of the total ingested iodine was concentrated in the thyroid gland (Riggs, 1952). Robbins and Rall (1960) pointed out that each thyroglobulin molecule contained approximately 110 tyroxyl residues. If the average molecule was 0.5% iodine, it held 26 iodine atoms per molecule. The complete protein was a tetramer of molecular weight of about 6,60,000 (Edelhock, 1960). The tyroxyl residues of the thyroglobulin were first iodinated to monoiodotyrosine(MIT) and diiodotyrosine (DIT) was then formed by a second iodination. The ratio of MIT/DIT remained relatively constant from the earliest minutes of hormonogenesis, suggesting that iodination was not a random process, but an orderly one. Bush (1969) reported that T_3 forms were in greater proportion in the total hormone. The mono and diiodothyrosine were coupled for the synthesis of triiodothyronine (T_3) and thyroxine (T_4). Three major steps were described in the synthesis of thyroxine. The first was the concentration of iodide by the gland from the blood; this iodide was

enzymatically oxidised to iodine. Then it combined with the protein, thyroglobulin and thus synthesis of thyroxine was initiated (Tong, 1971).

2.4. Functions of thyroid hormone

The primary function of the thyroid hormone was considered as regulation of cellular oxidation and stimulation of oxygen consumption for normal growth and development (Barker, 1951). In the laboratory more specific indications of augmented metabolism was demonstrated. Oxygen consumption was increased in the heart, liver, muscle, kidney and in white blood cells removed after administration of thyroxine. Some tissues such as brain did not share in this response (Barker and Schwartz, 1953). The activities of hexokinase, cytochrome reductase, cytochrome oxidase and other respiratory enzymes were augmented. The pentose phosphate pathway for glucose oxidation was stimulated (Necheles and Beutler, 1959). The thyroid hormone regulated the rate of energy turnover in vital organs and this helped in maintaining basal metabolic rate of the body. It also exerted influence on the development of hair and pigmentation in animals (Berman, 1960). In association with other hormones it exerted a control over growth and development of young animals, temperature regulation, intermediary metabolism and reproduction (Bush, 1969). Thyroxine exerted maximum growth rate in the presence of growth hormone. In the absence of thyroid hormone the effect of growth hormone

was greatly reduced (Jubb and Kennedy, 1970). Barker (1971) reported that thyroxine was essential for full translation of genetic message into the ribonucleic acid and ribosomal synthesis of protein. In addition, many metabolic processes were accelerated such as protein breakdown, carbohydrate and lipid turnover and calcium metabolism. Nervous functions at all levels were influenced by the thyroid. Microsomal protein synthesis was stimulated by thyroid hormones without dependence on cell nucleus. There was also apparent increase in RNA synthesis. There was considerable evidence to suggest that the numerous effects of thyroid hormones upon mitochondria resulted from a primary interaction with the mitochondrial membrane. Werner (1971) stated that thyroxine when given to normal animals not only caused mitochondria to swell but also produced an increase in both the number of mitochondria per cell and the number of cristae per mitochondrion. Only mitochondria from tissues which respond to T_4 with increased oxygen consumption were shown to swell *in vitro*. Anderson and Harness (1975) observed that for every unit increase in body weight there was a 69 unit increase in the thyroid hormone secretion rate. Louvet *et al.* (1979) pointed out that hypofunction of the thyroid, evidenced by low T_3 and T_4 caused anovulation and sterility.

2.5. Thyroid Pathology

The thyroid gland exhibited a variety of diseases and a larger spectrum of gross and microscopical pathological changes.

Thyroid diseases capable of producing clinical signs were classified (Bush, 1969) as:

1. Goitre
2. Hypothyroidism
3. Hyperthyroidism
4. Thyroiditis
5. Thyroid neoplasia

2.5.1. Goitre

The term "goitre" was defined as a non-inflammatory, non-neoplastic enlargement of thyroid gland (Cohrs, 1966). He classified goitre on the basis of the morphology.

1. Atoxic goitre which included most sporadic forms
2. Goitre with functional change which may be
   a) Athyroid or hypothyroid goitre
   b) Hyperthyroid goitre.

There were generally two types of goitre (Kaneko, 1970).

1. Non-toxic goitre, which produced normal amount of hormone (simple goitre) or less than normal amount of hormone (Hypothyroid).
2. Toxic goitre which was characterised by the excessive production of hormone (hyperthyroid).

Smith et al. (1972) classified goitre on the morphological basis into four patterns.

1. Colloid goitre
2. Hyperplastic goitre

3. Nodular goitre

4. Exopthalmic goitre

2.5.2. Endemic goitre

This has been considered as the most common condition to some degree from almost every country of the world. The geographical distribution of this condition has been well studied. In view of the occurrence of the disease, the terms endemic and sporadic atoxic goitre were often used. An absolute or relative deficiency of iodine was considered as the main factor for endemic goitre. McCarrison (1913) attributed goitre in the Himalayas to water pollution. Stott et al. (1930-31) pointed out the association between high goitre rates and dolmitic lime in India. It was concluded that drinking of hard water containing excessive amount of calcium was of etiological importance in Himalayan endemic zones. This supported the belief that calcium may be a goitrogenic factor. Levine et al. (1933) reported that the element iodine and inorganic iodine themselves in large doses have goitrogenic properties. Wilson (1941) suggested that excessive intake of flourine might be a causative factor of endemic goitre in Punjab. Murray et al. (1948) indicated that there was a relationship between the distribution of goitre and calcium concentration of drinking water in England. Several antithyroid substances have been isolated from plants and fodders responsible for endemic goitre. Greer (1950) made a comprehensive review of substances which were reported to be

goitrogenic to experimental animals and discussed their importance. The study by Stanbury et al. (1954) indicated that there was an inverse correlation between the quality of iodine excreted in the urine of patients in an endemic area and the radio active iodine uptake and concluded that lack of iodine in the diet was the most probable cause. The mountain slopes of Himalayas, Alps, Pyrenees, and Andes were considered as the world's most notorious foci for endemic goitre (Kelly and Snedden, 1960). In the Indian subcontinent the northern frontiers extending from Kashmir in the north, Bengal and Assam in the east formed the extensive Himalayan goitre belt (Ramalingaswami et al. 1961). Cold climate was reported to influence the prevalence of endemic goitre in regions of borderline iodine supply as a result of increased demands for thyroid hormone (Scrimshaw, 1964). Suzuki et al. (1965) reported endemic goitre in Japan due to excessive iodine intake. An endemic goitre area was defined as onein which more than 10% of the population showed clinical signs of thyroid enlargement (Koutras, 1971). Kochupillai et al. (1976) made an epidemiological study of the nodular lesion of the thyroid in a population living in an area of high background radiation in coastal Kerala. The results which were compared with those obtained from a comparable population living in an identical coastal strip without any high background radiation did not reveal any high incidence of nodular lesions or neoplasms in the area with high background radiation.

Halik and Zavadsky (1978) recorded mass outbreaks of goitre in breeding rams in an endemic goitre area. The thyroid glands were enlarged and diffuse colloid goitre was evident histologically. The goitre regressed after the administration of iodine preparations. Pandav and Kochupillai (1982) observed that in India, endemic goitre due to iodine deficiency affected an estimated 40 million people. In areas where goitre prevalence was high major attendant disabilities of endemic goitre such as endemic cretinism, deaf-mutism and feeble mindedness were present in about 4% of the population. Iodisation of salt was suggested as an effective prophylactic measure against the goitre (Pandav and Kochpillai, 1982). Studies conducted by Kochupillai et al. (1984) on the intrauterine and neonatal thyroid status among new borns from the Gonda district, an endemic area with environmental iodine deficiency revealed that the serum T_4 values of the newborns were significantly lower when compared to newborns from the non-endemic areas. This observation suggested that a significant population of newborn from areas with environmental iodine deficiency may be suffering from brain damage due to thyroxine deficiency at birth.

2.5.3. Colloid goitre

Colloid goitre was considered as an involutionary phase of hyperplastic goitre (Follis, 1959). Typically the goitre was asymptomatic. The gland was usually symmetrically enlarged and was soft or spongy to feel (Means et al. 1963). The

gland was unable to return to its normal size, when the demand for thyroxine was met or increased in size due to accumulation of colloid in quantities commensurate with the increase in thyroid epithelium. Follicles were packed with colloid, and there was greater variation in their size and sometimes they coalesced to form cysts. All follicles were filled with deeply staining colloid (Jubb and Kennedy, 1970). The follicular epithelium remained taller than normal and much of the increased vascularity persisted (Wilson, 1975).

2.5.4. Parenchymatous goitre

According to Jubb and Kennedy (1970) the morphological indication of increased stimulation of the thyroid gland by the thyroid stimulating hormone was hyperplasia of the thyroid epithelium. In this condition, the vascularity of the gland was greatly increased as also the total volume of the gland which some time became enormous. The follicular lumina were smaller and many disappeared and the colloid was reduced in amount with variable staining affinity or was completely absent. An early and characteristic sign of stimulation was the appearance of vacuoles around the periphery of the colloid or peripheral scalooping (Wilson, 1975).

2.5.5. Nodular goitre

According to Smith et al. (1972) this type was frequent in old animals. They described well defined nodules in one or both thyroid lobes. These nodules were clearly demarcated

from the rest of the thyroid tissue and the histological appearance of the nodular goitre usually varied from nodule to nodule. Many follicles were greatly distended with colloid; while others were small and devoid of colloid. In the simplest form, the epithelial cells were inactive and colloid was deeply stained. In some follicles the hyperplastic lining cells were thrown into small papillary folds. Retrogressive changes were reported to be common in nodular goitre (Smith et al. 1972).

2.6. Hypothyroidism

Congenital hypothyroidism occurring in an endemic iodine deficient area was termed endemic (enzootic) congenital hypothyroidism and when occurring elsewhere, sporadic congenital hypothyroidism (Marine and Lenhart, 1910). Deficient maternal hormone production resulted in defective foetal development and hypothyroidism (Marine and Lenhart, 1910). Ruminant hypothyroidism mainly occurs in areas of endemic goitre region (Hojer, 1931). Jubb and Kennedy (1970) reported that in domestic animals hypothyroidism was generally caused by congenital goitre and was encountered in the new born. Goats were considered particularly susceptible to both development of congenital goitre and associated effects of hypothyroidism (Mason and Wilkinson, 1973). They classified hypothyroidism into the following categories:

1. Primary hypothyroidism due to lack of functioning thyroid without its associated enlargement.

2. Secondary hypothyroidism due to pituitary insufficiency.

3. Hypothyroidism due to iodine deficiency

4. Hypothyroidism due to ingestion of goitrogens.

5. Hypothyroidism due to dyshormonogenesis

6. Hypothyroidism due to autoimmune thyroiditis

7. Hypothyroidism due to neoplasia.

Hypothyroidism had an adverse effect in the young growing animals as it caused interference with the overall development than in mature adults (Ferguson et al. 1956). Calderbank (1958) reported cases of infertility associated with iodine deficiency. Calderbank (1963) reported a close association between the thyroid and gonadal function and the loss of libido in males and suboestrum in females. Wallach (1965) pointed out that hypothyroidism was generally characterised by lowered body temperature, increased sensitivity to low environmental temperature and growth retardation. In hypothyroid domestic animals the gestation period was significantly prolonged. (Jubb and Kennedy, 1970). In domestic animals, hypothyroidism was generally associated with congenital goitre of the new born. But the dams of congenitally goitrous offspring usually showed no evidence of hypothyroidism and in adult domestic animals it was in the dog that hypothyroidism appeared as a predominant disorder of thyroid function (Jubb and Kennedy, 1970). New born goats showed myxoedema, alopecia, and high mortality rate (Jubb and Kennedy, 1970). Iodine

deficiency was associated with reproductive failure (Underwood, 1971). Retardation of growth and sexual development was a consistent finding in the young. Mason and Wilkinson (1973) observed that serum cholesterol determination was a valuable aid in the diagnosis of hypothyroidism since cholesterol synthesis was inversely proportional to the thyroid function. Wilson (1975) summarised the effects of hypothyroidism in ruminants as follows:

1. Retention of placenta
2. Infertility
3. Lowered milk production
4. Lowered resistance to infection
5. Increased susceptibility to ketosis
6. Late abortion, still birth and weak offsprings.

In goats, Sreekumaran (1976) and Reddy (1982) observed increased serum cholesterol and protein levels and decreased protein bound iodine in experimental hypothyroidism.

2.7. Hypothyroidism due to iodine deficiency

Simple hypothyroidism was attributed to the iodine deficiency in the environment (Southcott, 1945). However, four per cent of the total incidence of non-toxic goitre was due to other causes. Goldschmidt (1954) identified the factors influencing the iodine content of the soil. The iodine requirements were also influenced by the composition of diet as a whole (Scott et al. 1961). Calderbank (1963) suggested that

although soil is the source for iodine for both water and crop there may be little or no correlation between the iodine content of the soil and pasture growing on it. Soil in the neighbourhood of the sea was not always richer in iodine than those in land but was dependent upon the prevailing wind, the amount of precipitation and the nature and reaction of the soil. The iodine content of the drinking water was found to be low in endemic goitre region. The soils contained more iodine than the rocks from which they were produced. This was partly because of the retention of iodine by plant life growing on the soil. When the surface soil was eroded, as for example by glaciation, the iodine content of that soil was low, and the water derived from these regions was low in iodine content (Means et al. 1963). The effect of heavy rain was also a determining factor (Scrimshaw, 1964). Blokhina (1970) stated that some microelements may also influence the availability of iodine. High protein intake was found to interfere with utilization of iodine (Wilson, 1975). Walton and Humphrey (1979) reported that the iodine deficiency in the soil in the highlands of Papua New Guinea was due to high annual rainfall leaching the iodine from the soil. Environmental iodine deficiency as the causative factor of Himalayan endemic goitre was established by a series of investigative efforts. The level of iodine in the drinking water was extremely low in the endemic zone, no value being higher than 3 microgram/l and most values considerably below this figure (Pandav and Kochupillai, 1982).

2.8. Hypothyroidism due to goitrogen

Presence of goitrogen in the feed stuffs was described as an important cause for hypothyroidism in animals. Calderbank (1963) described two main types of goitrogen. A thiocyanate type that is a potent inhibitor of iodide transport and this blocking effect was overcome by simultaneous administration of iodine. Secondly a thiouracil type which inhibited the organification of iodide and this effect was reversed by the administration of thyroxine. Thiocyanate was about 25 times more potent than nitrate in inhibiting thyroid function (Greer and Walton 1961). The goitrogens like the thiocyanate were described as anionic goitrogens and those like thiouracil were designated as organic goitrogens (Catt, 1970).

2.9. Natural goitrogens

Chesney et al. (1928) first observed that rabbits fed on cabbage became hypothyroid and developed large hyperplastic goitres. A diet high in sowflower produces goitre in animals (McCarrison, 1933). Sharpless et al. (1939) demonstrated the goitrogenic action of soyabean flour meal in rats by producing enlarged thyroid by feeding soyabean flour. Kennedy and Purves (1941) produced goitre in rats fed Brassica seed. The weights of the glands were found to be increased by 300 times and histologically they observed hyperplasia of the thyroid glands. Examination of pituitary showed a rapid increase in the basophil cells and this was associated with

hyalinisation and vacuolation to form "signet ring" cells. There was also simultaneous loss of acidophil cells in the rats treated with Brassica seed diet (Griesbach et al. 1941). All the halogens if present in excess were capable of displacing iodine and caused iodine deficiency. Fluorosis was demonstrated to be one of the factors responsible for goitre in Punjab (Wilson, 1941). Sharpless and Metzger (1941) showed that arsenic at 0.02 level in the diet in rams caused decrease in growth, increase in thyroid weight and decrease in iodine concentration of the thyroid gland. Astwood et al. (1945) found in the seeds of Ochlearia and Conringia orientalis presence of 5,5-dimethyl-2-thiooxazolidone a goitrogenic substance. Astwood et al. (1949) isolated a goitrogen L-5-vinayl-2-thiooxazolidine (goitrin) from rutabagas and turnips. A marked decrease in follicular colloid and decrease of follicular epithelium were observed in sheep and goats fed cauliflower leaves (Spisni and Gravaglia, 1954). Clements and Wishart (1956) demonstrated that milk obtained from cows fed on narrow-stemmed kale or choumoellier (Brassica oleracea moellerii) interfered with the uptake of I^{131} both in the human subjects as well as in experimental animals. Butler et al. (1957) observed a decrease in total iodine content of thyroid and also inhibition of the conversion of inorganic iodine to organically bound iodine in sheep fed White clover. Sinclair and Andrews (1958) noted that a heavy diet of kale to pregnant ewes caused high incidence of goitre and hypothyroidism in born lambs. Goitrogenic action of perennial

grass has been reported (Setchell et al. 1960). Geer and Whalton (1961) derived 5-phenyl 2-thiooxazolidone from the seeds of Barbarea vulgaris and from the seeds, leaves, stem and roots of Resedea luteola by enzymic hydrolysis. This compound and 3-phenyl and 4-phenyl-2-analogues were shown to have antithyroid activity. Greer (1962) isolated an active agent called "Goitrin" from the yellow turnip and from Brassica seeds. In its natural state it was shown to exist as an inactive thioglycoside. The intestinal bacteria hydrolyzed the thioglycoside to the active compound. George et al.(1966) reported a high incidence of goitre in new born lambs of eves and sheep grazing on pasture dominated by white clover. Thyroid glands were shown to be heavier than normal and showed severe hyperplasia of the lining epithelial cells of the follicles and complete absence of colloid in lambs which had grazed rape (Russel, 1967). Simple goitre and hypothyroidism were observed in ruminants when fed Brassica seeds and Brussels sprints (Blood and Henderson, 1968). Akiba and Matsumato (1977) observed 2-5 times enlargement of the thyroids in chicks fed rapeseed meal compared to the controls.

2.10. Chemical goitrogens

Goitrogenic chemicals like thiourea and allied compounds have been often used as goitrogens to induce hypothyroidism experimentally.

$$\begin{matrix} H_2N \\ H_2N \end{matrix} \quad C = S \text{(Thiourea)}$$

Barker et al. (1941) found out that treatment of hypertension with thiocyanate produced goitre and hypothyroidism in man. Kennedy (1942) observed enlarged thyroids in rats treated with thiourea. The glands were three times heavier and follicles were almost devoid of colloid. Pituitary showed an increase in basophil cells and loss of acidophil cells. Baumann and Marine (1945) observed a decrease in adrenal size among rats fed thiouracil. Jones et al. (1946) noted resorption of foetus in rats fed thiouracil which indicated the significance of thyroid hormone for full utilization of estrogens and progesterone. Jones et al. (1946) observed hypertrophy of the thyroid, congestion of vessels and depletion of colloid in rats treated with repeated doses of 1000 mg/kg body weight of thiourea. Involution of the adrenal cortex occurred in rats treated with thiouracil (Zarrow and Money, 1949). The involution of the adrenal cortex after thiouracil treatment was both morphological and physiological in nature. Sellers and Ferguson (1949) observed exopthalmus in rats treated with thiouracil.

D'Angelo et al. (1951) reported thyroid hyperplasia in guinea pigs treated with propyl thiouracil. Histological changes were uniformly evident after 15 to 18 days treatment and were characterized by increased vascularity and increase in the height of the acinar epithelial cells; colloid resorption was in consistent feature. The microscopic changes in prolonged dosing with propyl thiouracil were colloid resorption

and high vascularity. The follicular epithelial cells assumed cord like formation. Hall (1952) reported reduction in serum cholesterol level in rats dosed with thiouracil. Swanson and Boatman (1953) noticed symptoms of hypothyroidism in two young and one old dairy bulls after treatment with thiouracil. The weight of the thyroid gland in the treated animals were twice the weight of the normal. Histologically the follicles were filled with colloid and lined by low cuboidal epithelial cells. Prolonged administration of thiouracil to rats resulted in macroscopical and microscopical changes simulating the late fibrolymphoid stage of struma fibrosa (Clausen, 1954). Durlach et al, (1954) observed an increase in the liver weight in guinea pigs treated with thiouracil. Harkness et al. (1954) recorded the effects of oral administration of thiouracil, on the collogen content of thyroid of rats. Increased weight and collogen content of the thyroid gland was noticed during the treatment.

Goldberg et al. (1957) observed in propyl thiouracil treated rats enlarged thyroid glands with tall columnar cells, numerous mitotic figures, scanty colloid, papillary infoldings and increased vascularity. In the pituitary, hyperplasia and hypertrophy of beta cells with a characteristic granularity and vacuolation and complete absence of granulated alpha cells were observed. Lascelles and Setchell (1959) administered methyl thiouracil at the dose rate of 0.5, 1.5 and 4.5 g daily to six Marino sheep after conception. The offsprings had goitre and retardation of ossification centres. A reduction

in protein bound iodine and increase in cholesterol values were reported. In hamster, colloid goitre was produced by thiouracil administration (Follis, 1959). Extensive thyroid hyperplasia and loss of colloid accompanied by an increase in vascularity were noticed in the first week after thiouracil administration. When thiourea was removed from the diet, the follicles got filled up with colloid and the epithelial cells became flattened but some had residual epithelial springs projecting into the colloid when compared to the normal. Most of the follicles were large in size. McCarthy et al. (1959) reported adrenal atrophy among rats fed goitrogens, thiouracil and tapzole. Besides, Lazovasen (1960) observed that in thiouracil fed rats there was thyroid and pituitary hypertrophy with concomitant reduction in the adrenocortico-trophic hormone (ACTH) level.

Mayberry and Astwood (1961) described the mode of action of thiourea and related compounds to inhibit the formation of iodotyronine and their coupling to form iodotyronine. They also diminished the inorganic iodide content of thyroid and had a slight inhibitory effect on iodide pump (Danowski, 1962).

Nangia et al. (1975) observed high blood levels of protein and cholesterol in methimazole treated birds. Nangia and Gulati (1976) recorded retarded growth in methimazole induced hypothyroid birds. Sreekumaran (1976) induced hypothyroidism in kids using thiourea. Histological picture was characterised by hypertrophy of the follicular epithelium in the thyroid,

hypertrophy and hyperplasia of the basophil cells in the pituitary and depletion of fat and petechiae in the adrenals.

Prasad and Singh (1979) reported decreased plasma protein bound iodine in methimazole treated hypothyroid birds. The effects of methimazole was counteracted by the administration of thyroxine. Burstein et al. (1979) observed a significant drop in the level of growth hormone in hypothyroidism induced by propyl-thiouracil with consequent loss of weight in rats. Davidson et al. (1979) demonstrated that in rats thiourea could inhibit in vivo protein bound iodine formation.

2.11. Dyshormonogenesis

Dyshormonogenesis a condition in which the thyroid fails to produce normal quantities of the thyroid hormone because of an inborn defect in synthesis, has been recorded in Merino sheep (Falconer, 1966). As a result large quantities of thyroid releasing hormone was released resulting in hyperplasia of the thyroid gland. High serum protein bound iodine concentration and low hormonal iodine were reported from goitrous sheep than the controls. The concentration of thyroid stimulating hormone was also significantly high in goitrous sheep. Rac et al. (1968) in their study on congenital goitre in Merino sheep observed enlargement of the thyroid glands in affected sheep. The enlargement was 25 times more than the control. There was significant increase in the cholesterol level in affected sheep. In the Merino the condition was due

to the formation of abnormal iodoproteins, probably controlled by an autosomal recessive gene showing high penetrance and variable expressivity (Mayo and Mulhearn, 1969).

2.12. Surgical thyroidectomy

Marston and Peirce (1932) noted a reduction in growth rate and metabolic rate in thyroidectomised Merino sheep. Zeckwer et al. (1935) reported degranulation of acidophil cells after thyroidectomy in rats. Silberg and Silberg (1940) indicated a delay in endochondral ossification in thyroidectomised immature guinea pigs. Contopoulos et al. (1958) reported that after thyroidectomy in rats there was atrophy of the pituitary and the plasma contained only decreased amounts of thyroid stimulating hormone, interstitial cell stimulating hormone and growth hormone. After thyroidectomy the acidophil cells underwent extreme granulation and with concomitant hyperplasia accompanying hypertrophy and reported a significant decrease in the acidophil cells in thyroidectomised rats. Yatvin et al. (1964) noticed a decrease in protein deoxyribo nucleic acid ratio in thyroidectomised rats. An increase in plasma globulin and sedimentation of red blood cells in thyro-para thyroidectomised Merino rams was reported by Belonje (1967). In thyroidectomised goats there was reduction in phosphorus excretion into long bones and endogenous excretion of phosphorus resulted in hypophosphatemia (Symonds, 1969, 1970). McIntosh et al. (1979) observed somatic changes like delayed osseous development in the limbs and

increase in the pituitary weight after foetal thyroidectomy. The thyroidectomised lambs failed to survive for more than few hours after birth.

2.13. Radio thyroidectomy

Radio thyroidectomy also resulted in the development of clinical signs and pathological lesions of hypothyroidism. Goldberg and Chaikoff (1951) produced an early state of hypothyroidism in rats by injecting various doses of I^{131} in rats. They observed hypertrophy and hyperplasia of the basophil cells accompanied by degranulation of the acidophils. Lewis (1956) observed a drop in protein bound iodine from 6.7 to 0.8 ug % in a Jersey bull after subcutaneous injection of carrier free I^{131}. Bustad et al. (1957) produced thyroid adenoma, fibroma and fibrosarcoma in sheep following daily administration of I^{131} at different levels. Inter follicular fibrosis, oedema and arterial damage were also reported (Marks et al. 1957). Potter et al. (1960) indicated papillary and follicular carcinoma in rats by single injection of I^{131}. Ayoub (1968) reported damage of the thyroid gland and reduction in the rate of radio active iodine uptake by the thyroid and its release into the blood plasma in goats on administration of radio iodine. Cons et al. (1975) recorded high plasma thyroid stimulating hormone level in radio thyroidectomised rats.

2.14. Thyroid status and male reproduction

McKenzie and Berliner (1937) observed that in the ram summer sterility was influenced by the thyroid. Turner and Cupps (1940) noted reduced anterior pituitary gonadotropin content in thyroidectomised male rats. Similarly a reduction in the gonadotrophic potency of the pituitary following thyroidectomy was reported among young male goats (Reineke et al. 1941). Peterson et al. (1941) observed that following thyroidectomy in bull calves there was normal development of gonads but there was complete lack of libido at the usual age of sexual maturity. Schultze and Davis (1946) noted a decrease in conception rate, sperm motility and greater resistance of spermatozoa in bulls fed iodinated casein.

Kumaran and Turner (1949) induced mild hypothyroidism in birds by feeding 0.6% thiouracil and observed a progressive depression of the secretion of interstitial cell stimulating hormone (ICSH) without having any effect on follicular stimulating hormone unlike mild hyperthyroidism. Maqsood (1952) demonstrated that the thyroid gland had an important role in the maintenance of male fertility. In his study on the effect of thyroxine supplementation recorded precocious sexual development, increased sex libido and improvement in semen picture. Hignett (1952) noted a decline in libido and deterioration of semen quality in iodine deficient bulls. Jovanovic et al. (1953) reported decreased libido and high percentage of sterility in males associated with enzootic goitre. Lenon and

Mixner (1958) observed better reproductive performance in Holstein cattle associated with high PBI values.

Brooks and Ross (1962) observed that exogenous administration of L-thyroxine in feed at 0.2, 0.3 and 0.4 µg % concentration failed to have any significant effect on the adverse influence of high ambient temperature on the semen quality in rams. Mahtiev (1966) recorded an improvement in fertility and semen picture following iodine supplementation in infertile rams maintained in iodine deficient areas.

Prasad and Singh (1971) observed the effects of propyl thiouracil and thyroxine on the testis of chicks. A four fold increase in the weight of the testis was noted when compared with normal chicks at 8 weeks of age. Testis of propyl thiouracil treated birds showed tightly arranged coils of seminiferous tubules as compared with the loose arrangement of such tubules in normal birds.

Sharma and Singh (1975) recorded more coiled seminiferous tubules lined with two or more layered germinal epithelium in hypothyroid birds as against single layer of epithelium in the control groups.

Sreekumaran (1976) observed that the seminiferous tubules contained only few primary and secondary spermatocytes in experimentally induced hypothyroid state in male kids. There was complete absence of spermatozoa and germinal layer in some tubules. The lumen of the tubules contained only a network of

fibres and scattered round cells. Peczely et al. (1979) reported that although thyroidectomy or treatment with thyroxine did not affect the basal testosterone concentration there was a marked inhibition of the growth of the testis.

Reddy (1982) observed a significant decrease in the relative weight of the testis in all the goats dosed with thiourea. There was also a diminution in the relative weight of epididymis and accessory sexual glands. Histologically the seminiferous tubules were small with almost complete absence of spermatogenesis.

2.15. Thyroid and infertility

Chu and You (1944) reported that feeding small doses of desiccated thyroid to thyroidectomised rabbits prevented the hypertrophy of ovarian follicles, while large doses had inhibitory action. Similar treatment in normal rabbits had no effect on the ovary and large doses caused incomplete inhibition of ovarian activity. In thyroidectomised females there was a failure to show external signs of estrus although physical examination revealed that these females to be cycling, ovulation occurring regularly (Spielman et al. 1946). Krohn (1947) found that daily subcutaneous injection of propyl thiouracil disturbed the oestrus rhythm of adult albino mice, causing lengthening, irregularity or complete disappearance of cycles. Krohn and White (1950) observed longer and variable

oestrus cycles in hypothyroid rats. Conception was normal with small litter size and high foetal resorption.

Thiouracil fed mice exhibited continuous oestrus while slightly hyperthyroid mice showed regular oestrus cycles. The thiouracil fed groups had ovaries packed with follicles but no corpora lutea (Soliman and Reineke, 1952).

Brownstand and Fowler (1959) reported that the ovulation rate tended to be lower among sows maintained on 0.15% thiouracil. Moberg (1959) described retention of placenta in bovines associated with sub-optimal iodine intake. Kovalskii et al. (1970) treated anestroum associated with iodine deficiency in dairy cows by supplementing potassium iodine. Oestrum with normal ovulation and fertilization was induced within an average period of 148 days, when treated with 1.75 mg of potassium iodide per kg. Barakat et al. (1971) observed no variation in the total thyroid iodine content in normal female buffaloes and in buffaloes with cystic ovary, inactive ovary, hydrosalphinx, mucometra, perimetritis with salp ingitis metritis, perimetritis with salpingitis and cophoritis.

Srinivas (1979) reported very low concentration of PBI among buffaloes with cystic ovarian degeneration. The mean value was 1.68 $\pm$ 0.04 ug %. The highest concentration of serum PBI was seen in animals with uterine infection. Vadodaria et al. (1980) observed that in ovulatory phase the thyroid gland follicles were less active compared to luteal phase follicles.

2.16. Incidence of goitre in sheep and goats

The incidence of goitre and hypothyroidism have been reported by several workers in sheep and goats. Love (1942) studied changes in the thyroid gland of four new born kids. He noticed alopecia in two cases out of four cases of parenchymatous goitre in still born kids. McIntosh (1943) reported that in hypothyroidism the new born lambs were weak and the wool growth was poor with focal area of denudation. Southcott (1945) recorded congenital goitre in lambs. The lambs were weak or born dead. Histologically follicles were depleted of colloid and were filled with finger like processes of the lining epithelium. The epithelial cells were low cuboidal or columnar type.

Baumann(1948) described goitre in new born kids. In six cases myxoedema and complete hairlessness were noticed. Histologically two were parenchymatous type and four mixed type. Andrews et al. (1948) noticed hyperplasia of the thyroid epithelium and depletion of colloid in the thyroid gland of new born lambs from the dams which were fed a diet free of iodised salt. Investigation on the incidence of goitre in domestic animals revealed that its incidence in animals and human was parallel. The number of animals affected with goitre was large. Otomatus (1954) made a study on the incidence of goitre in sheep in Japan. He recorded congenital goitre in 25% of new born lambs. In adults he documented an incidence of 33%. The gestation period of goitrous sheep

was much longer than that was observed for normal sheep. The new born lambs were weak and died within a few hours. The thyroid glands exhibited various forms of enlargement. Typical struma parenchymatosa was noted histologically.

Jovanovic (1955) investigated on the nature and incidence of goitre in domestic animals. The diffuse colloid type was the commonest in sheep and goats. The parenchymatous goitre was observed only in goats.

Setchell et al. (1960) reported neonatal mortality associated with thyroid enlargement in lambs. They observed that the affected lambs were weak and lethargic leaving coarse coat. The glands showed varying degree of colloid change histologically.

Growth (1962) reported hypothyroidism in sheep. He observed adverse effect on wool growth and increased incidence of alopecia and still born lambs and kids. Watson et al. (1962) observed increased size and weight of the thyroid glands and decrease in the iodine content of the glands in congenital goitrous Doorset Horn lambs. There was pronounced hyperplasia of thyroid epithelial cells in these animals.

Wallach (1965) reported goitrogenic hypothyroidism in feeder lambs. In the lambs he observed low basal metabolic rate, retardation of growth rate and increased sensitivity to low environmental temperature. There was significant enlargement of the glands and the enlarged thyroids were palpable.

Tall columnar epithelial cells were seen lining the acini. The acini contained scanty colloid and papillary structures were seen protruding into the acini. George et al. (1966) conducted histopathological study of thyroid glands of dead lambs in order to assess the incidence of goitre. He observed parenchymatous and transitional parenchymatous goitre in the affected glands of lambs. The follicles were devoid of colloid but showed large cystic spaces.

2.16.1. Incidence of goitre and hypothyroidism in sheep and goats in India

So far the endocrine system has not been a subject of detailed pathological investigation in this country. Only very few reports have appeared on the incidence of endocrine disorders in domestic animals from India.

Lall (1952) reported congenital goitre in three kids with enlarged thyroid. The thyroid glands histologically showed hyperplasia of the lining cells of the follicles. The acidophils of the pituitary were few in number and there was increase in the number of basophil cells. In the testis the seminiferous tubules were found immature.

Dutt and Kehar (1959) studied 1000 thyroid glands collected from sheep and goats from Barcily slaughter house. The incidence of goitre was common in goats (10%), particularly in female goats. Not a single case of goitre was observed in sheep. Dutt and Vasudeva (1963) described a case of hypothyroidism in a ram. There was loss of weight, irregular

appetite and intermittant diarrhoea. At autopsy the thyroid glands were found to be cystic and slightly enlarged. On histological examination the follicles were found to be atrophic and lined with low cuboidal epithelium. Seminiferous tubules in the testis were atrophic and some of the tubules showed multinucleated giant cells. Pituitary showed short cords of hypertrophied epithelial cells projecting as finger like processes in some places and also hyperplasia of the basophils.

Roy et al. (1964) made a comparative study of the thyroid glands of 25 human and 50 goats collected from a severely endemic area in the Himalayan belt. The thyroid glands of goats were large, pale and hyperplastic with intense lobular hyperplasia. In human thyroids, grossly visible, well circumscribed, greyish white multiple nodules were noticed. There was extreme reduction of organic iodine content of thyroids in man and animals from endemic area. Microscopically the epithelial cells were tall columnar type and they were thrown into papillary folds. Follicles contained little or no colloid. Human thyroid showed intense epithelial and stromal hyperplasia.

Rajkumar (1970) observed enlargement of the thyroid glands in 16 kids out of 29 Barbari kids in a Government Farm in Uttar Pradesh. Taking gross enlargement of the thyroid gland as the criterion for the diagnosis of goitre in village flock he recorded an incidence of 0.54% in local goats, 7.02% in Barbari x Local and 16.67% in Alpine x Local goats.

Sreekumaran (1976) described the clinico-pathological features of experimental hypothyroidism in kids. Histologically the thyroid gland showed formation of colloid depleted microfollicles and the pituitary was hypertrophied with hyperplasia of basophil cells. Both male and female gonads showed degenerative changes.

Reddy (1982) also studied the clinico-pathological features of experimental hypothyroidism in goats with particular reference to the reproductive organs. There was complete absence of spermatogenesis and degenerative changes in the tubules. But these testicular changes were found to be reversible. In the case of females there were smooth inactive ovaries. The weight of the uterus was significantly low with almost complete cessation of secretory activity. The increase in weight of the thyroid was associated with hyperplastic changes in the parenchyma.

Materials and Methods

3. MATERIALS AND METHODS

3.1. Survey studies

Blood samples (5 ml) from the jugular vein were collected from cows with non-infectious reproductive disorders maintained at the University Livestock Farm, Mannuthy and from cows which were confirmed to have reproductive disorders of non-infectious nature at the veterinary hospitals located at the different regions (Thodupuzha and Palghat). Besides this blood samples from cows with normal reproductive behaviour were also collected. The protein bound iodine (PBI) of all these animals were estimated to assess the thyroid status.

3.2. Experimental studies

3.2.1. Design of the experimental study:

a) Experimental calves: Fourteen, clinically healthy, two to three month old male cross-bred calves were randomly selected from the Livestock Research Station, Thiruvazhamkunnu for the study. The animals were housed separately in pens under hygienic conditions. All the animals were maintained on concentrates and paddy straw/green fodder. Water was provided ad libitum. The animals were divided into two groups consisting of a control group of four animals and experimental group of ten animals. All the calves were dewormed and kept under observation for a fortnight before the start of the experiment.

3.2.2. Dose of thiourea

Experimental hypothyroidism was induced by the daily oral administration of thiourea ($H_2NCS\ NH_2$ - Sarabhai-M-Chemicals) at a dosage level of 150 mg/kg body weight. The thiourea was administred as a bolus mixed with jaggery.

3.2.3. Clinical parameters studied

Body weight, hemogram (total RBC, WBC, differential leucocyte count, packed cell volume, hemoglobin content), serum cholesterol, serum protein bound iodine and total plasma protein of all the animals were recorded before the commencement of the experiment and subsequently at fortnightly intervals. The calves were observed daily and clinical symptoms, if any, manifested were observed and recorded. The study covered the following aspects.

a) Weight gain and growth rate
b) Observation of clinical symptoms
c) Haemogram values
d) Estimation of total protein content in blood serum
e) Estimation of serum cholesterol
f) Determination of protein bound iodine
g) Gross pathology
h) Histopathological changes in organs

3.2.4. Techniques

3.2.4.1. Clinical symptoms and weight gain

All the animals were observed daily for clinical symptoms, if any. Both the control and experimental group of animals

were weighed at the commencement of the experiment and thereafter at fortnightly intervals.

3.2.4.2. Collection of blood samples for laboratory estimation

Blood samples for analysis were collected using reagent grade Ethylene diamino tetra acetic acid (disodium salt EDTA) as the anticoagulant. EDTA at the rate of one mg for every one ml of blood was employed as the anticoagulant. Every time five millilitre of blood was drawn from the jugular vein for haematological studies with aseptic precaution. Five millilitre of blood was also collected separately in a sterile test tube without adding the anticoagulant. The blood was allowed to clot and then serum was separated by centrifugation for estimation of serum cholesterol and PBI values.

Packed cell volume: The method described by Wintrobe (1961) was adopted.

3.2.4.3. Haemoglobin

The method of Miale (1967) for the determination of haemoglobin was modified in this estimation. The cyan methemoglobin was prepared as detailed by Miale (1967) but the final readings were taken in a Erma Haemophotometer as against Spectronic 20.

3.2.4.4. Erythrocyte count

Erythrocyte counts were made following the technique of Schalm (1965).

3.2.4.6. Differential count

The technique of Schalm (1965) was adopted.

3.2.4.7. Total plasma protein

The Biuret assay method of Inchiosa (1964) was adopted for the estimation of total protein content in blood plasma.

3.2.4.8. Serum cholesterol

Serum cholesterol was estimated employing the method of Zak (1957).

3.2.4.9. Protein bound iodine

The protein bound iodine in the serum was estimated employing the method of Faulkner et al. (1961).

3.2.4.10. Post-mortem examination

The control and experimental animals which died/sacrificed by exsanguination at the end of experiment were subjected to detailed autopsy. They were weighed before autopsy. The method advocated by FAO/SIDA (1968) was followed for autopsy. The endocrine glands (thyroid, pituitary and adrenals) were dissected out and weighed after removing the loose fat and fascia. Testes were separated from the epididymis and their weights were recorded.

3.2.4.11. Histopathology

The endocrine glands and testis were incised and examined for gross lesions. Appropriate samples of tissues were collected in 10% buffered neutral formalin for histopathological

examination. Tissues were processed by routine paraffin embedding technique (Armed Forces Institute of Pathology, 1968). Paraffin sections cut at 5 to 6 microns thickness were stained routinely with haematoxylin and eosin method of Harris as described by Disbery and Rack (1970).

Results

4. RESULTS

4.1. Survey of thyroid status of cattle with normal and impaired reproductive performance

In this survey, the thyroid status of one hundred animals was assessed employing protein bound iodine as the marker. This included 53 cross-bred cows with the history of non-infectious infertility from Mannuthy (Trichur District), Palghat, Nemmara, Kozhinjampara and Alathur in Palghat district and Thodupuzha in Idukki district. These animals were brought to the local veterinary hospitals and they were given all the routine treatments by Veterinary Officer i/c of the hospital and they were declared as cases of non-infectious infertility not amenable for conventional treatment. The serum from 47 animals were also from these localities but with normal reproductive performance. The PBI levels recorded are listed out in Table 1. The mean PBI value of the animals with the history of infertility was 2.68 µg/100 ml. The means were tested for significance using Cochran's t-statistic and was found that the PBI level was significantly low in cows with non-infectious infertility. The PBI level of the dry cows belonging to the Livestock Farm, Mannuthy was relatively low and it ranged from 3.0 µg/100 ml to 4.6 µg/100 ml with a mean of 3.84µg/100 ml.

Table 1. PBI level in cows with non-infectious reproductive disorders

Area	Animal number	PBI levels in µg/100 ml
Palghat	1	2.2
	2	1.4
	3	1.2
	4	2.8
	5	2.4
	6	3.2
	7	2.4
	8	3.2
	9	2.8
	10	4.0
	11	2.4
	12	2.4
Nemmara	1	2.6
	2	2.4
	3	3.2
	4	2.8
	5	3.0
	6	2.0
	7	2.4
	8	2.6
	9	2.4
	10	2.0
	11	3.4
	12	2.4
	13	1.8
	14	1.2
	15	3.0
	16	3.4
	17	2.8
	18	2.4

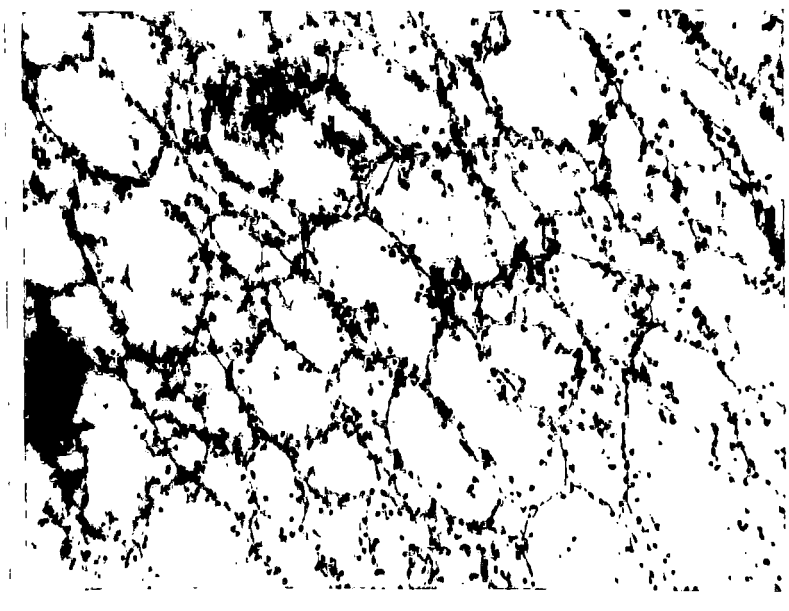
Place	Animal number	PBI levels in µg/100 ml
Nemmara (contd.)	19	2.2
	20	3.2
	21	2.2
	22	2.1
	23	2.4
	24	2.6
	25	2.4
Thodupuzha	1	2.4
	2	3.8
	3	2.6
	4	3.6
	5	3.2
	6	3.0
	7	5.2
	8	5.2
Alathur	1	2.6
	2	2.4
	3	2.4
	4	2.0
	5	2.8
	6	3.2
	7	2.6
	8	1.8
Livestock Farm, Mannuthy		
a) Lactating animals	1	5.2
	2	6.4
	3	6.4
	4	5.2
	5	6.8
	6	6.8

Place	Animal number	PBI levels in µg/100 ml
	7	5.2
	8	6.8
	9	5.0
	10	6.1
	11	6.8
	12	5.1
	13	4.8
	14	5.0
	15	4.8
	16	5.2
	17	5.2
	18	5.1
	19	4.8
	20	5.2
	21	5.2
	22	5.2
	23	5.0
	24	4.8
	25	5.2
	26	5.0
	27	4.8
b) Dry animals	28	4.1
	29	4.2
	30	4.2
	31	3.1
	32	4.0
	33	4.2
	34	3.6
	35	3.6
	36	4.2

Place	Animal number	PBI levels in µg/100 ml
Dry animals (contd.)	37	3.8
	38	3.0
	39	4.4
	40	4.2
	41	3.6
	42	3.2
	43	3.6
	44	3.2
	45	4.6
	46	4.4
	47	3.6

Fig. No.10. Experimental hypothyroidism - calf - severe degree of alopecia

Fig. No.11. Hypothyroidism - calf - Thyroid - colloid depleted follicles lined with scattered epithelial cells
H & E x 200



EXPERIMENTAL STUDIES

4.2. Experimental hypothyroidism in calves

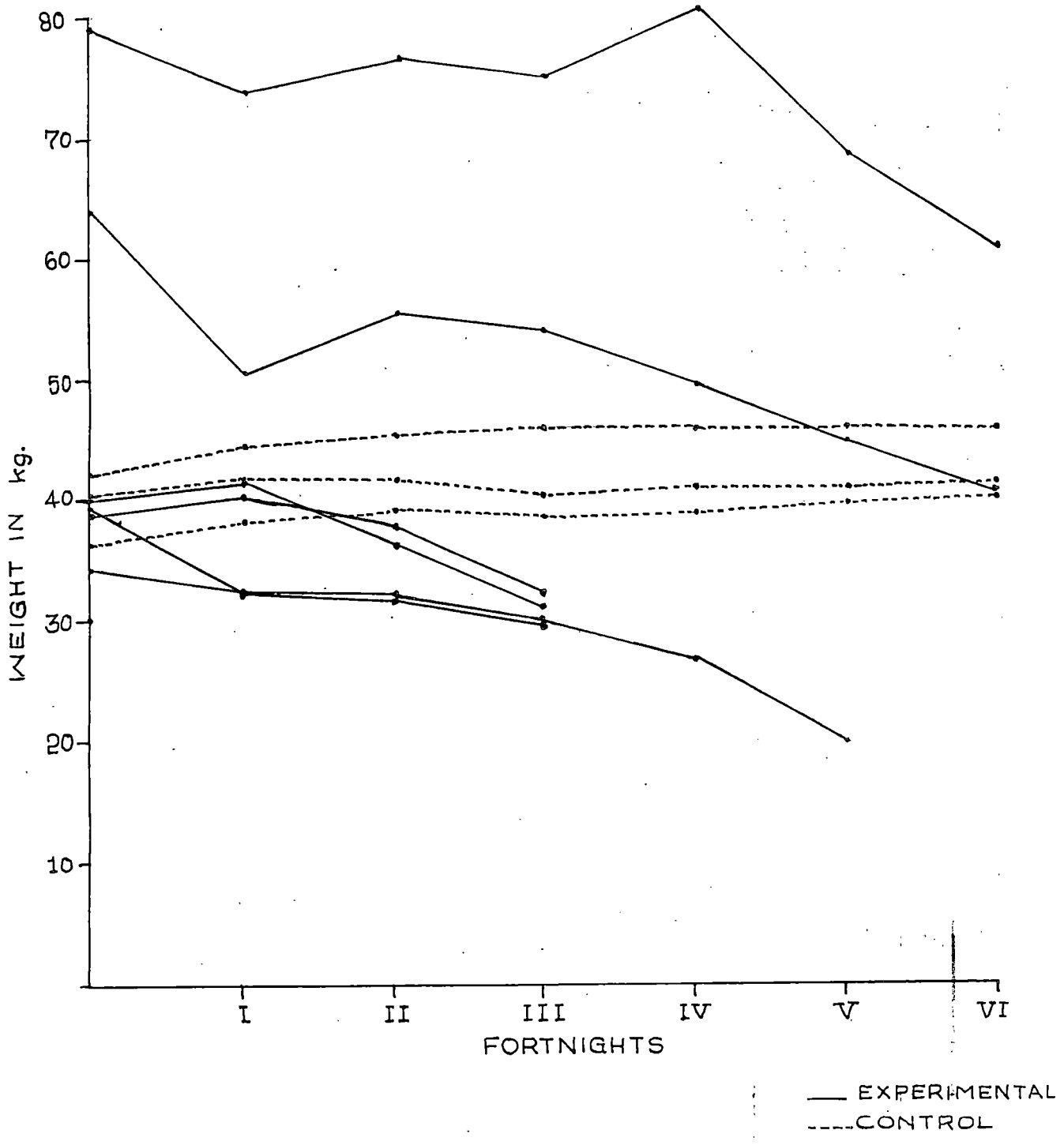
4.2.1. Clinical behaviour and symptoms

The experimental group consisted of 10 cross-bred male calves in the age group 2 to 4 months. They were fed thiourea at the rate of 150 mg/kg body weight daily. All the calves showed similar symptoms with slight degree of variation in their manifestation. The animals were apparently normal and healthy during the first fortnight. Subsequently there was progressive dullness and weakness. The hair coat was dry and coarse (Fig. 8). The eyes were sunken. There was irregular shedding of hair more markedly on the belly, thigh, neck and dewlap region and this resulted in patchy areas of alopecia (Fig. 9, 10). Moreover, there was considerable diminution of subcutaneous fat. They became lethargic, inactive and showed marked disinclination to move. They were mostly in recumbent posture. Feed consumption was greatly reduced. The gait was unsteady. They carried their head in drooping position. The muzzle was markedly dry. Slight to moderate oedema was noticed on the face below the eyelid, jaw region and lower parts of the body. There was slight to moderate watery discharge from the eyes.

4.2.2. Weight gain

The data on the weight of calves during the experimental period are given in Fig.1. Most of the calves gained body

FIG. 1. BODY WEIGHT OF CALVES DURING THE EXPERIMENTAL PERIOD



weight during the first fortnight and subsequently there was a gradual and progressive reduction in body weight. But in two of the calves initial sharp drop in body weight during the first fortnight was followed by moderate increase in body weight and subsequently there was steady reduction in body weight towards the termination of the experimental period. On the contrary, the control group of animals recorded a gradual but appreciable increase in body weight.

Table 2. Body weight of calves during experimental period

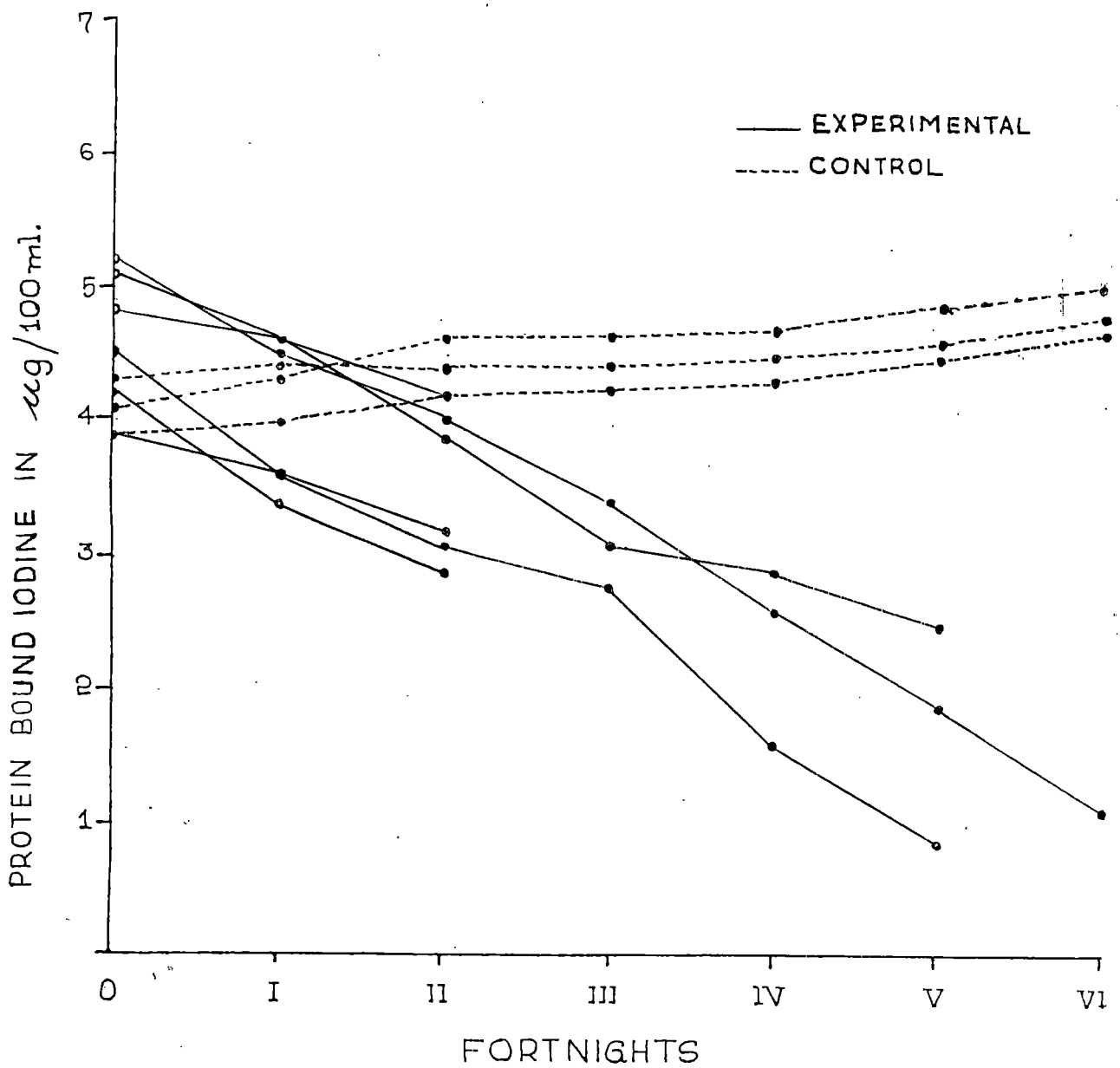
	Means	
	Control (Kg)	Experimental (Kg)
Initial	39.933	38.300
1st fortnight	41.533	36.675
2nd fortnight	42.466	34.175
3rd fortnight	41.900	30.525

By making use of the paired t test the mean weight of the control and experimental calves were compared and the reduction in weight in the experimental calves was compared to the control calves was found to be statistically significant at the end of second fortnight and during the third fortnight ($P < 0.05$).

4.2.3. Serum protein bound iodine

The data on serum protein bound iodine are presented in Fig. 2.

FIG. 2: PROTEIN BOUND IODINE LEVEL OF EXPERIMENTAL CALVES



Though the PBI level recorded a gradual decrease initially the decrease became statistically significant during the second fortnight in the animals dosed with thiourea as compared with the control animals which recorded a slow and gradual rise in PBI levels in the serum. Lowest level was noted in animals which survived the entire experimental period.

Table 3. Serum protein bound iodine level of calves

	Means	
	Control (µg/100ml)	Experimental (µg/100ml)
Initial	4.100	4.616
1st fortnight	4.233	4.050
2nd fortnight	4.400	3.366

The mean PBI levels of the control and experimental group of calves were compared by making use of paired t test and the reduction PBI level in experimental calves was found to be statistically significant at the end of the second fortnight.

4.2.4. Serum cholesterol

The data on serum cholesterol level of the animals are presented in Fig.3.

Table 4. Serum cholesterol level of calves

	Mean	
	Control (mg/100ml)	Experimental (mg/100ml)
Initial	163.420	160.005
1st fortnight	171.063	190.968
2nd fortnight	173.990	224.823

FIG.3: SERUM CHOLESTEROL LEVEL IN EXPERIMENTAL CALVES

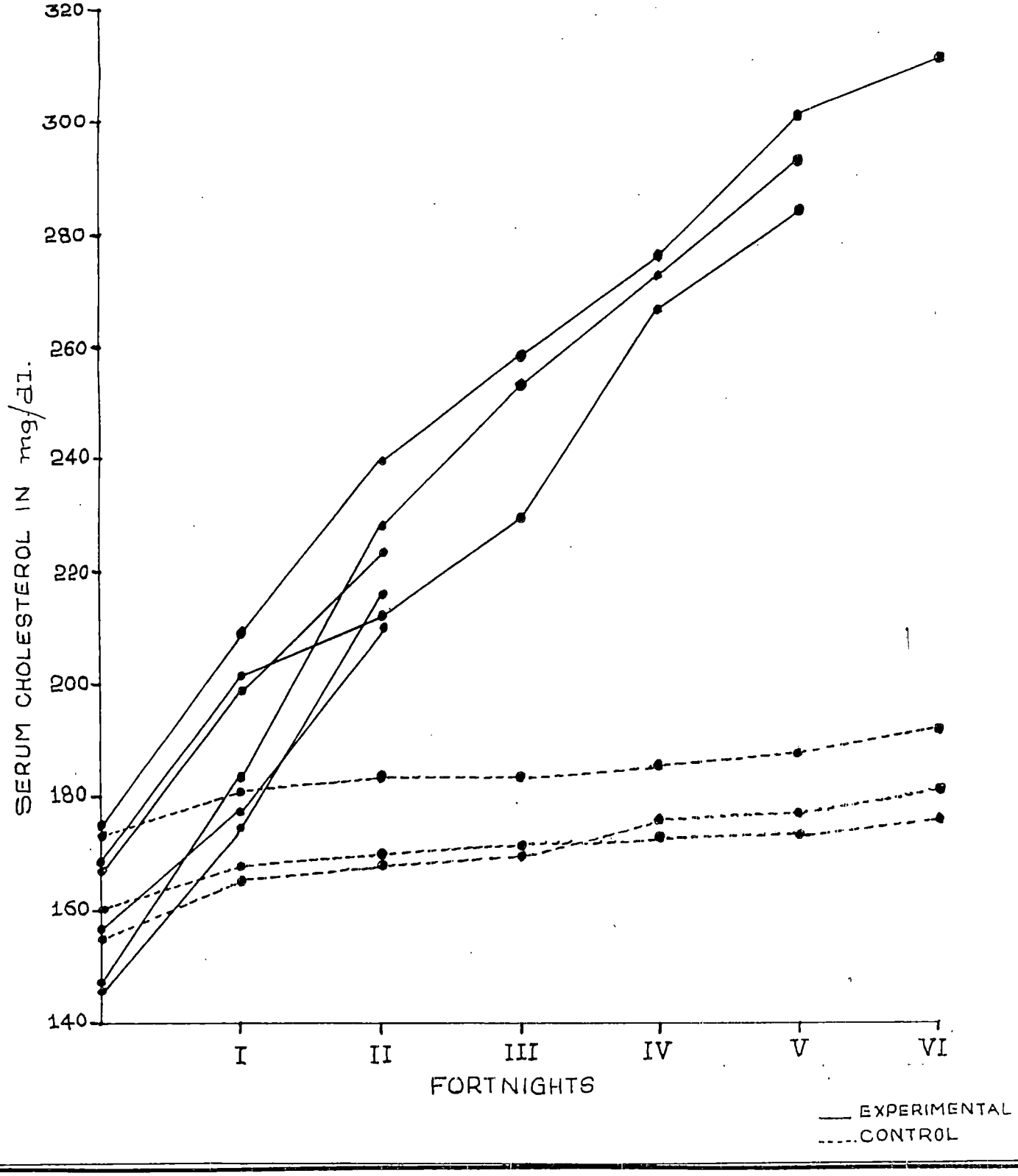
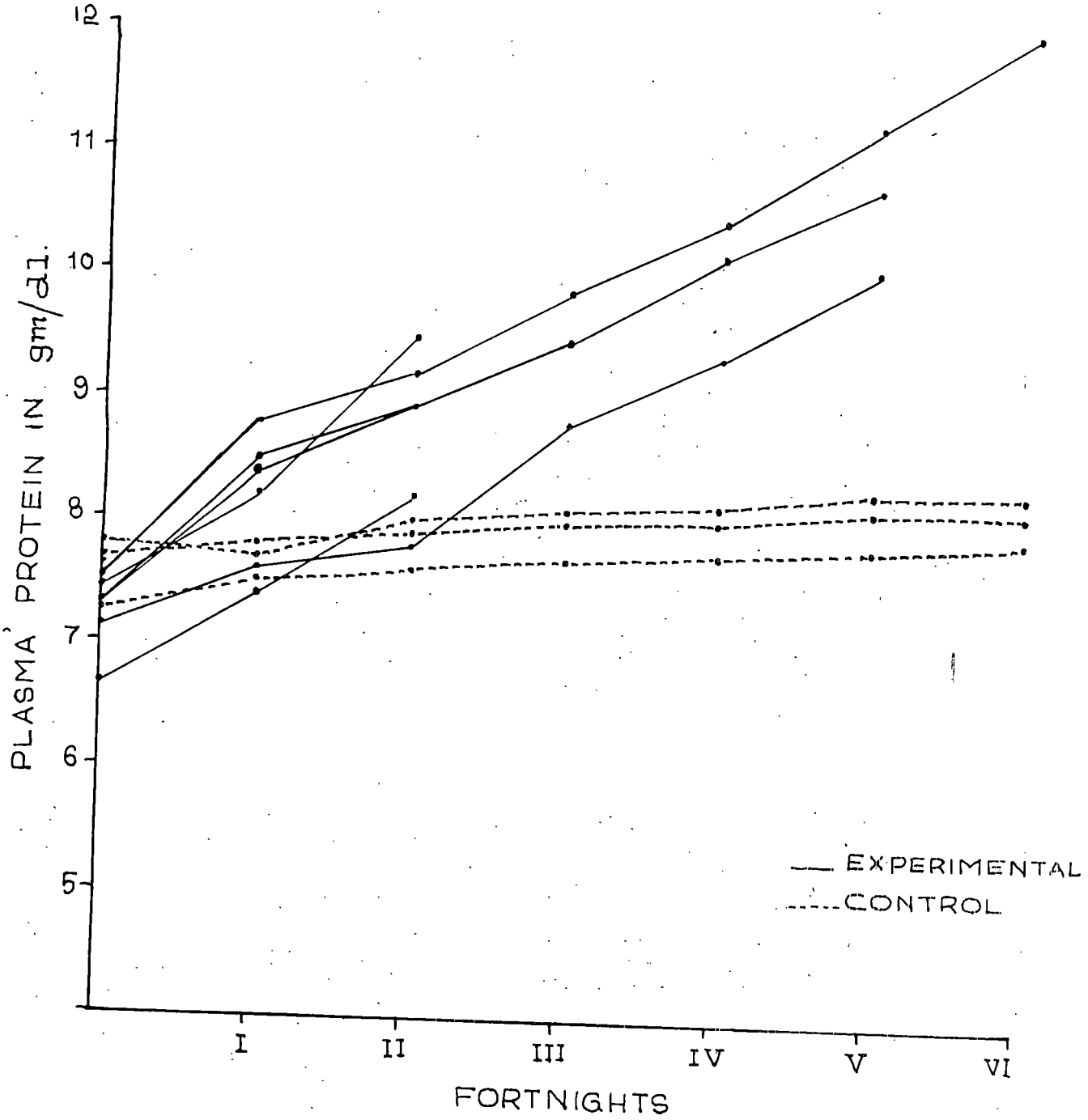


FIG.4: PLASMA PROTEIN LEVEL IN EXPERIMENTAL CALVES



Statistical analysis employing paired t test for the comparison of the mean of control and experimental group showed that the increase in the serum cholestrol level in experimental calves was statistically significant by the second fortnight.

Table 5. Plasma protein level of calves
(The data on plasma protein level of the animals are presented in Fig.4)

	Mean	
	Control (g/100ml)	Experimental (g/100ml)
Initial	7.566	7.266
1st fortnight	7.666	8.100
2nd fortnight	7.833	8.750

Comparison of the mean of the control and experimental group using paired t test showed that the increase in plasma protein level in experimental calves was statistically significant by the second fortnight.

Table 6. Haemogram of experimental animals

Calf No.M52 - 6(a)

Parameters	Before experiment	First fortnight	Second fortnight	Third fortnight	Fourth fortnight	Fifth fortnight	Sixth fortnight
RBC millions/µl	7.1	9.3	7.73	7.65	5.7	5.5	-
Hgb g/dl	9.4	9.5	7.2	7.6	5.7	4.8	-
PCV %	26	31	31	32	24	23	-
Leucocyte/µl	8.450	9.1450	5.800	10.350	11.350	9.775	-
DC							
Lymphocytes	84	76	88	60	62	72	-
Neutrophil (band)	1	3	-	-	-	2	-
Neutrophil (segment)	15	21	10	38	34	24	-
Monocyte	-	-	1	-	1	-	-
Eosinophil	-	-	1	2	3	1	-
Basophil	-	-	-	-	-	-	-

Calf No.750 - 6(b)

Parameters	Before experiment	First fortnight	Second fortnight	Third fortnight	Fourth fortnight	Fifth fortnight	Sixth fortnight
RBC million/µl	5.2	8.8	6.6	6.4	3.9	4.2	3.6
Hgb g/dl	8.4	8.6	7.8	7.5	6.4	5.0	4.2
PCV %	31	35	26	24	22	19	16
Leucocytes/µl	7100	6050	4900	5850	9950	3050	3450
DC							
Lymphocytes	82	87	88	58	54	56	62
Neutrophil (band)	3	2	1	-	-	-	-
Neutrophil (segment)	13	10	6	39	44	39	37
Monocyte	1	1	2	-	1	-	1
Eosinophil	-	-	3	3	1	4	-
Basophil	1	-	-	-	-	-	-

Calf No.485 - 6(c)

Parameters	Before experiment	First fortnight	Second fortnight	Third fortnight	Fourth fortnight	Fifth fortnight	Sixth fortnight
RBC million/µl	6.7	5.6	4.8	-	-	-	-
Hgb g/dl	12.4	9.8	8.2	-	-	-	-
PCV %	42	32	31	-	-	-	-
Leucocyte/µl	6250	5825	6595	-	-	-	-
DC							
Lymphocyte	87	89	77	-	-	-	-
Neutrophil (band)	1	-	3	-	-	-	-
Neutrophil (segmenter)	12	10	22	-	-	-	-
Monocyte	-	-	-	-	-	-	-
Eosinophil	-	1	1	-	-	-	-
Basophil	-	-	-	-	-	-	-

Calf No.486 - 6(d)

Parameters	Before experiment	First fortnight	Second fortnight	Third fortnight	Fourth fortnight	Fifth fortnight	Sixth fortnight
RBC million/µl	8.2	8.4	6.6	6.2	5.1	4.2	-
Hgb g/dl	12.2	12.4	8.2	6.2	5.8	5.6	-
PVC %	48	48	32	31	26	23	-
Leucocyte/µl	4450	6500	4900	5850	4670	4760	-
DC							
Lymphocyte	70	87	58	62	63	59	-
Neutrophil (band)	2	-	1	-	-	-	-
Neutrophil (segmenter)	27	12	37	35	34	39	-
Monocyte	1	-	-	-	2	2	-
Eosinophil	-	1	4	3	1	-	-
Basophil	-	-	-	-	-	-	-

Calf No.775 - 6(e)

Parameters	Before-experiment	First fortnight	Second fortnight	Third fortnight	Fourth fortnight	Fifth fortnight	Sixth fortnight
RBC million/µl	7.4	5.4	5.2	-	-	-	-
Hgb g/dl	13.8	10.4	7.2	-	-	-	-
PCV %	47	42	36	-	-	-	-
Leucocyte/µl	6150	3600	5700	-	-	-	-
DC							
Lymphocyte	71	78	82	-	-	-	-
Neutrophil (band)	1	2	2	-	-	-	-
Neutrophil (segmenter)	78	20	13	-	-	-	-
Monocyte	-	-	1	-	-	-	-
Eosinophils	-	-	2	-	-	-	-
Basophil	-	-	-	-	-	-	-

Calf No.776 - 6(f)

Parameters	Before experiment	First fortnight	Second fortnight	Third fortnight	Fourth fortnight	Fifth fortnight	Sixth fortnight
RBC million/µl	6.4	6.2	6.1	-	-	-	-
Hgb g/dl	10.4	9.2	7.4	-	-	-	-
PCV %	39	78	25	-	-	-	-
Leucocyte/µl	6590	6210	6920	-	-	-	-
DC							
Lymphocyte	62	58	56	-	-	-	-
Neutrophil (band)	1	-	-	-	-	-	-
Neutrophil (segmenter)	34	38	42	-	-	-	-
Monocyte	1	2	-	-	-	-	-
Eosinophil	2	2	2	-	-	-	-
Basophil	-	-	-	-	-	-	-

CONTROLS

Calf No.082-M52 - 6(g)

Parameters	Before experiment	First fortnight	Second fortnight	Third fortnight	Fourth fortnight	Fifth fortnight	Sixth fortnight
RBC million/µl	5.2	4.8	5.6	-	-	-	-
Hgb g/dl	9.8	9.6	9.8	-	-	-	-
PCV %	36	38	39	-	-	-	-
Leucocyte/µl	6550	8920	7625	-	-	-	-
DC							
Lymphocyte	78	64	82	-	-	-	-
Neutrophil (band)	2	3	-	-	-	-	-
Neutrophil (segmenter)	18	30	15	-	-	-	-
Monocyte	-	1	1	-	-	-	-
Eosinophil	1	2	2	-	-	-	-
Basophil	-	-	-	-	-	-	-

Calf No.081-M52 - 6(h)

Parameters	Before experiment	First fortnight	Second fortnight	Third fortnight	Fourth fortnight	Fifth fortnight	Sixth fortnight
RBC million/µl	6.1	6.2	6.2	-	-	-	-
Hgb g/dl	8.6	9.2	10.4	-	-	-	-
PCV %	27	29	32	-	-	-	-
Leucocyte/µl	7520	6545	6670	-	-	-	-
DC							
Lymphocyte	75	69	72	-	-	-	-
Neutrophil (band)	-	1	1	-	-	-	-
Neutrophil (segmenter)	23	29	27	-	-	-	-
Monocyte	-	-	-	-	-	-	-
Eosinophil	2	1	-	-	-	-	-
Basophil	-	-	-	-	-	-	-

Calf No.189 M52 - 6(i)

Parameters	Before experiment	First fortnight	Second fortnight	Third fortnight	Fourth fortnight	Fifth fortnight	Sixth fortnight
RBC million/µl	6.4	6.6	6.6	-	-	-	-
Hgb g/dl	10.2	9.8	10.4	-	-	-	-
PCV %	31	31	32	-	-	-	-
Leucocyte/µl	5700	8600	8970	-	-	-	-
DC							
Lymphocyte	72	62	67	-	-	-	-
Neutrophil (band)	-	1	1	-	-	-	-
Neutrophil (segmenter)	25	36	30	-	-	-	-
Monocyte	-	-	1	-	-	-	-
Eosinophil	3	1	1	-	-	-	-
Basophil	-	-	-	-	-	-	-

FIG. 5 : FORTNIGHTLY HAEMOGLOBIN LEVEL

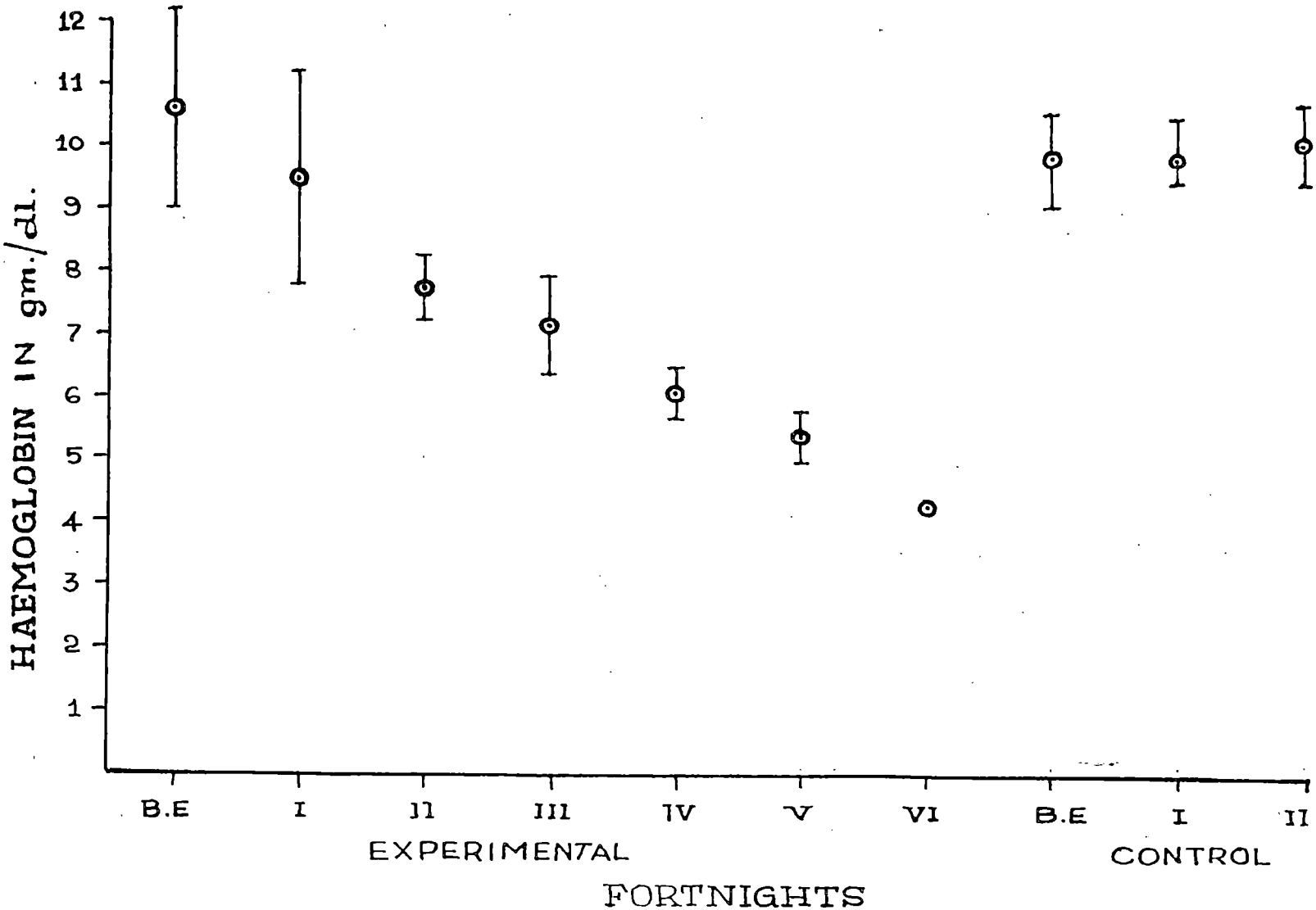


FIG.6: RELATIVE WEIGHT OF THE ENDOCRINE GLANDS OF THE EXPERIMENTAL CALVES

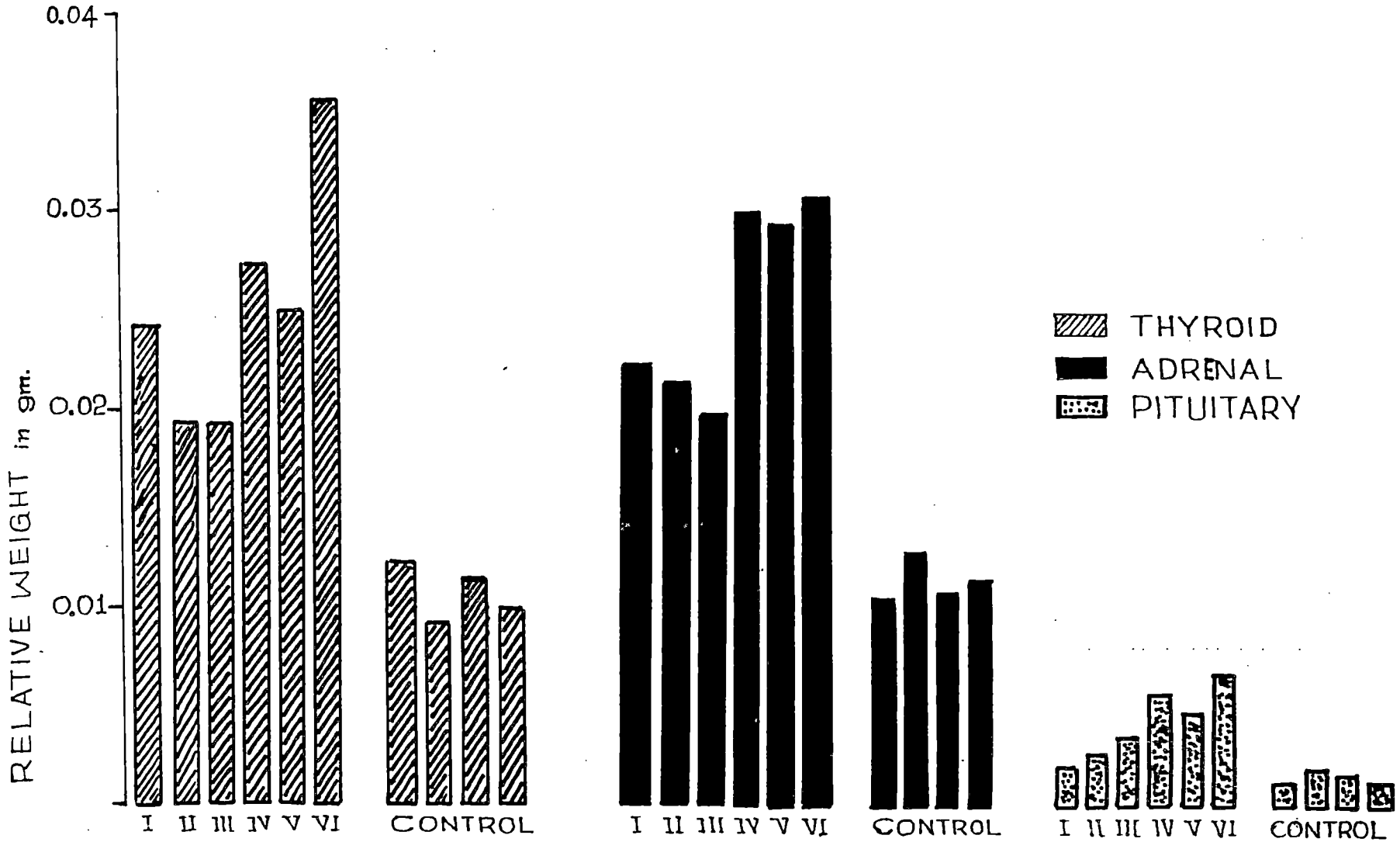
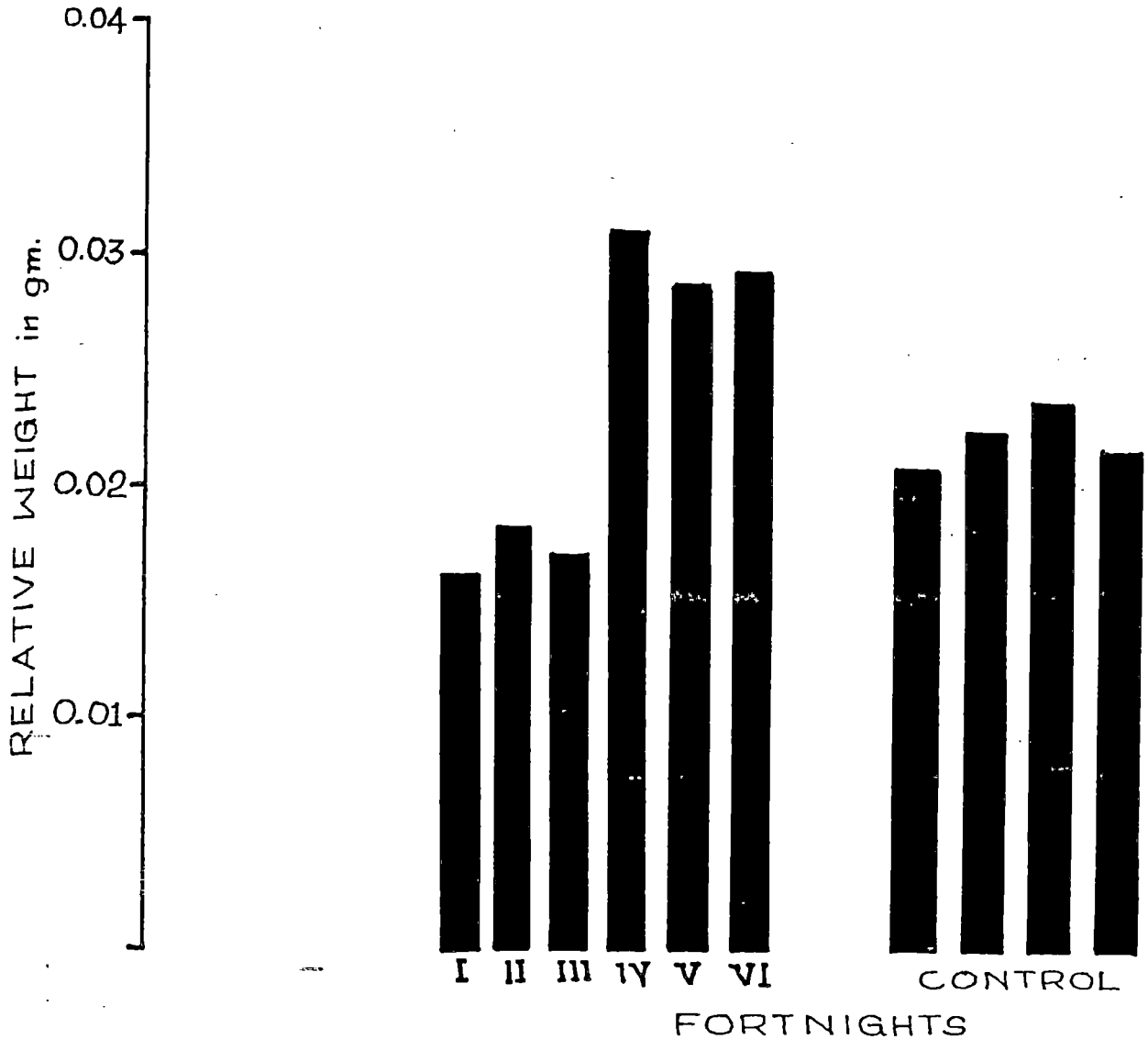


FIG. 7: RELATIVE WEIGHT OF THE TESTES OF EXPERIMENTAL CALVES



4.2.5. Haemogram

The haemogram of the experimental animals is presented in table 5. The experimental animals showed anaemia as indicated from the progressive reduction in hemoglobin (Fig.5), PCV and total erythrocyte counts. The other values did not show any significant change.

4.2.6. Relative weight of the Endocrine glands

The relative weights of the endocrine glands including the testis are presented in the figure 7.

In the experimental calves the thyroid, adrenal and pituitary showed an increase in their relative weights when compared to the control. On the contrary, a reduction in the relative weight of the testis was noticed in the experimental calves when compared to the control.

4.2.7. Autopsy findings

Four of the calves died during the second and third fortnight, one died before the completion of the sixth fortnight and the remaining one was sacrificed at the end of the sixth fortnight.

The carcases were very much dehydrated and poor in condition. There was depletion of fat in the eye socket and the eyes appeared sunken. The visible mucous membrane was pale. The hair coat was rough and dry. Throughout the body there was marked gelatinisation of subcutaneous fat. Gelatinisation of

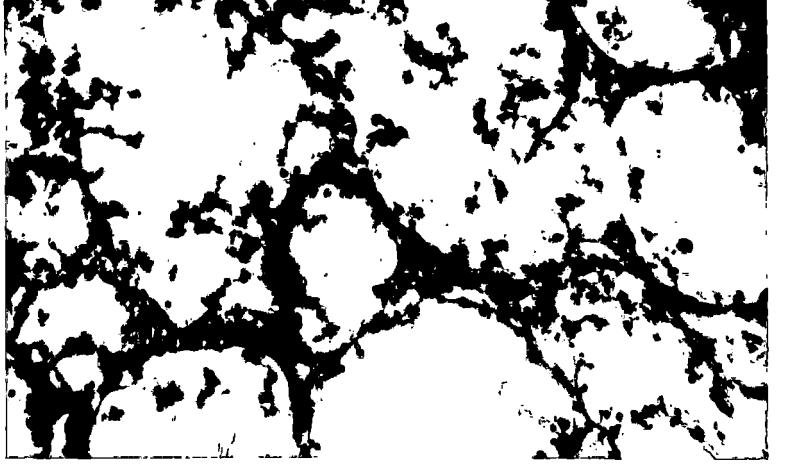


Fig. No.14. Hypothyroidism - calf - Follicles lined with tall columnar epithelial cells.
H & E x 400

Fig. No.15. Control - calf - Thyroid - Regular colloid filled follicles lined with cuboidal epithelial cells.
H & E x 200

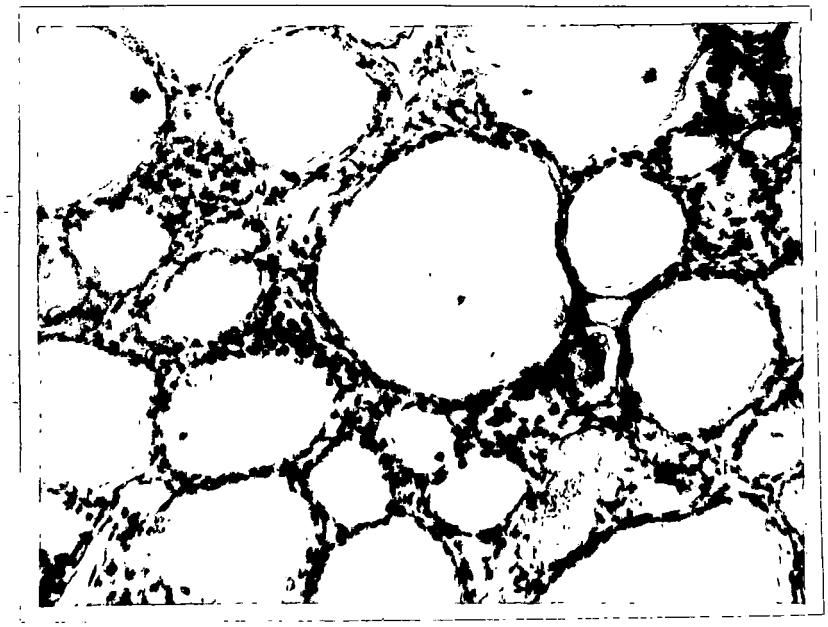
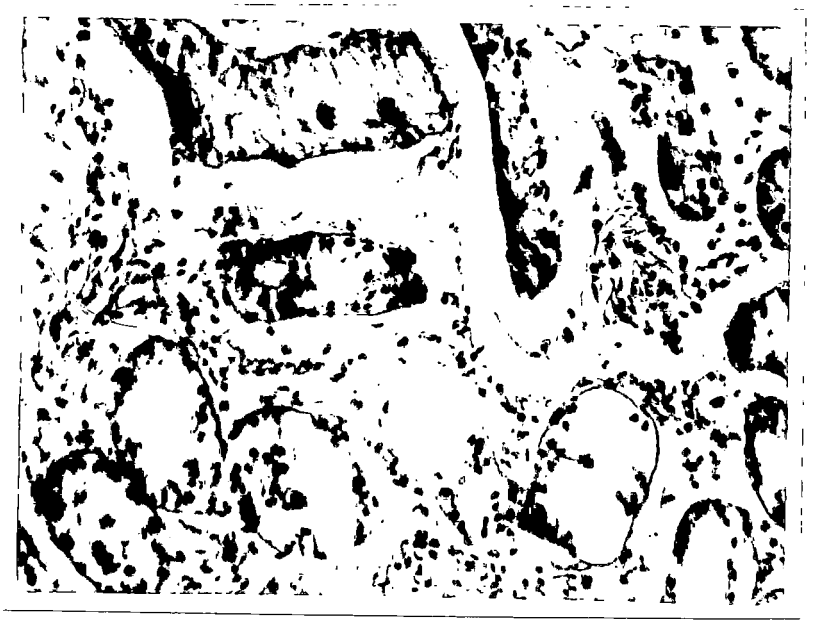


Fig. No.16. Hypothyroidism - calf - Adrenal gland - proliferating zona fasciculata cells containing abundant fat

Fig. No.17. Hypothyroidism - calf - Testes - Interstitial oedema, degenerated and desquamated epithelial cells. The lining cells of the tubules are sparse. No evidence of spermatogenesis.
H & E x 200



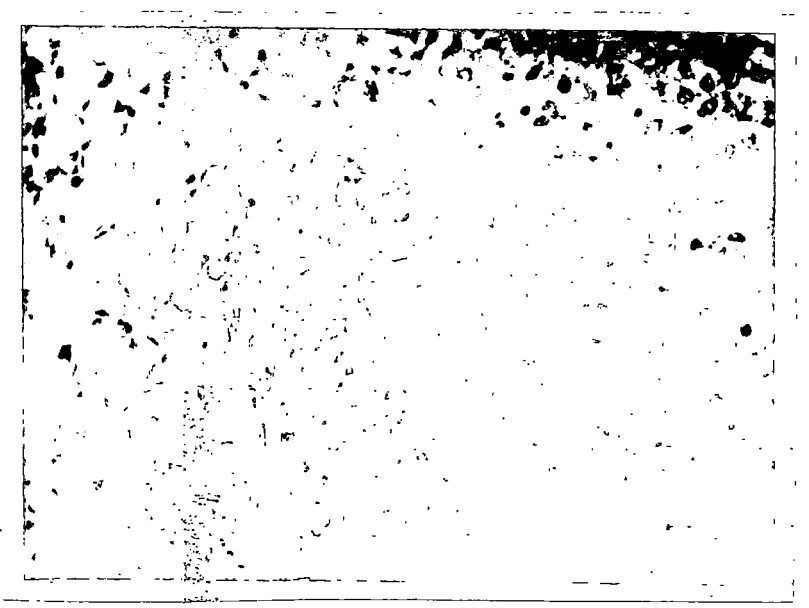
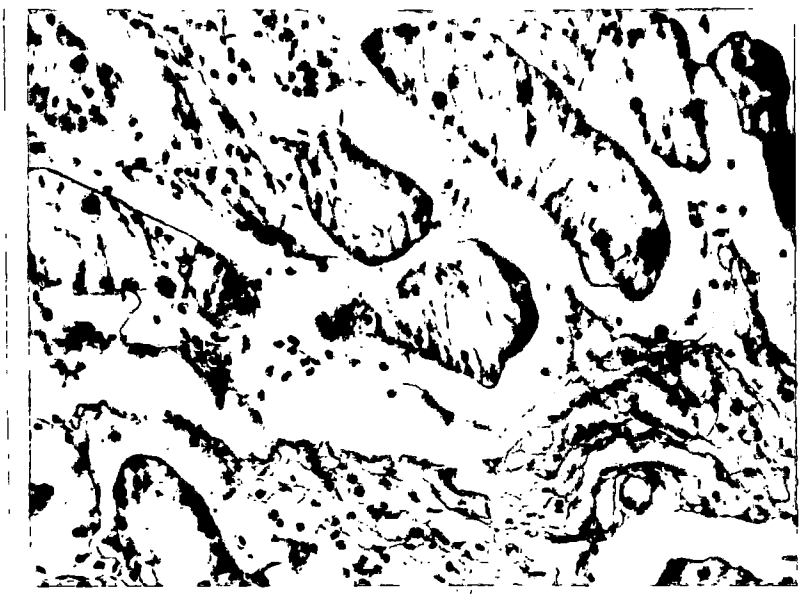
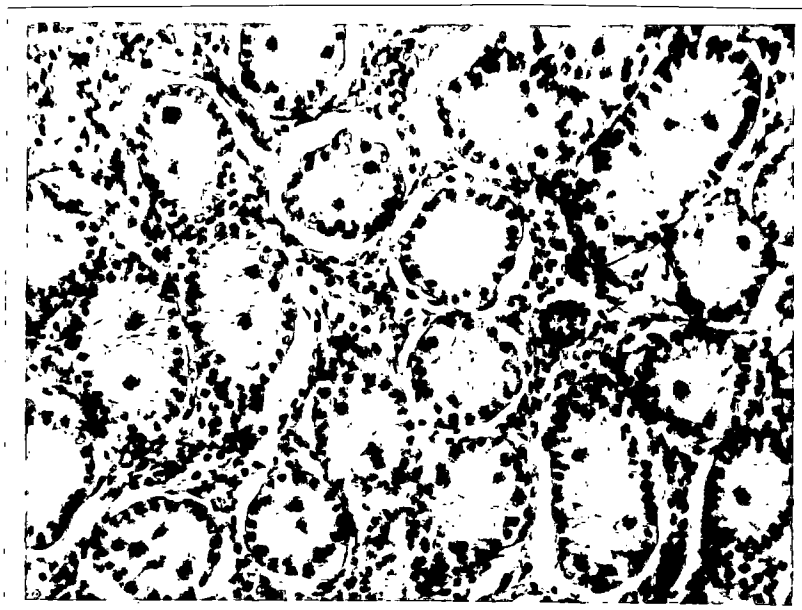


Fig. No.18. Hypothyroidism - calf - Testes - Interstitial oedema - degenerated and desquamated epithelial cells in the tubules - No evidence of spermatogenesis.

Fig. No.19. Control - calf - Testes - Seminiferous tubules containing cells with active spermatogenesis. H & E x 300





the coronary and renal fat was also evident. Except for the animal that survived all the six fortnights, the lungs of all the others showed lesions of pneumonia marked by areas of congestion and consolidation. The bronchi and bronchioles contained colourless froth. There was ventricular dilatation and the heart presented a rounded appearance. The liver was pale yellow, with rounded borders and was slightly oily to touch. The thyroid glands were enlarged and pale brown in colour and the adrenal glands were also markedly enlarged. The testes were relatively smaller in size. The relative weight of the testes is shown in figure 7.

4.2.8. Histopathology

I. The calves which survived for only two fortnights showed the following lesions:

Thyroid: The follicles were of more or less uniform in size. There was congestion of the blood vessels. Most of the follicles did not contain colloid. However, a few of them contained pale thin colloid. They were lined with scattered cuboidal to columnar epithelial cells (Fig.11). In some of the follicles the lining epithelial cells had degenerated and desquamated into the follicles. A few follicles had ruptured (Fig.12).

Adrenal glands: Depletion of fat in the region of fasciculata. Congestion of blood vessels.

Pituitary glands: Slight vacuolation of basophil cells. In focal areas hyperplasia of basophils were seen. Moderate congestion of blood vessel was also seen.

Testis: Most of the seminiferous tubules were lined with single layer of epithelial cells. No mitotic activity was seen. Some of the tubules showed degenerated and desquamated epithelial cells. There was moderate interstitial oedema (Fig.17).

II. The calves which survived for a period of five to six fortnights showed the following lesions.

Thyroid: The follicles were of varying sizes. Most of the follicles did not contain any colloid and they were relatively small in size. Tall columnar epithelial cells were seen lining the follicles. In some of the follicles two or three layers of tall columnar cells were seen lining the follicle. Some of the follicles were filled with proliferating epithelial cells and the follicular lumen was not evident (Fig. 13, 14). However, a few had very little pale thin colloid. Some of the follicles contained a few desquamated epithelial cells. The thyroid gland of the control animals contained regular colloidal follicles lined by cuboidal epithelial cells (Fig. 15).

Adrenal glands: There was slight proliferation of the zona fasciculata cells in focal areas and the cells contained abundant fat (Fig.16), and the zona appeared congested. Blood vessels in the cortico-medullary junction appeared congested.

Pituitary gland: In focal areas, vacuolation of beta cells was evident. Varying degree of congestion of blood vessels was also noticed.

Testis: The seminiferous tubules were slightly smaller in size and the lining cells were very scanty. In a few, scattered spermatocytes were seen. There was no evidence of proliferation of the spermatogonial cells. In some of the tubules a few degenerated and desquamated cells were seen along with a few giant cells (Fig.18). Moderate degree of interstitial oedema was seen. Interstitial cells were very few and appeared loosely scattered in the interstitium. The interstitial tissue was slightly oedematous and appeared relatively prominent.

The testis of the control calves contained seminiferous tubules lined with intact spermatogonial cells and spermatogenesis was evident. The interstitial cells were also active (Fig.19).

Discussion

5. DISCUSSION

The survey studies on the thyroid status of cattle with non-infectious reproductive disorders employing protein bound iodin (PBI) as the marker have therefore indicated that hypothyroidism is prevalent in animals with non-infectious reproductive disorders namely, repeat breeding and it could be an important factor which causes non-infectious subfertility and infertility in cattle. The results of the investigation carried out have given proof to the fact that there is significant reduction in the PBI level of repeat breeder animals compared to the control.

The dry animals of the Livestock Farm, Mannuthy had relatively a low PBI level as compared with the lactating and pregnant animals. In dry cattle a low PBI was recorded when compared to animals in late pregnancy and lactation. While a significant increase in the PBI level was documented in the latergroup (Sorensen, 1956; Vzaimosuyaz, 1973). This increase in the PBI level during pregnancy and lactation was attributed to increased metabolic demand. A subnormal thyroid function has been recorded in anoestrous buffaloes as revealed by low plasma PBI level in the blood, and the PBI level in cycling animals manifested a downward trend towards dioestrus stage of oestrous cycle (Dhoble and Gupta, 1980). The results of the present survey study have indicated that the reproductive performance in animals could be impaired by hypothyroidism.

This observation is in agreement with the observation made by Reddy (1982) in goats. He recorded significant reduction in PBI level in goats with experimental hypothyroidism. These observations made have clearly brought to light that hypothyroidism is an important contributing factor for reproductive failures and there is need to look into this factor also while diagnosing and instituting therapy for reproductive disorders.

In order to study the clinico-pathological features of hypothyroidism in cattle, hypothyroidism was experimentally induced in male calves employing thiourea as the goitrogen. The dose of thiourea employed brought about hypothyroidism as indicated by the low PBI level. However, four of the calves died by the second fortnight. These were relatively young animals of the experimental group and they were susceptible to the amount of thiourea administered. This conclusion is supported by the fact that there was sudden drop in PBI level when compared to relatively more aged calves, which continued to live in a hypothyroid state for the entire experimental period.

Experimentally induced hypothyroid state was characterized clinically by disturbance in growth. There was stunted growth and progressive weight loss in all the calves dosed with thiourea. Similar observations were made in hypothyroid goats by Sreekumaran (1976) and Reddy (1982). However, in the case of dogs dosed with thiourea no undesirable effects were observed

on growth (Lombardi et al. 1962). This was attributed to the fact that metabolic processes in the dog are less dependent on the thyroid hormone. The skin of experimental animals was thick and hair coat was dry and coarse. There was also irregular shedding of hair especially on the neck and dewlap region. These observations fall in line with those made by Sreekumaran (1976), Rignberk et al. (1977) and Reddy (1982) in goats and in horses (Lowe et al. 1914) and in pigs (Bussian, 1975). In the dogs, breed was found to be more influential than hypothyroidism in determining the predominant phase of hair growth (Aslam et al. 1983). The epidermal layer was considered to be an important target organ for the action of thyroxine (Freedberg, 1971) and hence pathological changes in the skin can be expected in hypothyroidism.

Slight to moderate oedema was noticed on the face and lower parts of the body. This is in conformity to the finding of Lowe et al. (1914), Sreekumaran (1976) and Reddy (1982) in goats.

The poor condition and marked gelatinisation of the subcutaneous and coronary fat are indicative of the adverse effects of hypothyroidism. Similar observations were reported in experimental hypothyroidism in goats by Sreekumaran (1976) and Reddy (1982). This may be on account of the reduced feed conversion in the absence of thyroxine. Russell (1943) noted that most energy demands were met from preformed lipid in hypothyroid rats. Consequently the gelatinisation of the

body fat may be inferred to be due to the utilization of fat for vital functions and energy needs of the body.

It may be pointed out that all the animals in the experimental group showed marked ventricular dilatation with the heart assuming a rounded appearance. This observation is similar to that reported in goats by Sreekumaran (1976) and Reddy (1982) in goats. Cardiac dilatation could be considered as a pathological change resulting from the effort on the part of the heart to compensate the reduced function in the face of reduced cardiac output and decreased velocity of blood flow in hypothyroidism.

Most of the animals carried their head in drooping position. Sreekumaran (1976) and Reddy (1982) attributed this symptom in goats in hypothyroid state to the development of cerebral oedema. The observations made in this study have clarified that the symptomatology in hypothyroidism in calves is in no way different from that seen in hypothyroid goats.

Serum cholesterol level was one of the parameters studied. The cholesterol level was found to be abnormally high in all the experimental calves as compared to the controls. This goes to confirm the observation made by Mason and Wilkinson (1973) in dogs that cholesterol synthesis is inversely proportional to the thyroid function. Munson and Belshaw (1966) considered cholesterol values of 275 mg/100 ml and above as indicative of hypothyroidism when coupled with appropriate clinical signs. Increase in the serum cholesterol level has

been reported in experimental hypothyroidism in sheep (Lascelles and Sctchele, 1959; Belonje, 1967) and in chicken (Nangia et al.; 1975) and in goats (Sreekumaran, 1976; and Reddy, 1982). The increase in serum cholesterol level was considered to be an indication of a specific change in lipid metabolism in hypothyroidism in human myxoedema by Peters and Man (1950). Fletcher and Myant (1958) indicated that in hypothyroid rats the hepatic synthesis and release of cholesterol from acetate was subnormal but the peripheral breakdown and biliary excretion is lowered and this they attributed as the reason for hypercholesteraemia.

Hypothyroid state in the animals fed thiourea was also accompanied by an increase in the total plasma protein level. Similar increase in plasma protein level was reported in hypothyroidism in human beings (Lamberg and Grasbeck, 1955). An increase in plasma globulin was reported in thyroidectomised Merino ram (Belonje, 1967), in poultry (Nanjia et al., 1975) and in goats (Sreekumaran, 1976; Reddy, 1982). Nanjia et al. (1975) attributed the accumulation of blood proteins to the defective utilization of nutrients in the absence of thyroxine. Crispell and Wilson (1964) reported reduction in both anabolism and catabolism of protein in hypothyroidism; catabolism being more reduced than anabolism and this accounted for a rise in plasma protein level.

The animals dosed with thiourea to induce hypothyroidism showed a significant decrease in the serum PBI level. This

observation conforms to the results of experimental hypothyroidism in sheep (Lascelles and Setchell, 1959), in bull (Lewis, 1956), in spontaneous hypothyroidism in sheep (Watson et al.; 1962), and in goats (Sreekumaran, 1976; Reddy, 1982). From the observation it could be inferred that the estimation of PBI level could be taken as a reliable marker for the identification of hypothyroid state, along with high serum cholesterol and protein levels. Thiourea was observed to cause hypothyroidism by inhibiting the organification of iodide and the subsequent formation of iodothyrosine and their coupling to form iodothyronine (Mayberry and Astwood, 1961). The inorganic iodide content of the thyroid was also diminished and there was slight inhibitory effect on iodide pump (Danowaski, 1962).

All the experimental animals recorded a decrease in total erythrocyte count, hemoglobin value and packed cell volume. This observation is consistent with that of Sreekumaran (1976) and Reddy (1983) indicating that a deficiency of thyroxine would lead to anaemic state. Adamon and Finch (1966) demonstrated a decrease in erythropoietin production in hypothyroidism. Rivlin (1971) observed a reduction in intestinal absorption of vitamin B12 as a significant effect of hypothyroidism in man. These factors may play a role in causing anaemia in hypothyroidism.

There was an appreciable increase in the relative weight of the thyroid gland in all the experimental calves dosed with thiourea. This was characterized by hyperplasia of the

follicles and hypertrophy of the lining epithelium, with depletion of the colloid. The later is a proof to show that there has not been any synthesis of thyroxine, although hyperplastic reactions were evident in the gland. This observation is a reflection on the compensatory hyperplastic reaction of the thyroid gland mediated through the pituitary in response to low thyroxine levels. The enlargement of the thyroid was found to be progressive. There was notable correlation between the time interval and the degree of enlargement of the gland. As the duration of the experimental period increased there was also proportionate increase in the weight of the gland. Though the hyperplasia of the thyroid gland was a compensatory response, functionally it was not found to be compensatory, since the PBI level of the thiourea dosed animals was much lower when compared to euthyroid animals.

Although there was significant increase in the weight of the thyroid, the enlargement was not appreciable either by outward manifestation or by clinical palpation. This finding points to the fact that the subclinical form of hypothyroidism could exist in animals without any gross appearance of thyroid enlargement. Therefore, estimation of PBI level provides a valuable tool for the identification of hypothyroid state. However, appreciable thyroid enlargement has been reported in experimental hypothyroidism in different species of animals (Kennedy, 1942; Jones et al., 1946; Harkness et al., 1954; Goldberg et al.; 1957; Lascelles and Setchell, 1959;

Lazo-Wasem, 1960; Sreekumaran, 1976; Reddy, 1982) and in spontaneous hypothyroidism in sheep and goats (Southcott, 1945; Lall, 1952; Dutt and Kehar, 1959).

The most important histological observation was the complete absence of colloid in most of the follicles of the thyroid and hyperplasia of the lining follicular cells. Although there was stimulation by thyroid stimulating hormone (TSH) and hyperplasia of the thyroid epithelium, there was no synthesis of thyroglobulin due to non-availability of iodine in the presence of thiourea. This goes to support the finding that thiourea has effectively blocked the thyroglobulin production and has lowered the PBI level.

There was an increase in the relative weight of the pituitary gland in all the animals dosed with thiourea. This observation reconciles with the reported finding of increased weight of the pituitary in experimentally induced hypothyroidism in laboratory animals (Kennedy and Purves, 1941; Grieshbach et al.; 1941; Goldberg et al., 1957; Lazo-Wasem, 1960) in goats (Sreekumaran, 1976; Reddy, 1982) and in spontaneous hypothyroidism in goats (Lall, 1952). Thiourea interferes with organic binding of iodine and consequent deficiency of thyroxine (T_4) stimulates basophil cells to produce more TSH. This stimulation leads to hyperplasia of the basophil cells to meet the increased demand for TSH. This hyperplastic response was reflected in the increased weight of the pituitary.

In the pituitary there was marked hyperplasia of the

basophils. Basophil cell hyperplasia is a physiological response to stimulate the thyroid by releasing TSH in large quantities in the absence of adequate thyroxine (T_4) with increasing thyroid dysfunction the hypertrophy of basophil cells associated with storage of granules was followed by the loss of granules in many cells and finally complete degranulation and vacuolation of basophil cells. These vacuolated basophils have been described in rats as "Thyroidectomy cells" (Zeckwer et al., 1935). According to them the cytoplasmic vacuolation represents an exhaustion stage in the reactive hyperplastic process. These changes have been described in experimental hypothyroidism in dogs (Lippincott et al., 1957), in rats (Goldberg and Chaikoff, 1951) and in goats (Sreekumaran, 1976; Reddy, 1982). The hypertrophy of basophil cells has also been reported in spontaneous hypothyroidism in goats and sheep (Lall, 1952; Dutt and Vasudeva, 1963).

There was significant increase in the relative weight of the adrenal gland in animals treated with thiourea. This observation is in contrast to the reports of atrophy of adrenal glands in laboratory animals dosed with thiouracil and allied compounds by Baumann and Marine (1945), Zarrow and Money (1949) and McCarthy et al. (1959). However, the present observation is in agreement with the findings of Durlach et al. (1954) who have reported an increase in the adrenal weight in guinea pigs dosed with propylthiouracil. Similar observations have been reported in experimental thyroidectomy in laboratory

animals (Gley, 1923) and in experimental hypothyroidism in goats (Sreekumaran, 1976; Reddy, 1982). The animals with induced hypothyroid state was under the influence of stress and this stress might have been responsible for the enlargement of adrenal glands.

Microscopic findings like depletion of fat and congestion of blood vessels and hemorrhages in the zona fasciculata region seen in the adrenal glands are all histological features described by Symington (1969) in stress reaction in the adrenal gland.

There was significant decrease in the relative weight of the testis in all the calves dosed with thiourea. On the contrary Prasad and Singh (1971) and Sharma and Singh (1975) recorded increase in the weight of testis in chicks dosed with thiourea. However, the present findings are in agreement with those observed in quails by Peczely et al.(1979) who attributed low level of testosterone in hypothyroidism. Reddy (1982) also documented a decrease in the weight of the testis in experimental hypothyroidism in goats.

Histologically the seminiferous tubules were slightly smaller in size and the lining cells were scanty. A few tubules revealed the presence of scattered spermatocytes. There was no evidence of mitotic activity of spermatogonial cells. In some of the tubules a few degenerated and desquamated cells were seen along with a few giant cells. Interstitial cells were very few and appeared loosely scattered.

These observations provides histological evidence to conclude that all the cellular components of the testis are affected in hypothyroid state. Similar changes have been reported in experimental hypothyroidism induced by thiouracil in rams and rabbits (Maqsood, 1951) and thiourea induced hypothyroidism in goats by Reddy (1983). According to Gorbman and Bern (1974) thyroxine has a priming effect on the action of hormones on cells and they indicated that in the absence of thyroxine, the gonadotrophic hormone of the pituitary will not function effectively and this may lead to degenerative changes in the testicular tissue. All these observations underscores the adverse effect, hypothyroidism is bound to have on the reproductive performance in cattle by bringing about degenerative changes in the gonadal tissue. The results of the studies indicated that marginal hypothyroidism could lead to significant reduction in growth rate, lowered productivity and fertility in cattle as reported in the case of goats and this could lead to serious economic loss. It may be pointed out that the Government of India has recognized the fact that the country is endemic for iodine deficiency and it has decided to provide iodised salt to the human population in future.

The observations made in this study have pointed out significant biological effects in calves due to hypothyroidism. The survey and experimental studies undertaken in calves have conclusively shown that hypothyroidism could contribute to reproductive disorders and hypothyroidism. This points out

the need for taking measures to control hypothyroidism in domestic animals as in the case of human beings in order to reduce economic loss due to hypothyroidism in livestock.

Summary

6. SUMMARY

Employing serum protein bound iodine as the marker a survey study was conducted to assess the thyroid status of cattle affected with non-infectious reproductive disorders. Hypothyroidism characterized by low PBI level was encountered in all the repeat breeder cows examined.

Experimentally, hypothyroidism was induced in male calves employing thiourea at the rate of 150 mg/kg body weight with the object of delineating the sequence of clinico-pathological changes in sub-clinical hypothyroid state.

Clinically, all the experimental calves manifested progressive weakness, dullness, marked reduction in feed intake, irregular shedding of hair and subcutaneous oedema.

Stunted growth was consistently observed in all hypothyroid animals. After an initial increase during the first fortnight, there was progressive reduction in the growth rate thereafter.

The serum PBI level of the experimental calves recorded a significant decrease. The serum cholesterol and plasma protein levels were high when compared to the control animals. The hypothyroid animals were anaemic. The hemoglobin level registered a progressive decrease when compared to the euthyroid control calves.

The lesions observed in hypothyroid calves were cardiac

dilatation, gelatinization of subcutaneous and other depot fat, alopecia and catarrhal enteritis.

There was a significant increase in the weight of the thyroid gland in hypothyroid animals. The thyroid enlargement could not be appreciated by external palpation. Most of the microfollicles did not contain any colloid. In some of the follicles, the lining epithelial cells were hyperplastic, while others showed degenerative changes and desquamation.

There was an increase in the relative weight of the pituitary glands in all the animals dosed with thiourea. The predominant histological picture was vacuolation and hyperplasia of the basophil cells in focal areas.

The adrenal glands of all the animals dosed with thiourea showed significant increase in weight. Histologically the cells of zona fasciculata showed slight proliferation and depletion of fat. Blood vessels were also found to be congested.

A significant decrease was observed in the relative weight of the testis of the hypothyroid calves. Histologically the seminiferous tubules were obliterated slightly and the lining tubular cells were very scanty with no evidence of mitotic activity. In some of the tubules a few degenerated and desquamated cells were seen along with giant cells. Moderate degree of interstitial edema was also noticed.

The degenerative changes affecting the testicular tissue

in the hypothyroid state has brought out the important role that hypothyroidism plays in inducing subfertility and infertility in cattle. It was demonstrated by this investigation that hypothyroidism would lead to reduction in growth rate and reproductive disorders.

References

REFERENCES

*Adams, A.E. (1946). Variations in the potency of thyrotrophic hormone of the pituitary in animals. Quart. Rev. Biol. 21: 1-32.

*Adamson, J.W. and Finch, G.A. (1966). Mechanism of erythroid marrow activation. Trans. Ass. Am. Physns., 73: 419.

Akiba, Y. and Matsumato, T. (1977). Effect of graded doses of a goitrogen in rape seed, on synthesis and release of thyroid hormone in chicks. Japanese J. Zootechnical Sci., 48: 757-765.

Anderson, R.R. and Harness, J.R. (1975). Thyroid hormone secretion rates in growing and mature goats. J. Anim. Sci., 40: 1131-1135.

*Andrews, F.N., Shrewsbury, C.L., Harper, C., Vestal, C.M. and Doyle, C.P. (1948). Iodine deficiency in new born sheep and swine. J. Anim. Sci., 7: 298-310.

Armed Forces Institute of Pathology (1968). Manual of Histology staining Methods. McGraw-Hill Book Company, New York, 3rd Ed. pp. 12-184.

Aslan, S.H., Mackenzic, C.P., Brown, R. and Baxter, J.T. (1983). The phase of hair growth in hypothyroidism in the dog. Vet. Res. Commun., 6(1): 51-58.

*Astwood, E.B., Bissdi and Hughes, M. (1945). Further studies on the chemical nature of compounds which inhibits the sanction of the thyroid gland. Endocrinology, 37: 456-481.

*Astwood, E.B., Greeder, M.A. and Ethlonger, M.G. (1949). The antithyroid factor of yellow turnip. Science, 109: 631.

*Ayoub, L.A.L.L. (1968). Effect of radioiodine in thyroid function in goats. Dt. tier. arztle wsch., 75: 341-344.

*Barakat, M.Z., Osman, A.M., Salah, M.K. and Abdel-Aziz, F.T. (1971). A study on the thyroid iodine content of buffalo. Zentrablall vet. Med., 18A: 731-737.

*Barker, M.M., Lindbug, M.A. and Wald, M.M. (1941). Further experience with thiocyanate, clinical and experimental observation. J. Am. Med. Assoc., 117: 1591-1594.

Barker, S.W. (1951). Mechanisms of action of thyroid hormone. Physiol. Rev., 31: 205.

*Barker, S.B. and Schwartz, M.S. (1953). Further studies on metabolism of tissues from thyroxine injected rats. Proc. Soc. Exptl. Biol. Med., 83: 500.

Barker, S.B. (1971). "Chemistry and cellular effects". The Thyroid. A fundamental and clinical Text. Edited by Warner, S.C. and Jugbar, S.M., Harper and Row, New York, 3rd Ed. pp. 79-90.

*Baumann, E.J. and Marine, D. (1945). Involution of adrenal cortex in rats fed thiouracil. Endocrinology, 36: 400.

*Baumann, R. (1948). Goitre and Myxoedema of new born goats. Wien. Tierarztt. Mschr., 35: 585-592.

*Belonje, P.C. (1967). A report of some long term effect on thyroparathyroidectomy in a Merino wether. J.S. Afr. vet. Med. Ass., 38: 225-226.

Berman, A.J. (1960). Peripheral effects of L-thyroxine on hair growth and colouration of cattle. J. Endocr., 22: 288-292.

*Blockhina, R.I. (1970). Proc. Ist Int. Symp. Trace Elements metab. Anim. Edited by C.F. Mills. Livingstone, Edinburg, pp. 426.

Blood, D.C. and Henderson, J.A. (1968). Veterinary Medicine. The english language book society and Bailliere, Tindall and Cassell, London. 3rd Ed. pp. 711.

Brooks, J.R. and Ross, C.V. (1962). Effect of ambient temperature and thyroxine therapy on semen quality of rams. J. Anim. Sci., 21: 700-705.

Brownstand, G.E. and Fowler, S.H. (1959). Thyroid status and embryonic mortality in swine. Am. J. Physiol., 196:287-290.

Burstein, P.J., Draznin, B., Johnson, J. and Schalch, D.S. (1979). The effect of hypothyroidism on growth, serum growth hormone the growth hormone dependent somatomedin, insulin like growth factor and its carrier protein in rats. Endocrinology, 104: 1107-1111.

Bush, B.M. (1969). Thyroid disease in the dog. A Review. J. Small Anim. Pract., 10: 95-109.

Bussian, E. (1975). Iodine deficiency in unweaned piglets kept under intensive conditions. Monatshefte fur veterinarmedizin, 30(5): 182-190.

Bustad, L.K., Marks, S., George, C.A. and Seigneur, L.J. (1957). Thyroid adenomas in sheep administration of I^{131} daily. Nature, Lond. 179: 677.

*Butler, G.W., Flux, D.S., Peterson, G.B., Wright, E.W., Glenday, A.C. and Johnson, J.M. (1957). Goitrogenic effect of white clover. N. Z. J. Sci. Tech. Sect. A. 38: 793-802.

Calderbank, G. (1958). Thyroid dysfunction and infertility. Vet. Rec., 70: 731.

Calderbank, G. (1963). "Iodine", Animal Health, Production and Pasture. Longman's Green Co., London, pp. 250.

Catt, K.J. (1970). ABC of Endocrinology. II. The Thyroid gland. Lancet., 1: 1383-1389.

*Chensey, A.M., Clawson, T.K. and Webster, B. (1928). Endemic goitre in rabbits. Incidence and characteristic. Bull. John Hopekin Hospital, 43: 261-277.

*Chu, T.P. and You, S.S. (1944). The role of thyroid gland and oestrogen in regeneration of gonadotrophic activity of anterior pituitary. J. Endocrinol., 46: 115.

*Clausen, H.J. (1954). Experimental production of struma fibrosa. Archs. Path., 58: 222-226.

Clements, F.W. and Wishart, J.W. (1956). A thyroid blocking agent in the etiology of endemic goiter. Metabolism, 5: 623.

Cohrs, P. (1966). Text book of the special pathological anatomy of the domestic animals. Pergamon Press, Oxford, pp. 919-922.

Cons, J.M., Umezu, M. and Timiras, P.S. (1975). Developmental and patterns of pituitary and plasma TSH in normal and hypothyroid female rats. Endocrinology, 63: 643-653.

Contopoulos, A.N., Simpson, M.E. and Koneff, A.A. (1958). Pituitary function in thyroidectomised rat. Endocrinology, 63: 643-653.

Crispell, K.R. and Wilson, E.C. (1964). "Pathology of Myxoedema". The Thyroid. Edited by Hazard, J.B. and Smith, D.E. Williams and Wilkins Company, Baltimore, pp. 236-238.

D'Angelo, S.A., Paschkis, K.E., Cantarow, A., Siegal, A.N. and Rivero Fontan, J.C. (1951). Hyperplasia of Thyroid during and after chronic propyl thiouracil treatment. Endocrinology, 49: 624-636.

D'Angelo, S.A., Paschkis, K.E., Cantarow, A., Siegal, A.N. and Rivero Fontan, J.C. (1961). Hyperplasia and I^{131} uptake of guinea pig thyroid during and after chronic propyl thiouracil treatment. Endocrinology, 49: 624-636.

Danowski, T.S. (1962). Clinical endocrinology. Thyroid. Williams and Wilkins, Baltimore, Vol.II, pp. 223.

Davidson, B., Soodak, M., Strout, H.V., Neary, J.T., Nakamur, C. and Maloof, F. (1979). Thiourea and cyanamide as inhibitors of thyroid peroxidase. The role of iodide. Endocrinology, 104: 917-924.

De Robertis, E. (1941). Proteolytic enzyme activity of colloid extracted from single follicles of the rat thyroid. Anat. Rec., 80: 219-230.

Dhoble, R.L. and Gupta, S.K. (1980). Protein bound iodine in blood plasma of buffaloes in relation to post-partum anoestrum. Indian J. Dairy Sci.; 33: 124-125.

Disbrey, B.D. and Rack, J.H. (1970). Histological Laboratory Methods. E & S Livingston, Edinburgh, pp. 99-101.

Durlach, J., Levillian, R. and Bouvett, P. (1954). Action of propyl Thiouracil on the guinea pig liver, structures on the liver in hypothyroidism. Annls. Endocr., 15: 158-162.

Dutt, B. and Kehar, N.D. (1959). Incidence of goiter in goats and sheep in India. Brit. Vet. J., 115: 176-179.

Dutt, B. and Vasudeva, B. (1963). A clinical case of hypothyroidism in a Ram. Indian Vet. J., 40: 505-508.

*Edelhock, M. (1960). The properties of thyroglobulin. I. The effects of alkali. J. biol. Chem., 235: 1326-1334.

FAO/SIDA (1968). A guide to Autopsy Techniques. Stockholm, Vol.IV, pp. 1-10.

Falconer, I.R. (1966). Studies on congenitally goitrous sheep. The iodinated compound of serum and circulatory TSH. Biochem. J., 100: 190-196.

Faulkner, L.W., Levy, R.P. and Leonards, J.R. (1961). Simplified technique for the determination of serum protein bound iodine. Clin. Chem., 7: 637-645.

*Ferguson, K.A., Schinckel, P.G., Carter, H.B. and Clarke, W.J. (1956). The influence of thyroid and wool follicle development in the lamb. Aust. J. Biol. Sci., 9: 575-585.

Fletcher, K. and Myant, M.B. (1958). Influence of thyroid on the synthesis of cholesterol by liver and skin vitro. J. Physiol., 144: 361-372.

Follis, R.H. Jr. (1959). Experimental colloid goitre produced by thiouracil. Nature, Lond., 183: 1817-1818.

Freedberg, I.M. (1971). 'Skin'. The Thyroid. A fundamental and clinical text. Edited by Warner, S.C. and Jugbar, S.H. Harper and Row, New York, pp. 722.

George, J.M., Farleigh, E.A. and Harris, A.N.A. (1966). Occurrence of goiter in Summer Born lambs of different breeds. Aust. Vet. J., 42: 1-4.

*Gley, E. and Bourcet, P. (1900). Presence de l'iode dans le sang. C.R. Acad. Sci., Paris, 130: 1721.

*Gley, E. (1923). The physiology of Adrenals. Rev. med., 40: 193-221.

*Goldberg, R.C. and Charkoff, I.L. (1951). On the nature of hypertrophied pituitary gland induced in mouse by I^{131} injections and mechanism of its development. Endocrinology, 48: 1-5.

*Goldberg, R.C., Wolff, J. and Greep, R.O. (1955). The mechanism of depression of plasma protein bound iodine by 2,4-dinitrophenol. Endocrinology, 56: 560.

Goldberg, R.C., Wolff, J. and Greep, R.O. (1957). Studies on the nature of thyroid pituitary on the relationship. Endocrinology, 60: 38-52.

Goldschmidt, V.M. (1954). Geochemistry. Calaenon, Oxford, pp. 602-620.

Gorbman, A. and Bern, H.A. (1974). A Text Book of Comparative Endocrinology. Wiley Eastern Private Limited, New Delhi. pp. 159.

*Gottschalk, A. and Ada, G.L. (1954). The separation and quantitative determination of component sugars of mucoproteins. Biochem. J., 62: 681-685.

*Greer, M.A. (1950). Nutrition and goitre. Physiol. Rev., 30: 513-548.

Greer, M.A. (1962). The natural occurrence of goitrogenic agents. Recent Progr. Hormone Res., 18: 187-212.

Greer, M.A. and Whalton, J. (1961). Antithyroid effects of barbarian (phenylthiooxa zolidone) a naturally occurring compound from barbares. Proc. Soc. Expt. Biol. Med., 107: 802-804.

*Griesbach, W.E., Kennedy, T.H. and Purves, H.D. (1941). Studies on the experimental goitre. II. The effect of goitrogenic diet on hypophysectomised rat. Brit. J. Exp. Path., 22: 249-254.

*Gross, J. and Pitt-Rivers, R. (1952). The identification of 3,53-L-Triiodothyroxine in human plasma. Lancet., 1: 439-441.

*Growth, W. (1962). Pathology of goitre and thyroid tumours in domestic animals. Dtsch. tierarztt.

*Halik, J. and Zavadsky, I. (1978). Mass outbreak of goitre in breeding. Veterinarslvi, 28: 68-69.

*Hall, W.T.K. (1952). Effect of methyl thiouracil on liver, vitamin A, spermatogenesis and calcium metabolism of Merino rams. Qd. J. Agric. Sci., 9: 301-306.

*Harington, C.R. and Barger, G. (1927). Chemistry of thyroxine. III. Constitution and synthesis of thyroxine. Biochem. J. 21: 169-183.

*Harkness, M.L.R., Harkness, R.D. and Santler, J.E. (1954). Changes in collogen content of thyroid in rats treated with thiouracil. J. Physiol., 125: 51-55.

Hignett, P.G. (1952). Animal health production and posture. Edited by Calderbank, G. Longman Green and Co., London. pp. 250.

*Hojer, J.A. (1931). Goitre studies. The prevalence of endemic goitre in Sweden. Svenska. lak. Sallak. Forthandl., 57: 1-104.

Inchiosa, M.A. (1964). Direct Biuret determination of total protein in tissue homogenates. J. Lab. Clin. Med., 63: 319-324.

*Jones, G.E.S., Delfa, E. and Foote, E.E. (1946). The effect of thiouracil hypothyroidism on reproduction in rat. Endocrinology, 38: 337-344.

*Jovanovic, M., Pantic, V. and Markovic, B. (1953). Goitre in domestic animals in Serbia. Acta vet. Belgrade, 3: 31-51.

*Jovanovic, M. (1955). Histology of thyroid in endemic goitre in domestic animals. Acta. vet. Belgrade, 5: 13-52.

Jubb, K.V.F. and Kennedy, P.C. (1970). Pathology of Domestic Animals. Academic Press, New York, 2nd Ed. Vol.I, pp. 407-415.

Kaneko, J.J. (1970). "Thyroid Function". Clinical Biochemistry of Domestic Animals. Edited by Kaneko, J.J. and Cornelius,C.E Academic Press, New York, 2nd Ed. Vol.I, pp. 27-233.

*Kelly, F.C. and Snedden, W.W. (1960). "Prevalence and geographical distribution of endemic goitre". Endemic goitre. World Health Organization Monograph series. No.44, Geneva, pp. 27-233.

*Kendall, E.C. (1915). The isolation of crystalline form of the compound containing iodine which occurs in the thyroid. Its chemical nature and physical activity. Trans. Ass. Am. Physns., 30: 420-449.

*Kennedy, T.H. and Purves, H.P. (1941). Experimental goitre. I. The effects of brassica seed diets on rat. Brit. J. Exp. Path. 22: 241-244.

*Kennedy, T.H. (1942). Thiourea as goitrogenic substance. Nature, Lond., 150: 233-234.

*Kimbal, O.P. (1937). Prevention of goitre in Michigan and Ohio. J. Am. Med. Ass., 108: 860-864.

*King, T.W. (1836). Observation on the thyroid gland. Guy. Hosp. Rep. 1: 429-447.

*Kocher (1983). Ueber das hormale vorkommen Von Jod in Thiekorper. Z. Physiol. Chem., 21: 319.

Kochupillai, N., Thangavelu, M. and Ramalingaswami, V. (1976). Nodular lesions of the thyroid in an area of High Background Radiation in Coastal Kerala, India. Indian J. Med. Res., 64: 437-544.

Kochupillai, N., Godbole, M.M., Pandav, C.S., Karmarkar, M.G., and Ahuja, M.M.S. (1984). Neonatal thyroid status in iodine deficient environments of the sub-Himalayan region. Indian J. Med. Res., 80: 293-299.

Koutras, D.A. (1971). "Non-toxic goitre". The Thyroid. A fundamental and clinical text. Edited by Warner, S.C. and Jugbar, S.H. Harper and Row, New York, 3rd Ed. pp.409.

*Kovalskii, V.V., Struck, M.J. and Loan, A.I. (1970). Role of iodine in eradicated cow infertility in area of iodine deficiency in the Amur region. Dokl. Uses. Akad. Sel. Khoz. Nauk, VI. Lenina. pp. 24-25.

*Krohn, P.L. (1947). The effect of propyl thiouracil on estrus cycle in mice. J. Endocr., 5:33.

Krohn, P.L. and White, H.C. (1950). The effect of hypothyroidism on reproduction on female albino rat. J. Endocr., 6: 375-385.

Kumaran, J.D.S. and Turner, C.W. (1949). The endocrinology of spermatogenesis in birds. Effect of hypo and hyper thyroidism. Poultri. Sci., 28: 653-665.

Lall, H.K. (1952). A case of congenital goitre in kids. Indian Vet. J., 29: 133-135.

Lamberg, B.A. and Grasbeck, R. (1955). The serum protein pattern in disorders of thyroid function. Acta Endocr., 19: 91-100.

Lascelles, A.K. and Setchell, B.P. (1959). Hypothyroidism in sheep. Aust. J. Biol. Sci., 12: 455-464.

*Lazo-Wasen, E.A. (1960). Pituitary ACTH level during adrenal involution following thiouracil administration. Proc. Soc. exp. Biol. Med., 103: 300-302.

Lennon, H.D. Jr. and Mixner, J.P. (1958). Relationship between plasma protein bound iodine and certain measures of reproductive and lactational performance in dairy cattle. J. Dairy. Sci., 42: 327-332.

*Levine, H., Remington, R.E. and Von Kolnitz, H. (1933). Studies of diet to goitre. I. A dietary technic for the study of goitre rat. J. Nutr., 6: 325-345.

Lewis, R.C. (1956). The effect of thyroidectomy and iodine supplementation on plasma PBI of a Jersey bull. J. Dairy Sci., 39: 610-611.

*Lippincott, S.W., Lewallen, C.G. and Sheelabarger, C.J. (1957). Pathology of radio isotopic ablation of the thyroid in the dog. Archs. Path., 63: 540-556.

Lombardi, M.A., Comar, C.L. and Kirk, R.W. (1962). Diagnosis of thyroid gland function in dog. Am. J. Vet. Res., 23: 412-420.

Louvet, J.P., Govarre, M., Solandin, A.M. and Boulard, C. (1979). Hypothyroidism and anovulation (letter). Lancet. 8124: 1032.

*Love, W.G. (1942). Parenchymatous goitre in newborn goats and kids. J. Am. Vet. med. Ass., 101: 481-484.

Lowe, J.E., Baldwin, B.H., Foote, R.H., Hillman, R.B. and Kallfelz, F.A. (1914). Equine hypothyroidism: the long term effects of thyroidectomy on metabolism and growth in mares and stallions. Cornell Veterinarian, 64(2): 276-295.

Mahtiev, S.M. (1966). The effect of potassium iodide and iodo-microfertilizer on reproduction in sheep in iodine deficient zones. Dokl. Vser. Akal. Sel. Kohoz. Nauk. VI. 1: 38-41.

Maqsood, M. (1951). Influence of thyroid status on spermatogenesis. Science, 114: 693-694.

Maqsood, M. (1952). Thyroid function in relation to reproduction of mammals and birds. Biol. Rev., 27: 281-319.

*Marine, D. and Lenhart, C.H. (1910). On the occurrence of goitre (active thyroid hyperplasia) in fish. Bull. John Hopkins Hosp., 21: 95-98.

*Marks, S., Dockum, N.C. and Bustad, C.K. (1957). Histopathology of thyroid gland of sheep in prolonged administration of I^{131}. Amer. J. Path., 33: 219-249.

*Marston, H.R. and Peirce, A.W. (1932). The effect of following thyroidectomy in a Merino sheep. Aust. J. Exp. Biol. med. Sci., 10: 203-213.

Mason, R. and Wilkinson, J.S. (1973). Thyroid gland - A review. Aust. Vet. J., 49: 44-49.

Mayberry, W.E. and Astwood, E.D. (1961). Advances in Thyroid Research. Pergamon Press, New York, pp. 42.

*Mayo, G.M.E. and Mulhearn, C.J. (1969). Aust. J. agric. Res., 20: 533.

McCarrison, R. The Etiology of Endemic goitre. Lancet, 219: 1913.

*McCarrison, R. (1933). Recent researches on the aetiology of goitre. Report of second international conference Berue. Verlag. Hans. Hubor. Berne. pp. 354-403.

McCarthy, J.C., Corley, R.C. and Zarrow, M.X. (1959). Effect of goitrogen and adrenal gland of rat. Am. J. Physiol. 197: 693-698.

McIntosh, R.A. (1943). Goitre of new born. Can. J. Comp. Med., 7: 263-265.

*McIntosh, G.H., Baghurst, K.I., Potter, B.J. and Hatzel, B.S. (1979). Foetal thyroidectomy and brain development in the sheep. Neuropath. Appl. Neuro. biol., 5: 363-376.

*McKenzie, P.F. and Berliner, V. (1937). The reproductive capacity of rams. Missouri Agr. Expt. Sta. Res. Bull. pp. 265.

Means, J.M., Degroot, L.J. and Stanbury, J.B. (eds). "The thyroid and the External Environment", in the thyroid and its diseases (3rd ed.). New York, McGraw-Hill, 1963. pp. 105-120.

*Miale, J.B. (1967). Laboratory Medicine Haematology. The C.V. Morby Company, Saint Louis, 3rd Ed. pp. 1143-1144.

*Moberg, R.(1959). Possible influence of iodine-deficiency in reproductive performances in cattle with special reference to retained placenta. Proc. III World Cong. on Fertand Steril, Amsterdam.

*Munson, T.O. and Belshaw, B.E. (1966). "Current veterinary therapy", Saunders Co., Philadelphia, pp. 326.

*Murray, M.M., Rule, J.A., Simpson, B.W. and Wilson, D. (1948). Thyroid enlargement and other changes related to the mineral content of drinking water (with a note on goitre prophylaxin). Medical Research Council Memorandum, No.18, London.

Nangia, O.P., Dixit, U.P. and Agarwal, V.K. (1975). Studies on blood constituents in chicken with modified thyroid activity. Haryana Agric. Univ. J. Res., 5: 254-259.

Prasad, O. and Singh, A. (1979). Effect of altered thyroid status on PBI blood glucose, plasma cholesterol, liver glycogen and oxygen consumption by liver and gonads of poultry. Indian J. Anim. Sci., 49: 446-450.

Rac, R., Hill, G.N., Pain, R.W. and Mulhearn, C.J. (1968). Congenital goitre in Merino sheep due to an inherited defect in the synthesis of thyroid hormone. Res. Vet. Sci., 9: 209-233.

Ragowitsch, N. (1869). The thyroid. Edited by Beach Hazard, J. and Smith, D.E. The William and Wilkins Company, Baltimore, pp. 76.

Rajkumar, S.S. (1970). A case of congenital goiter in kids. Indian Vet. J., 29: 133-135.

Ramalingaswami, V., Subramanian, T.A.V. and Deo, M.G. (1961). The aetiology of Himalayan endemic goitre. Lancet, 1: 791-794.

Reddy, N.M. (1982). Pathology of the Reproductive organs in experimental hypothyroidism in goats. M.V.Sc. Thesis, Kerala Agricultural University.

*Reineke, E.P., Bergman, A.J. and Turner, C.W. (1941). Effect of thyroidectomy of young male goats upon certain anterior pituitary hormones. Endocrinology, 29: 306-312.

*Riggs, D.S. (1952). Quantitative aspects of iodine metabolism in man. Pharmacol. Rev., 4: 284-370.

Rijnberk, A., Vijlder, J.J.M.De., Eijk, J.E. Van, Jorna, T.J., Tegeleers, W.H.H. (1977). Clinical aspects of iodine metabolism in goats.with congenital goitre and hypothyroidism. Br. Vet. J. 133(5): 495-503.

Nangia, O.P. and Gulati, D.P. (1976). Influence of thyroid status on growth of chickens. Indian Vet. J., 53: 185-189.

Necheles, T. and Beutler, E. (1959). The effect of triiodothyronine on the oxidative mechanism of erythrocytes. I. Cellular studies. J. Clin. Invest., 38: 788.

Niepce, B. (1851). The Thyroid. Edited by Beach Hazard, J. and Smith, D.E. The Williams and Wilkins Company, Baltimore, pp. 76.

Otomatus, T. (1954). Studies on goitre of farm animals in Japan, studies on goitre in sheep. Jap. J. vet. Sci., 16: 53-64.

Pandav, C.S. and Kochupillai, N. (1982). Endemic goitre in India: Prevalence, etiology, attendant disabilities and control measures. Indian J. Pediat., 50: 259-271.

Peczely, P., Astier, H. and Jallageas, M. (1979). Reciprocal interaction between testis and thyroid in male Japanese quail. Gen. Comp. Endocr., 37: 400-403.

*Peters, J.P. and Man, E.B. (1950). The significance of serum cholesterol in thyroid disease. J. Clin. Invest., 29:1-7.

*Petersen, W.E., Spielman, A.A., Pomeroy, B.S. and Boyd, W.J. (1941). Effect of thyroidectomy upon sexual behaviour of the male bovine. Proc. Soc. Expt. Biol. & Med., 46:16.

*Peterson, R.E. (1958). The influence of the thyroid on adrenal cortical function. J. Clin. Invest., 37: 736.

*Potter, G.D., Lindsay, S. and Chaikoff, I.L. (1960). Introduction of neoplasms in rat by low doses of radio iodine. Archs. Path., 69: 257-269.

Prasad, O. and Singh, A. (1971). Thyroid gland relationship in desi chicken under hypo and hyper thyroid conditions. Indian J. Anim. Sci., 41: 867-872.

Rivlin, R.S. (1971). 'Vitamins'. The Thyroid. A fundamental and clinical text. Edited by Warner, S.C. and Ingbar, S.H. Harper and Row, New York, 3rd Ed. pp. 751.

Robbins, J. and Rall, J.E. (1960). Proteins associated with thyroid hormones. Physiol. Rev., 40: 415.

Roy, S., Deo, M.G. and Ramalingaswami, V. (1964). Pathologic Features of Himalayan endemic goiter. Am. J. Path., 44: 839-851.

*Russell, J.A. (1943). "The relationship of the anterior pituitary of the thyroid and adrenal cortex in control of carbohydrate metabolism". Essays in Biology. Edited by Farquhar, S.T., Leake, C.D., Lyons, W.R. and Sympson, M.E. University of California Press, Berkely, pp. 507-527.

Russel, A.J.F. (1967). A note on goitre in lambs grazing rape (Brassica napus). Anim. Prod., 9: 131-133.

Schalm, C.W. (1965). Veterinary Haematology. Lea and Febiger, Philadelphia, 2nd Ed. pp. 62, 80, 95-100.

*Schuttze, A.B. and Davis, H.P. (1946). The influence of feeding synthetic thyroprotein on fertility of bulls. J. Dairy. Sci., 29: 534-535.

Scott, P.P., Greaves, J.P. and Scott, M.C. (1961). Nutrition of cat. 4. calcium and iodine deficiency on a meat diet. Br. J. Nutr. 15: 35-51.

Scow, R.O. and Greer, M.A. (1955). Effect on the thyroid gland of experimental alteration of the level of circulating thyroxine in mice with heterotropic pituitaries. Endocrinology, 56: 590.

Scrimshaw, N.S. (1964). "Geographical pathology of thyroid disease". The Thyroid. Edited by Hazard, J.B. and Smith, D.E. William and Wilkins Company, Baltimore, pp. 107-111.

*Sellers, E.A. and Ferguson, J.K.W. (1949). Exopthalmus in rats after prolonged administration of propyl thiouracil. Endocrinology, 45: 345-346.

Setchell, B.P., Dickinson, D.A., Lascelles, A.K. and Bonner, R.B. (1960). Neonatal mortality in lambs associated with goitre. Aust. Vet. J., 36: 159-164.

*Silberg, M. and Silberg, R. (1940). The effect of thyroidectomy and anterior pituitary extract of cattle. Am. J. Path., 16: 505-524.

Sharma, D.N. and Singh, Y. (1975). Histological observation on thyroid gland relationship in male cross-bred (New Hampshire x White cornish) chicken. Indian J. Anim. Health, 14: 147-149.

*Sharpless, G.R., Pearson, J. and Prato, G.S. (1939). Production of goitre in rats with raw and treated soyabean flour. J. Nutr., 17: 545-555.

Sharpless, G.R. and Metzger, M. (1941). Arsenic and goitre. J. Nutr., 25: 119-127.

*Sinclair, O.P. and Andrews, E.D. (1958). Prevention of goitre in new born lambs from kale-fed ewes. N.Z. Vet. J., 6: 87-95.

*Smith, P.E. and Smith, I.P. (1922). The repair and activation of the thyroid in hypophysectomised tedpole by parenteral administration of fresh anterior lobe of the bovine.hypophysis. J. Med. Res. 43: 267-283.

Smith, H.A., Jones, T.C. and Hunt, R.D. (1972). Veterinary Pathology. Lea and Febiger, Philadelphia, 4th Ed. pp. 1357-1363.

Soliman, F.A. and Reineke, E.P. (1952). Influence in variation in environmental temperature and thyroid status on sexual function of young female mice. Am. J. Physiol., 168: 400-405.

Solomon, J. and Greep, R.D. (1959). The effect of alteration in thyroid function on pituitary growth hormone content and acidophil cytology. Endocrinology, 65: 158-164.

Sorensen, P.H. (1956). Variations in the concentration of PBI in blood of cattle and pigs. K. Vet. Hojsk. Aarsskr. 15: 64-76.

Southcott, W.H. (1945). Congenital goitre in lamb in Tasmania. Aust. Vet. J., 21: 35-36.

*Spielman, A.A., Peterson, W.E., Fitch, J.B. and Pomeroy, B.S. (1946). General appearance, growth and reproduction of thyroidectomised bovine. J. Dairy Sci., 29: 329.

*Spisni, D. and Garavaglia, G. (1954). Histology of thyroid gland of cattle fed on cauliflower. Vet. Ital., 5: 883-895.

Sreekumaran, T. (1976). Pathology of experimental hypothyroidism in goats. M.V.Sc. Thesis, Kerala Agricultural University.

Srinivas, V. (1979). Studies on protein bound iodine in the blood serum of the buffaloes in certain physiological and pathological conditions of reproduction. M.V.Sc. Thesis, University of Agricultural Sciences, Bangalore.

*Stanbury, J.B., Brownell, C.L., Rigss, D.S., Perinetti, H., Hoiz, J. and del Castillo, E.B. (1954). Endemic goitre. The adaptation of Man to iodine deficiency. Cambridge, Mass, Harward University Press.

*Stott, H., Bhatia, B.B., Lal, R.S. and Rai, K.C. (1930-31). The distribution and cause of endemic goitre in United Province. Indian J. Med. Res., 18: 1059-1086.

*Suzuki, H., Higuchi, J., Sawa, K., Ohatakis, S. and Horiuchi, Y. (1965). Endemic coast goitre in Kokkaido, Japan. Acta Endocr., 50: 161-176.

Swanson, E.W. and Boatman, J.P. (1953). The effect of Thiouracil Feeding upon the seminal characteristic of Dairy Bulls. J. Dairy Sci., 36: 246-252.

Symington, T. (1969). Functional Pathology of Human Adrenal gland. E & S Livingstone Limited, Edinburgh, pp. 70.

Symonds, H.W. (1969). The effect of thyroidectomy and parathyroidectomy on ascertion of phosphorus into the skeleton of young goats. Res. Vet. Sci., 10: 218-224.

Symmonds, H.W. (1970). The effect of thyroidectomy and parathyroidectomy upon phosphorus homeostasis in goat. Res. Vet. Sci., 11: 260-269

Tong, W. (1971). "Thyroid hormone synthesis and release". The Thyroid: A fundamental and clinical text. Edited by Werner, S.C. and Jugbar, S.H. Harper and Row, New York, 3rd Ed. pp. 24-40.

*Turner, C.W. and Cupps, P.L. (1940). Effect of certain experimental conditions on the thyrotrophic hormone content of albino rat. Endocrinology, 26: 1042-1047.

Underwood, E.J. (1971). Trace Elements in Human and Animal Nutrition. Academic Press, New York, 3rd Ed. pp. 300.

Vadodaria, V.P., Janakiraman, K. and Buch, N.C. (1980). Studies on thyroid follicles in relation to phases of oestrus cycle and breeding periods. Indian Vet. J., 57: 985-990.

Vale, W., Burgus, R. and Guillemin, R. (1967). Competition between thyroxine and TRF at the pituitary level in the release of TSH. Proc. Soc. Exp. Biol. Med., 125: 210.

*Vzaimosuyaz, U.S.B.I.S. (1973). Relation of protein bound iodine to reproduction in cows. Nutr. Abstr. Rev., 43: 917, 7593.

Wallach, J.D. (1965). Goitrogenic hypothyroidism in feeder lambs. Vet. Med. Small Anim. Clinician, 60: 1051-1053.

Walton, E.A. and Humphrey, J.D. (1979). Endemic goitre of sheep in highlands of Papua New Guinea. Aust. Vet. J. 55: 43-44.

Watson, W.A., Boadbent, G.D. and Kilpatrick, R. (1962). Congenital goitre in Dorset horn lambs. Vet. Rec., 74: 506-509.

*Werner, S.C. (1971). "Male Reproductive System". The Thyroid: A Fundamental and Clinical Text. Edited by Werner, S.C. and Ingbar, S.H. Harper and Row, New York, 3rd Ed. pp. 785.

*Wharton, T. (1956). The Thyroid. A Fundamental and Clinical Text. Edited by Werner, S.E. and Ingbar, S.H., Harper and Row, New York, 3rd Ed. pp. 5.

*Wilson, D.C. (1941). Flourine in the aetiology of endemic goitre. Lancet., 1: 211-212.

Wilson, J. (1975). Hypothyroidism in ruminants with special reference to foetal goitre. Vet. Rec., 97: 161-164.

*Wintrobe, M.M. (1961). Clinical Haematology. Lea and Febiger, Philadelphia. 5th Ed. pp. 381.

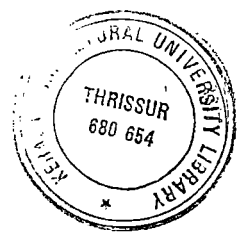
Yatvin, M.B., Wannemacher, J.R. and Banks, W.L. Jr. (1964). Effects of thiouracil and thyroidectomy on liver protein metabolism. Endocrinology, 74: 874-884.

Zak, B. (1957). A simple lipid micro technique for serum total cholesterol. Am. J. Clin. Path., 27: 583-588.

*Zarrow, M.K. and Money, W.L. (1949). Involution of the adrenal cortex of rats treated with thiouracil. Endocrinology, 44: 345-357.

*Zeckwer, I.T., Davison, L.W., Keller, T.B. and Livingood, C.S. (1935). The pituitary in experimental cretinism. I. Structural changes in pituitaries of the thyroidectomised rats. Am. J. med. Sci., 190: 145-147.

* Originals not consulted.



INCIDENCE AND PATHOLOGICAL FEATURES OF HYPOTHYROIDISM IN CATTLE

By

MAMMEN J. ABRAHAM

ABSTRACT OF A THESIS

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Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Pathology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy - Trichur

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ABSTRACT

A survey study conducted indicated that hypothyroidism was one of the major etiological factors responsible for non-infectious reproductive disorders in cattle. The reason for repeat breeding cases in certain instances could be traced to hypofunction of the thyroid gland. Besides, this, hypothyroidism was experimentally induced in six male calves using thiourea as the goitrogen at a single dosage schedule of 150 mg/kg body weight with a view to delineate the sequence of clinicopathological changes in the sub-clinical hypothyroid state. The haemogram, plasma protein level, serum cholesterol level and serum protein bound iodine level of these animals were estimated at fortnightly intervals.

Clinically, the experimental animals manifested progressive weakness, dullness, reduction in feed intake, irregular shedding of hair, subcutaneous edema and above all a marked stunting in growth. Gelatinization of subcutaneous and other depot fat and cardiac dilatation were the chief lesions encountered at autopsy. The calves became progressively anaemic and registered high serum cholesterol and plasma protein levels but significantly low PBI level as compared with control calves. There was significant increase in the relative weights of the thyroid, adrenal and pituitary glands but reduction in the relative weight of the testis.

Histologically the thyroid follicular epithelial cells exhibited hyperplastic changes and varying degrees of degeneration along with significant depletion of colloid. Microscopically pituitary gland revealed vacuolation and hyperplasia of the basophil cells in focal areas. Adrenal glands showed slight proliferation of cells of zona faciculata and depletion of fat. Microscopic picture of the testis showed slight obliteration of the seminiferous tubules with no evidence of mitotic activity in the tubular cells. A few degenerated and desquamated cells were seen along with giant cells. The varying degrees of degenerative changes observed in the testis accounted for the reproductive disorders in hypothyroidism.