THE CLINICOPATHOLOGIC EFFECTS OF THYROIDECTOMY ON REPRODUCTIVE AND OTHER BODY FUNCTIONS IN THE MALE GOAT

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By

JAMES ALFRED WILLIAMS

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
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DOCTOR OF PHILOSOPHY

Department of Veterinary Pathology

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JAMES ALFRED WILLIAMS

ABSTRACT

The clinical and pathological effects of complete and partial thyroidectomy and thyroidectomy plus iodinated protein therapy on reproductive and other body functions in young goats were studied. Twenty male goats of mixed breeding were surgically thyroidectomized at two weeks to one month of age. Eight were left as controls.

Clinical studies included growth rates, libido, semen production (by electrical ejaculation), sperm morphology, hematology; and blood sugar, plasma calcium, and plasma inorganic phosphorus determinations. Post-mortem examinations were conducted on control and experimental goats after 15 to 18 months of age to determine gross and histologic lesions if any.

When thyroidectomy was complete typical cretinoid symptoms including lethargy, edema, stiffness, hyperkeratosis, alopecia, bloating and stunting of growth appeared. The athyroid goats failed to show libido, produced significantly fewer motile spermatozoa, and significantly more abnormal spermatozoa per ejaculate than did the controls.

No decrease in testicular size relative to body weight was observed in the athyroid goats, but the mean

testicular weight was less than half that of the controls.

Histologically there was more vacuolation and hyaline-like change among secondary spermatocytes and spermatids and more sudanophilia in the seminiferous tubules of the completely thyroidectomized goats than in the controls. Cytoplasmic atrophy, absence of granulation, and pyknosis of the Leydig cells were more pronounced in the athyroid than in the euthyroid goats.

Pronounced stereocilial loss, epithelial vacuolation, and the formation of bodies resembling corpora amylacea in the epithelium of the head of the epididymis were observed in the athyroid goats.

Prolonged athyroidism resulted in pronounced degranulation and vacuolation of the large basophils in the anterior lobe of the pituitary gland. The adrenal glands of cretinoid goats showed decreased cortical depth and increased medullary diameter when compared to non-thyroidectomized goats.

Marked hyperkeratosis of the epidermis and hair follicles along with vacuolar degeneration of the smooth muscle in the dermis was a constant feature of the untreated thyroidectomized goats. Shallow erosions of the articular cartilages of the tibiotarsal joint occurred in the athyroid goats but not in the controls.

Reproductive and other body functions were not impaired in partially thyroidectomized goats nor in athyroid goats which were fed iodinated protein before cretinoid symptoms developed. Replacement therapy after pronounced cretinoid symptoms developed did not restore normal growth nor reproductive functions but did alleviate other symptoms of cretinism.

Accessory thyroid tissue was found in ten of nineteen goats by I^{131} uptake studies. It was concluded that this procedure is essential in determining the thyroid status of goats used in athyroid experimentation.

Testicular biopsy provided an adequate means of assessing testicular histology but resulted in some local necrosis and calcification along the path of needle entry. It was found that extreme care should be taken to prevent puncture of the epididymis; otherwise a sperm granuloma invariably results.

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VITA

The author was born in Michigan March 23, 1919, and raised on a farm near Riverdale.

High school training was completed at Riverdale High School in 1936.

He attended Michigan State College from 1937 to 1941, receiving the B.S. degree in Animal Husbandry and Agricultural Education.

During World War II he was commissioned a naval aviator and was discharged as a lieutenant in 1945.

He re-entered Michigan State College in the School of Veterinary Medicine in 1946 and received the D.V.M. degree in 1950.

Upon joining the staff of the Department of Veterinary Pathology in 1950 graduate study undertaken in 1946 was continued and the M. S. degree in Dairy Husbandry received in 1951.

Since joining the staff of the Department of Veterinary Pathology he has engaged in research on diseases of infertility, particularly in cattle.

Dedicated

to

Kala, Melanie, and Jama

TABLE OF CONTENTS

TABLE OF CONTENTS

I. INTRODUCTION

Infertility in livestock constitutes a major economic loss to farmers in Michigan and elsewhere in the nation. The annual Michigan Dairy Herd Improvement Association reports have listed sterility and delayed fertility as the second most frequent reason for removal of cows from dairy herds. Approximately seven percent of the cows in herds participating in the DHIA - IBM program are removed annually because of sterility or delayed fertility.

Sterility and delayed fertility result from many causes either individually or collectively. These causes include congenital or hereditary defects of the reproductive organs, infectious disease, hormonal disturbances, nutritional imbalances, and faulty management practices. Experiments conducted in recent years have pointed out the need for studying the relationships of non-reproductive hormones to reproductive function.

One of the hormones known to affect reproductive function, particularly in the male, is thyroxine, the secretory substance produced by the thyroid gland. Decreased sperm output and lowered fertility are known to result when hypothyroid states occur, whether naturally or experimentally induced.

It is often difficult to determine whether reduced spermatozoan production and reduced libido are due to gonadotrophic hormone failure *per se* or whether it may be complicated by a secondary thyroid hormone disturbance. From experimental studies of thyroid inhibition in the rabbit and ram characteristic histologic changes in the testis have suggested the possible relationship of thyroxine to sperm production.

The primary purpose of the study presented in this thesis was to gain information relative to clinical and pathologic effects of supplemented and unsupplemented athyroidism in the male goat, with particular regard to reproductive function. An attempt was also made to determine whether testicular biopsy might be a feasible applied technique in correlating testicular function with the thyroid status. Goats were used in this study because of their immediate availability and their ruminant nature. It was hoped that the information gained would be useful in extending these studies to bulls. The seasonal variation in sperm production in goats appears to compare favorably to that of bulls.

II. REVIEW OF LITERATURE

A. Relation of Thyroid Gland to Reproductive Function in the Male

1. General considerations. Several literature reviews during the late 1940's and early 1950's touch on the role of the thyroid gland and its relation to reproductive function. Beach (1947) reviewed studies relating to sexual behavior in mammals. He concluded that, "thyroid secretions appear to affect sexual activity primarily if not exclusively by way of systemic changes in metabolic function". Blaxter et al. (1949) and Dyrendahl (1949) reviewing the effects of thyroid materials on fertility in male ruminants concluded on the basis of evidence available up to 1949 that thyroid hormone affected reproduction both directly and indirectly. Maqsood (1952) summarized his experiments with rabbits and rams and critically reviewed the literature relating male reproductive function to thyroid secretory status. He has clearly shown that thyroprotein or thyroxine prevents seasonal decline in ram sperm production. It was important to administer thyroactive compounds, however, at or near physiologically optimum levels to obtain maximum reproductive response.

Evidence has accumulated that thyroid gland activity varies considerably under varying environmental conditions and physiological states. Some of these thyroid changes have also been associated with changes in reproductive function in animals. McKenzie and Berliner (1937) early considered that "summer sterility" in rams was due to high environmental temperature. It was not known at that time whether the decreased reproductive function was due to reduced metabolic rate or to high scrotal temperatures.

That marked depression of thyroid secretion rates do occur as environmental temperatures increase was shown by Henneman, Reineke, and Griffin (1955). By observing the rate at which injected radioactive iodine (I^{131}) disappears from the thyroid glands of sheep they estimated an approximately fourfold decline in thyroid secretion rate between May and July in Michigan. Branton et al. (1955) found that there was a highly significant negative correlation between the serum protein bound iodine (PBI) in bulls and the average monthly maximum temperature in Louisiana. Fertility and percent of usable ejaculates declined with the fall in PBI. Under controlled environmental conditions Dutt and Bush (1955) found that in ewes and rams housed at 45-48°F. only 1.9 services per conception were required. On the other hand rams and ewes housed under temperatures simulating summer conditions required 5.3 services per conception. Dutt and Simpson (1957) bred separate groups of ewes with Southdown rams housed at 45-48°F. and rams kept at ordinary summer environmental temperatures. Cleavage occurred in 64 and 26 percent of the ova resulting from the treated and control ram matings respectively. In those ewes allowed to complete gestation 50 percent of the treated and 13 percent of the control groups dropped lambs. Although thyroid activities were not measured in these studies they tend to substantiate the earlier observations by Maqsood and Reineke (1950) that $30^{\circ}C$. environmental temperature caused significant testicular atrophic and degenerative changes in male mice. Thyroprotein in physiologically optimum amounts in the diet prevented these degenerative changes. Thiouracil, an antithyroid compound, produced testicular changes similar to the high environmental temperature, which were also prevented by thyroprotein.

Age plays a role in thyroid activity and consequently reproductive function. A decrease in body metabolism is known to be associated with advancing age (Brody, 1945). Maqsood (1951b) reported reduced spermatogenic function in aged male rabbits. Reineke (1946) reported that aged bulls responded with improved sperm production and shorter ejaculation time following the administration of thyroprotein.

Among farm animals, effects of light on thyroid function in the male have received little study. Puntriano and Meites (1951) found that continuous light decreased and continuous darkness increased thyroidal I^{131} uptake in mice.

Limited genetic observations between and within animal species have revealed differences in thyroid activity. Meites and Chandrashaker (1948), finding that injected thyroxine increased the response to gonadotrophin, concluded that normal mice are slightly hypothyroid. Silberberg et al. (1955) have reported that certain strains of mice become hypothyroid and characteristically show joint lesions early in life. Mixner and Upp (1947) observed that thyroxine secretion rates were much higher in hybrid chicks resulting from mating inbred Rhode Island Reds to White Leghorn inbred lines than in single crossed chicks produced by mating two inbred lines of the same breed. These authors postulated that "hybrid vigor" may be due to increased thyroid activity.

Level of food intake may affect thyroid activity. Armstrong and Hansel (1956) reported that the pituitary glands from heifers fed 60-70 percent of Morrison's recommended total digestible nutrient (TDN) intake had lower thyroid stimulating potency than those fed at 140-160 percent of necessary TDN. Recently Sorenson et al. (1959) reporting on another phase of the above experiment found the low level heifers to have significantly smaller thyroid glands than did the high level heifers. In addition the thyroid acinar epithelial cell heights averaged 12.4 microns for high level heifers and 8.7 microns for low level heifers, a statistically significant difference. Similar feeding level trials were reported in bulls by Bratton et al. (1959) and Flipse et al. (1953). Although thyroid activities were not observed, onset of puberty was greatly delayed by low TDN intake. Maqsood (1951b) reported a similar delay in the onset of semen production in young rams fed thiouracil continuously.

2. Libido. Sexual desire is decreased in hypothyroid animals. Thyroidectomy in young rats results in a failure to develop sexual maturity (Rickey, 1925). Maqsood (1951d) reported that aged buck rabbits showing poor sexual desire responded to thyroxine therapy. Thyroidectomy in a four month old bull did not prevent spermatogenesis but did prevent libido (Petersen *et al.*, 1941). When thyroxine was fed to this bull at sexual maturity normal libido was restored. Maqsood (1950a) also found that thyroxine treated rams showed greater sexual interest than control rams during the warm summer months, when thyroid secretion rates are usually low. Oloufa *et al.* (1951) reported that high temperature (90-92°F.) either continuously or intermittently, high temperature plus thyroprotein, or low temperatures (55-65° F.) plus thiouracil caused buck rabbits to be slower in their sex drive than control rabbits kept at 55 to 65°F. without other treatment.

3. Semen characteristics and fertility. The effects of the thyroid secretion on semen quality, quantity, and fertility vary among species and environmental conditions. Oloufa *et al.* (1951) reported that, in rabbits, temperatures above 90°F. or thiouracil caused a decrease in semen volume, motility, sperm concentration, percent of live sperm and normal sperm. Thyroprotein as .01 percent of the diet given to rabbits kept continuously in a high temperature room decreased semen quality. However it alleviated to some extent

the adverse effect produced when rabbits were alternated between a warm room and one kept at 55°-65°F. Maqsood (1951d) administered thyroxine to male rabbits in amounts estimated to be necessary to meet physiological requirements. Both libido and semen quality were considerably improved over that obtained in litter mate controls. Maqsood (1951d) also reported that a peculiar deformity on the head of spermatozoa obtained from rabbits showing poor semen quality, disappeared after thyroxine treatment. In rams, thyroidectomy hot summer temperatures, or thiouracil treatment, has decreased semen volume, number of motile sperm, and often increased the number of abnormal spermatozoa, (Berliner and Warbritton, 1937; Bogart and Mayer, 1946; Maqsood, 1952). Dutt and Simpson (1947), as stated previously, found increased embryonic death to be associated with high environmental temperature in rams.

In the bovine male, Petersen _et al_. (1941) found that thyroidectomy at four months halted sexual desire, but did not prevent spermatogenesis. Libido was restored by giving thyroxine. Branton _et al_. (1955) found semen quality to be poorer when PBI values were lowest during the hot summer months. Few data are available on actual effect on fertilizing capacity of spermatozoa produced by bulls in a hypothyroid or hyperthyroid state.

Eaton and Simmons (1952) reported the results of monthly collections of semen from Toggenburg and Common

American goats during a three year period. Their results appear to be more comprehensive and provide a better basis for average values than those reported by other authors (Möckel, 1937; Phillips et al., 1943; Perry, 1945). Average values for all seasons were: semen volume, .65 cc.; motility, 1.51 (1 = maximum motility; 6 = no motility); concentration, 2.724 billion spermatozoa per milliliter; total spermatozoa per ejaculate, 1.658 billion; percentage of abnormal spermatozoa, 8.46. Variations in values were greater among bucks than for seasons of the year. Highest values for all of the characters listed above except percent of abnormal spermatozoa corresponded to the normal breeding season. Only semen volume and concentration were statistically significantly different.

Abnormalities of spermatozoa occurred most frequently in the neck and tail. Heads separated from tails and sharply bent middle pieces constituted the most frequent types of abnormality.

4. Testicular size. Maqsood and Reineke (1950) found that testicular weight in mice increased when thyroprotein was given in physiologic doses. On the other hand, temperatures of 30°C. and thiouracil decreased the testicular size from that recorded for control mice housed at 24°C. Young rats thyroidectomized a day or two after birth had testes which were smaller both absolutely and in proportion to body size than did normal rats of the same age, (Scow and

Simpson 1945). Jones, Delfs and Foot (1946) studied the effect of thiouracil feeding in rats and found that it did not decrease testicular size. Kirkpatrick (1955) has reported that among wild mammals the testis of the adult fox-squirrel undergoes marked changes in size and histology during the year. The testis weights were greatest during the principal mating seasons, December to March, and June to August. Testes weights ranged from 2.6 - 12.6 gm. (average 6.9) during periods of full spermatogenic activity and between 1.7 and 5.5 (average 3.9) during the redevelopment phase. Maqsood (1950b) has found that thiouracil treatment or thyroidectomy results in significantly lower weights of the sex organs of rabbits. The effects of thyroid inhibition were more marked in the growing animal than in the adult animal.

Several investigators have reported a decrease in size of the testes, comb, and wattle, in the cockerels of several breeds of chickens following thyroidectomy. (Greenwood and Chu, 1939; Payne, 1944; Blivaiss, 1947).

5. <u>Histologic changes in the male reproductive organs</u>. Histologic changes in the male gonads and accessory sex glands which are associated with thyroid activity have been reported by several investigators in laboratory animals. Unsupplemented thiouracil treated young mice show marked atrophy and degenerative changes in the seminiferous tubules of the testis (Maqsood and Reineke, 1950). Maqsood (1950b)

investigated the effects of prolonged thyroid depression with thiouracil in rabbits. Rabbits starting at four weeks of age received .1 percent thiouracil in their diet for 16 weeks. Control rabbits received thyroxine injected at optimum levels for their weight. The seminiferous tubules from the testes of the thiouracil treated rabbits exhibited extensive degeneration of the spermatogenic cells. Fluid containing protoplasmic debris occupied the lumina of many of the tubules. Marked atrophy and degenerative changes along with a decrease in number of interstitial cells also were noted. In the thyroxine treated rabbits there was active spermatogenesis with many maturing spermatids present in the tubules. In addition the epididymal ducts contained masses of normal appearing spermatozoa and tall epithelial cells compared to numerous degenerated spermatogenic cells and a lower epithelial height in the thiouracil treated rabbits. Fiaccavento (1952) reported that testicular changes did not occur in guinea pigs 10 days after starting thiouracil treatment, but after 40 days spermatogenesis was definitely impaired. Leydig cell changes were not consistent even after the longer treatment period.

Among wild mammals Kirkpatrick (1955) described profound cyclic changes in the testis of the fox-squirrel. Degenerative changes of the spermatogenic cells are most pronounced during the non-mating season from June to August. The annual cycle of epithelial regeneration corresponds generally to the appearance of the development of the testis from birth to puberty. Though thyroid status was not considered in this study the period of decreased spermatogenesis corresponds closely with that noted in the ram when lowered thyroxine secretion is known to occur. Mossman _et al_. (1955) noted marked epithelial degeneration in the accessory genital organs of the fox-squirrel during the late summer months. Regeneration of the epithelium followed this period.

Among farm mammals the sheep has received principal attention in regard to the histologic structure of the gonads in varying thyroid states. Berliner and Warbritten (1937) observed a fall in sperm numbers and an increase in abnormal spermatozoa in thyroidectomized and partially thyroidectomized lambs. Thyroidectomy was accompanied by testicular edema, atrophy of interstitial tissues, and sloughing and pyknosis in the seminiferous tubules. Again Maqsood (1951c) has reported that thiouracil given to young rams for up to twelve months caused progressive impairment to spermatogenesis and decreased Leydig cell activity.

B. Effects of Thyroidectomy on Other Endocrine Glands

1. _Pituitary gland_. Jubb and McEntee (1955a) extensively reviewed the literature pertaining to the general problem of adenohypophyseal functional cytology. Halmi (1950), using the Gomori (1950) aldehyde-fuchsin stain on sections of pituitaries from rats subjected to various

endocrine disturbances, found two distinct basophilic cell types which he described as beta and delta cells. The cells he called delta cells become progressively hyperplastic and vacuolated after castration and thyroidectomy. In a later study by Halmi (1952) thyroxine therapy increased the beta cell count and decreased the delta cell count in hypothyroid rats. Vacuolation of the delta cells disappeared. His findings suggested that the beta cells might be the site of thyrotrophin storage.

Griesbach and Purves (1945) found that the depletion of granules from acidophils in the pituitary following thyroidectomy, reported by earlier investigators, only occurred in extreme thyroxine deficiency. When physiological levels of thyroxine were given to young thyroidectomized rats, acidophils were present in normal numbers and there were no "thyroidectomy cell" basophils present. Slightly lower than optimal thyroxine levels caused "thyroidectomy cells" to appear without disappearance of acidophils. A thyroxine secretion rate of 2.25 micrograms/100 gm. body weight in young rats was determined by this technique. Purves and Griesbach (1951a, 1951b), employing periodic acid Schiff stain (PAS) to detect glycoprotein in the pituitary cells of rats, found two types of cells present. A round cell type located adjacent to the pars intermedia and to the lower surface of the pars distalis stained intensely with PAS. This cell type was inhibited by injected estrogen, which led to the conclusion that these were the "gonadotroph" basophils corresponding to delta cells of Halmi (1952). A second basophilic cell type, large polyhedral cells with vesicular nuclei, was inhibited by thyroxine and formed thyroidectomy cells in thyroxine deficiency. The intensity of the PAS stain correlated with the thyrotrophic hormone content of the gland. These "thyroidectomy cells" corresponded to the beta cells of Halmi (1952).

Griesbach (1951) reported having seen vacuolated basophils in the pituitary glands of rabbits, dogs, and sheep suffering from thyroid hyperplasia. Jubb and McEntee (1955b) reported the histologic architecture of the bovine pituitary in various physiologic and pathologic states. In 14 naturally occurring cases of thyroid epithelial hypertrophy or hyperplasia, the pituitary beta cells were largely degranulated and vacuolated. These cells occupied the medullary zone of the anterior lobe primarily, although some were found throughout the anterior lobe. These investigators conclusively demonstrated with PAS staining techniques that thyroidectomy of a young bull results in degranulation of the beta basophils. Six weeks following complete thyroidectomy they began to degranulate without any definite pattern. Some areas contained normal granulated acidophils while in other areas the granules were replaced with vacuoles. The delta basophils apparently remained unaltered or, at least, retained their PAS staining granules. Prolonged thyroidectomy and its possible effects on the "gonadotroph" cells in the bovine were not reported.

Reineke _et al_. (1941) reported lowered gonadotrophic potency in the pituitary glands of thyroidectomized goats than in the controls. Histologic examination of the pituitary glands of these goats was not reported.

2. _Effect of thyroid secretion on adrenal gland_. Changes in thyroid gland secretion affect the gross and histologic structure of the adrenal gland. Bauman and Marine (1945) noted that the adrenal glands involute to one-half their normal size in rats fed three to four months on thiouracil. The involution was primarily due to shrinking of the cortex accompanied by a decrease in lipid staining material. Occasionally hemorrhage and cyst formation was noted in the reticular zone. Several investigators (Wallach and Reineke, 1945 ; Maqsood, 1954) have shown that giving thyroxine or thyroprotein above physiologic levels increases the size of the adrenal glands in laboratory animals. Deane and Greep (1947), studying the effects of hypothyroid and hyperthyroid states on adrenal gland histology in rats, found that thyroidectomy or thiouracil decreased sudanophilic ketosteroid substances in the zona fasciculata. At the same time ketosteroids in the zona glomerulosa increased.

These investigators found that thyroxine given above physiologic levels stimulated the zona fasciculata at first but later depleted both the fascicular and glomerular zones of the adrenal cortex. Maqsood (1950a) fed thyroprotein to mice at different levels in their ration and kept them in environmental temperatures of 24° or 30°C. At 24°C. thyroprotein above .1 percent of the ration decreased adrenal weight while at 30°C., .025 percent thyroprotein was the upper limit which the ration could contain without decreasing adrenal weight.

Functional studies in rats (Timiras, 1955) and humans (Levin and Daughaday, 1955) have revealed that hypothyroid states decrease the amount of 17-hydroxy-corticosteroid excretion.

C. Pathological Changes in Extra-endrocrine Tissues

1. Skin. There have been few detailed reports on gross and histologic changes which occur in the skin of thyroidectomized ruminant animals. Perhaps this is because more attention was paid to growth and to other pathologic changes by investigators in the past. Simpson (1924b) reported that wool became coarse and easily pulled out in sheep and horn growth was greatly retarded. Marston and Pierce (1932) noted delayed wool growth in sheep also. Bustad et al. (1957) report that adult ewes showed pronounced loss of hair, especially over the poll and carpus, two months or more after continously feeding 240 or 1800 microcuries I^{131} per day. Lambs born from ewes receiving

high doses of I^{131} also exhibited variable alopecia. Some showed extreme denudation except for small patches of wool and hair about the face, legs, and ventral midline. Description of the skin in experimental thyroprivia of goats was not found in the literature studied.

2. Subcutaneous tissues. Myxedema is a pathologic change commonly noted in hypothyroidism. Marked subcutaneous edema was noted by Goldberg (1927) in two thyroidectomized male sheep. To a lesser extent edema was present in most of the rest of 16 sheep and three goats which were thyroidectomized between eight and 105 days after birth. Bustad et al. (1957) noted edema in sheep radiothyroidectomized with I^{131}, particularly in the neck region around the thyroid gland.

3. Cardiovascular changes. Goldberg (1927) found aortic calcification in 11 of 17 sheep that lived over 244 days after thyroidectomy. The arteriosclerosis attendant to the calcification was unlike that noted in man. Histologically the aorta and pulmonary arteries were thin walled, showed hyaline degeneration of the elastic fibers, and production of new tissue mostly between the calcified plaques. Three cretinoid goats did not show aortic calcification. Cardiac dilatation was present in both sheep and goats which was attributed to imperfect or arrested muscular development. The cardiac muscle was constantly pale and flabby as compared to the controls. Histologically,

calcification was not seen in the thyroidectomized goats but cross striations were absent. Bustad et al. (1957) noted cardiac enlargement and calcific plaques in the aorta, jugular vein, and coronary vessels of adult ewes receiving 240 and 1800 millicuries of I^{131} per day. Offspring of these radiothyroidectomized ewes also showed calcification of the large vessels close to the heart.

4. Renal pathology. All experimental sheep and goat cretins examined by Goldberg (1927) showed renal lesions. Degeneration of tubular epithelial cells and colloid casts were constantly present while hyaline degeneration occurred in 10 of the 21 thyroidectomized sheep and goats.

5. Skeletal muscle. Goldberg and Simpson (1925) reported that both skeletal and cardiac muscle were pale and flabby in thyroidectomized sheep. Striations in the skeletal muscle fibers had almost completely disappeared. In addition the sarcolemmal nuclei were smaller and more numerous than in the control animals.

Simpson, (1927a) found muscular changes in thyroidectomized sheep. She found that the muscle cell nuclear count remains high in the cretin as compared to that of normal sheep. She concluded that early thyroidectomy prevents normal development of the sarcoplasm since the cross sectional area of the fibers is much less than in control sheep. Cross striations remain in the muscle fibers of both the controls and the thyroidectomized animals. In a later communication (1927b) the same author concluded that the lack of muscle development in the athyroid sheep was due to lack of exercise. Millikan and Haines (1953) have found impaired muscular function in human patients who suffer from hyperthyroidism or hypothyroidism separately or in conjunction with other conditions. Muscular atrophy with or without other degenerative processes has been found in these cases.

6. Osseous changes. Skeletal changes involving the long bones in sheep and goats have been reported by Goldberg and Simpson (1925). Todd and Wharton (1938) found that the diaphyso-epiphyseal junction of the long bones failed to develop properly in athyroid sheep. Obvious pathological changes did not become evident until the sheep were two or more years old. Features of the changes included: a) irregular exuberances resembling proud flesh on the shaft, b) inturned, clawed, trachoma-like epiphyseal margins, and c) poorly modeled, ill fitting epiphysis scarcely covering the shaft end. Failure of the head to grow properly has been observed by Liddel (1925) and Todd and Wharton (1934).

More recently Silberberg and Silberberg (1954, 1954a, 1954b, 1955) have reported that non-degenerative joint lesions occur in radiothyroidectomized mice. The incidence of this lesion is higher in young than old mice, higher in females than males, and higher in some strains of mice than others.

D. Thyroid Secretion Rates

1. Bioassay techniques. Thyroid secretion rates in varying metabolic states in normal animals are fundamental to determining when the thyroid activity is abnormal. Basal metabolism rates commonly employed in measuring human thyroid activity are generally impractical in animals. Reineke and Turner (1945) fed thiouracil to chicks to inhibit the thyroid gland and injected thyroxine in graded doses to determine the thyroid secretion. That weight of thyroid tissue per 100 gm. body weight which corresponded with the control thyroid weight determined the thyroid secretion rate. By this method it was found that thyroid secretion rates in female white Plymouth Rock chicks were higher in all seasons than were the secretion rates in the male. The secretion in the male varied between a low of .75 micrograms d.l. thyroxine per day in March to a high of 2.45 micrograms per day in October and November. Using the same method in rabbits, Maqsood (1950c) determined that although thyroid secretion rates increase with age there is a decrease in secretion in proportion to the body weight increase. Rabbits four and 48 weeks old secreted 8.4 and 21.9 micrograms of thyroxine respectively daily. He also worked out an equation for determining the secretion rate in rabbits:

$$y = .43\, X^{.48}$$

where y is the number of micrograms of thyroxine and X is the body weight in grams.

2. <u>Radio-active iodine (I^{131}) measurements</u>. Henneman (1953) developed a technique for the measurement of thyroid secretion rate in sheep which involved injecting tracer doses of I^{131}. Counts were made after seven days as the zero time and gradually increasing doses of l-thyroxine were injected daily until no further decrease in counts due to thyroid gland output of thyroxine occurred. The level of thyroxine injection at which 100 percent of the previous thyroid count was obtained determined the thyroxine secretion rate. By this technique Henneman <u>et al</u>. (1955) determined that the secretion rate during the summer in ewes was .078 mg. per day, which was significantly different from .26 mg. per day, the next higher secretion rate obtained in May. Lactation increased the daily output of thyroxine while pregnancy had no effect. Two-year-old ewes had significantly higher thyroid secretion rates than four-year-old ewes in all months except July.

Lodge (1957) and Lewis, Lodge and Reineke (1957) studied the thyroid secretion rate in four dairy breeds of calves. They used the technique of Henneman (1953), but found that cows are able to recycle I^{131} to the thyroid gland. To overcome this they gave thiouracil, a goitrogenic agent which prevents uptake of iodine. Daily thyroxine secretion rates on 42 calves was .57 mg. per 100 lb. body weight. Seasonal variations were not noted but calves were housed inside.

A seasonal variation in secretion rate in cows and calves was obtained by Premachandra *et al.* (1957) and Pipes *et al.* (1957). Using a technique similar to that employed above, thyroxine levels ranged from .2 to .7 mg. per 100 lb. body weight during the winter season with a mean of .5 mg. per 100 lb. The summer level was .1 to .3 mg. with a mean of .17 mg. per 100 lb. body weight.

3. Protein bound iodine. Plasma protein bound iodine (PBI) level has been advocated as a measure of thyroid activity and Lewis (1952) and Lewis and Ralston (1953) found that there is considerable variation in PBI levels in cattle of various ages. Values ranged from a maximum of 18.0 micrograms percent (μg%) in calves under 48 hours old to a minimum of 3.0 (μg%) in cows over two years old. More recent investigations of PBI levels indicate varying plasma levels associated with various physiological states (Lennon and Mixner, 1958; Asplund *et al.*, 1959; Branton, 1955).

E. Therapy with Thyroactive Compounds in Male Infertility

1. Thyroxine. Somewhat conflicting reports have arisen from the use of thyroactive substances in the treatment of male infertility. Maqsood (1951d) treated male rabbits that had low sexual desire, poor semen quality and arrested spermatogenesis. Also a peculiar protoplasmic mass was present on the heads of a small percentage of the spermatozoa produced by these rabbits. Thyroxine administered

in optimal physiologic doses improved spermatogenesis, libido, and fertility. The sperm abnormality also disappeared in the treated rabbits.

2. Synthetic iodinated proteins. Reineke (1946) reported that thyroprotein given orally at the rate of .5 to 1 gm. per 100 lbs. of body weight improved the libido in 10 of 14 bulls. It also improved the fertility in four bulls which had previously exhibited poor sexual desire or low fertility or both. Schultze and Davis (1946) gave seven bulls used in artificial insemination 1.0 to 1.24 gm. of thyroprotein per 100 lbs. body weight. Fertility increased from 51 percent to 55.7 percent in the treated bulls. Five of the seven bulls showed definite improvement in fertility. Thyroxine restored libido in a male goat which had shown a definite lack of interest in doe goats (Turner et al., 1943). Bogart and Mayer (1946) and Maqsood (1951c) have reported that thyroprotein prevents "summer sterility" in rams. There was marked improvement in libido, normal spermatozoa, and sperm motility. Bogart and Mayer (1946), however, found that therapy did not prevent gradual loss of volume of semen and concentration of spermatozoa.

On the other hand, no improvement in semen concentration or abnormal sperm was obtained by Williams (1956) from feeding 10 gm. of thyroprotein per day to two bulls which produced low quality semen during two successive summers prior to therapy. Thyroid I^{131} uptake and decay counts in these bulls were similar to two bulls of similar age and breed that produced normal semen. Spontaneous recovery of quality had occurred in the autumn in the two years prior to treatment. It also occurred in the autumn following the summer in which treatment was administered.

Eaton et al. (1948) failed to obtain improvement in ram sperm concentration, motility, volume, or abnormal forms by feeding one gm. or two gm. thyroprotein daily from May to September. In fact two gm. thyroprotein per day increased the number of abnormal forms significantly from that found in the controls. Aged rams were used in the study however. Warwick et al. (1948) obtained similar results in rams. These investigators found that thyroprotein decreased semen quality when given during April and May. At levels of .5 and 1.5 gm. of thyroprotein per day there was a slight increase in motility and methylene blue reduction time, but not in sperm concentration. Some rams tolerated six gms. of thyroprotein per day while others showed increased heart rate, lost weight and became lethargic. No beneficial effects to reproductive efficiency resulted from thyroprotein therapy to rams in work reported by Black et al. (1950). Maqsood (1952) pointed out that treatment of rams should start before the beginning of the seasonal decline and continue through the season. Also, dosage should be adjusted to meet the ram's demands for thyroxine, depending on the environmental temperature.

Wilwerth *et al.*, (1954) found that thyroprotein in excess of .04 percent of the ration to Rhode Island Red cockerels significantly decreased both semen volume and sperm concentration. It is apparent that treatment of hypothyroidism will be dependent on the relative need for thyroid-active substances, which is in turn dependent on such factors as age, size, environmental factors (such as temperature and light), genetic makeup and, possibly, nutrition.

From the review of literature there are several aspects of the relation of the thyroid gland to reproductive function on which data are still lacking. From the information available it has been inferred that it is possible to prevent seasonal changes in semen quality by administration of thyroid-active compounds. However in most instances there has not been a clear cut appraisal of the actual thyroid function.

Thiouracil has been used to depress thyroid function but some question has arisen as to whether there is complete block of the thyroid gland. Possible side effects of thiouracil on spermatogenic function have not been adequately investigated.

Where thyroidectomy has been employed in studying reproductive function, little or no evidence has been presented as to whether thyroidectomy was complete. For these reasons this experiment was set up to determine the effects of complete thyroidectomy on the reproductive functions in the male goat. The extent to which thyroidectomy was complete was carefully checked by measuring thyroid uptake of radioactive I^{131} and by post-mortem examination.

Survey of the literature indicates that spermatogenesis or libido or both may be related to the level of thyroid function. In this experiment an attempt was made to differentiate between these two factors by clinical and pathologic studies.

III. PROCEDURES

A. Source of Goats

Male offspring of mixed breeding, born in 1957 and 1958 and maintained by the Departments of Veterinary Pathology and Physiology and Pharmacology at Michigan State University, were used in these experiments. No outside breeding had entered these goat herds since 1954. The bucks were uniform in size. The majority of the goats had horns, only five of thirty being polled.

B. Thyroidectomy Procedures

1. Surgical procedures. Thyroidectomy was performed between two and four weeks after birth. Modifications of surgical procedures outlined by Reineke *et al.* (1941) in goats and George *et al.* (1957) in sheep were followed in removing the thyroid glands. Some difficulty was encountered in providing sufficiently deep anesthesia to permit manipulation of the thyroid gland during surgery.

Fasting the goats for 12 to 24 hours lessened complications due to regurgitation. It was found that sodium pentobarbital administered intravenously at the rate of one grain per three pounds body weight provided adequate anesthesia. Occasionally ether was needed to supplement the barbiturate anesthesia when tension was placed on the thyroid gland in separating it from its connective tissue attachments. Two percent procaine hydrochloride was injected locally in some goats. The extra fluid made it more difficult to locate the isthmus of the thyroid and occasional strands of accessory thyroid tissue.

Following recovery from surgery the goats were returned to their dams to nurse or weaned and fed milk until they were at least six weeks of age. All goats were weaned from their dams by two months of age.

A grain ration containing 38.5% corn, 38.5% oats, 19% soybean oil meal, 2% steamed bone meal, and 2% iodized salt was fed along with alfalfa-brome grass hay. The goats were fed ad libitum twice daily.

Some of the goats were allowed to nurse their dams while on pasture prior to weaning in 1957. A heavy helminth infestation resulted and therefore all goats in the 1958 experiment were kept inside after weaning.

In order to determine whether thyroidectomy was complete, radioactive iodine (I^{131}) was administered. A tracer dose of 30 microcuries (μc.) was injected subcutaneously. Forty-eight to 72 hours later, thyroid uptakes of the I^{131} were measured with a scintillation tube and a disintegration count rate meter. A number of the goats were found to have thyroid tissue present which went undetected at surgery. The disintegration rate detected in these goats when compared with that of the controls gave the approximate percent of thyroid tissue remaining.

2. Radioactive iodine I^{131} administration. Approximately 60 percent of the surgically thyroidectomized goats were found to have unremoved accessory thyroid tissue. Seven bucks were injected subcutaneously with eight millicuries of I^{131} each to destroy the remaining tissue. This procedure proved effective as determined by subsequent I^{131} uptake.

Repeat surgery was attempted in two goats using I^{131}, a scintillation tube, and count rate meter to determine the location of the accessory thyroid tissue. Because of the diffuse distribution of the tissue in one of these goats it was deemed inadvisable to use this procedure routinely to effect complete thyroidectomy.

C. Organization of Experimental Groups

In the spring of 1957 thirteen young male goats were available for the experiment and it was planned to thyroidectomize nine of these and leave four as controls. All nine of the thyroidectomized goats were to be allowed to develop symptoms of cretinism; then four of them were to be given thyroprotein to determine whether recovery could be effected. The other four were to remain cretinoid as long as they would live or until the experiment was terminated.

The original plan could not be followed since two of the goats died during surgery. Accessory thyroid tissue, undetected at surgery, remained in five of the seven experimental goats, as detected by radioactive iodine uptake measurements. One of the two completely thyroidectomized bucks was injured while still nursing its mother in pasture and had to be destroyed. The other developed such severe abdominal distension and dyspneic symptoms three months following thyroidectomy that supplemental thyroprotein was administered to keep him alive.

When it was learned that accessory thyroid tissue remained in the other surgically treated goats it was decided to destroy the remaining tissue in three of the five bucks with radioactive iodine I^{131}. Two were left partially thyroidectomized.

In 1958 the experiment was repeated and extended. It was planned to have four groups of four male goats each: (1) control group, (2) thyroidectomy plus immediate thyroid replacement therapy, (3) thyroidectomy plus delayed therapy after thyroid symptoms became marked, (4) prolonged athyroidism without treatment. Although extreme care was taken to remove all suspected accessory tissue only five of 13 goats operated proved to be completely thyroidectomized. Of the eight remaining, four were given thyroid destroying doses of I^{131}, two were reoperated to remove the remaining tissue, while two were left partially thyroidectomized. As indicated in Table 1, six groups of goats evolved as a result of the delay encountered in thyroidectomy at a uniform age.

TABLE 1. Showing Experimental Grouping of Goats in Various Thyroid States.

Group	Year 1957-58 No. goats	Year 1958-59 No. goats	Total	Treatment
A	4	4	8	Control group - no treatment.
B	2	2	4	Thyroidectomized 2 weeks to 1 month of age. Accessory thyroid tissue remained. Left as partially thyroidectomized.
C	3**	2	5	Thyroidectomized 2 weeks to 1 month of age. Accessory thyroid present. Allowed to go without further treatment until 6 months of age then rest of tissue removed surgically or by radiothyroidectomy.
D	-	2	2	Thyroidectomized 2 weeks to 1 month of age. One re-operated surgically. Both started on thyroprotein(1) as soon as athyroidism established (.04 gm. per 10 lb. body wt.).
E	1	3**	4	Thyroidectomized 2 weeks to 1 month. Allowed to show cretinoid symptoms then treated with .04 gm. per 10 lb. body wt. thyroprotein daily.
F	1*	4**	5	Thyroidectomized 2 weeks to 1 month. Remained cretinoid until death or killed at end of experiment.

*1 goat died during experiment.
**2 goats died during experiment.
(1) "Protamone" brand of iodinated casein having thyroid properties, kindly supplied by Cerophyl Laboratories, Kansas City, Mo.

D. Clinical Observations

Goats were observed daily for onset of symptoms of hypothyroidism. Notes regarding appetite, horn growth, hair coat, skin condition, edema, gait when walking, bloating, respiratory distress, aggressiveness, riding, scrotal development, and weakness were recorded. Photographs were made of goats representative of each group during and at the end of the experiment.

Weights were recorded weekly during the first three months, biweekly until eight or nine months old, then monthly. Blood was collected at irregular intervals during 1957-58 for detection of possible hematological changes. During 1958-59 monthly blood collections were made for hematological and biochemical study.

White blood cell total and differential counts were made. Circulating eosinophils were stained with .1 percent phloxine dissolved in 50 percent propylene glycol and counted in a Spiers-Levy eosinophil counting chamber. Averages of counts made in each end of the chamber were recorded. Hemoglobin was measured as oxyhemoglobin using a Bausch and Lomb "Spectronic 20" spectrophotometer. Blood sugar was determined by the method of Somogyi (1945) after precipitation of the blood proteins with a saturated solution of barium hydroxide and five percent zinc sulphate. Smears of fresh heparinized blood were made and stained with Wrights' stain for differential white blood cell counting.

Biochemical determinations of serum calcium, phosphorous, sodium, and potassium were made infrequently during the latter part of the 1958-59 experiment. A Coleman model 21 flame photometer coupled with a Coleman junior spectrophotometer was used to make the sodium, potassium and calcium determinations. The procedure used in these determinations was that outlined in the manual accompanying the instrument. Serum phosphorus was determined by the method of Fiske and Subbarrow (1925).

E. Replacement Therapy

To determine whether normal growth and reproductive function could be maintained in recently thyroidectomized young goats a thyroactive iodinated casein called "Protamone" was fed by capsule daily to two thyroprival goats. This material contains approximately one percent of thyroxine, and approximately 10 percent of the active principle is absorbed by the ruminant, according to Reineke (1946). On the basis of a thyroxine secretion rate of .4 mg. per 100 lbs. in immature goats reported by Flamboe and Reineke (1957, 1959) a dosage rate of .04 gm. iodinated protein per 10 lbs. body weight per day was determined. The dose was increased by .04 gm. per 10 lb. as body weight increased.

One goat in the 1957-58 group and two goats in the 1958-59 group were given iodinated protein after they had developed severe symptoms of cretinism. Two other thyroidectomized goats in the 1958-59 group were started on iodinated casein as soon as symptoms were noted and it was established that they were free of accessory thyroid tissue.

F. Testicular Biopsy

Testicular biopsies were performed on control and experimental goats at intervals during prepuberal and post-puberal development. The procedure followed was that described by Barker (1949) for use in bulls. In this technique the skin over the posterior aspect of the scrotum was cleaned, local anesthetic was injected subcutaneously, and a small incision through the skin made with a scalpel. A Vim-Silverman biopsy needle was used to obtain the specimen. Knudsen (1954) found that the dorsal surface of the testis lateral to the head of the epididymis has a minimum number of small blood vessels. This was the needle insertion site chosen for testicular biopsy in the goat. The outer needle and cannula were inserted parallel to the long axis through the tunica albuginea. The cannula was withdrawn and the divided cutting needle inserted through the outer trocar needle. Gentle pressure was exerted on both needles to penetrate the testicular parenchyma approximately two centimeters. Holding the inner needle in position the tip of the outer needle was pushed past the tip of the cutting needle. With a tubular piece of testicular tissue thus entrapped in the inner cutting needle, both were withdrawn from the testis. The excised tissue was immediately fixed in Bouin's fluid. No significant hemorrhage following biopsy was detected. A single suture was used to close the skin wound.

G. Semen Collection and Evaluation

Thyroidectomized goats showed little or no libido at the outset of the experiment. In view of this fact electroejaculation was selected as the means of obtaining semen for study. The electroejaculation apparatus and technique used were essentially those of Dzuik et al. (1954). In order to provide consistent stimulation for ejaculation the number of stimuli necessary to produce a satisfactory ejaculate in the control goats, was selected. Weak stimuli were applied intermittently until erection occurred and seminal fluids appeared. Stronger stimuli were then initiated to obtain ejaculation. A special stand was erected to provide suitable goat restraint and convenient collecting.

Volume of semen was determined by collecting it in a graduated 15 cc. conical centrifuge tube. A plastic test tube containing water at approximately 35°C. was fitted over the collecting test tube to protect the semen from cold shock while collecting.

Semen evaluation was carried out immediately after collection for total and progressive straight line motility of spermatozoa. Egg yolk-citrate, (one part egg yolk - one part 2.9 percent sodium citrate), heated whole homogenized

milk, and reconstituted skim milk were tried as diluters but these failed to promote survival of goat semen on storage. This prevented the study of the effect of thyroidectomy on the livability of stored goat semen. A stage incubator thermostatically set at 37°C. was placed on the microscope stage prior to microscopic evaluation. A drop of 2.9 percent sodium citrate was placed on one end of a clean warmed glass slide with a dropping pipette. To the citrate a drop of semen was added from a clean chromel wire loop. Sufficient dilution was obtained by this technique to evaluate total and progressive sperm motility. On the other end of the slide a drop of raw semen was placed to estimate the amount of swirling and eddying present. A rating of 0 to 5 was given to the raw semen according to the procedure outlined by Hermann and Madden (1947). A two-millimeter chromel wire loop was used to lift a drop of raw semen from the collection tube for making a smear of each sample. An eight-millimeter wire loopful of distilled water was placed on a clean glass slide prior to mixing the loopful of semen for smearing. The semen was smeared by touching the tip of a second glass slide to the drop of semen, and with the two slides at an angle of approximately 30° to each other, a thin film was formed. This procedure allowed better separation of spermatozoa for study of abnormal morphology. No adverse effects were noted as a result of using the water-diluted semen when these smears were compared with those of undiluted semen. Slides were air dried and a number one coverslip was mounted with clear fingernail polish over the smear.

Examinations for morphological abnormality were made with a phase contrast microscope. Two hundred spermatozoa were counted using the 95X oil immersion objective.

A green Kodak no. 58 filter gave better contrast to the cells than unfiltered illumination. Tabulation of abnormal forms was done with a hematological differential counter.

H. Libido Measurements

Mature does were brought into estrus by giving five mg. diethylstilbestrol daily or two to four mg. estradiol (Haver-Lockhart) on two successive days. The latter resulted in more certain and prompt appearance of estrus. These does were placed in a pen singly with each buck. In the early trials one half hour per buck was permitted for mounting to occur. However, it developed that if a buck did not mount the estrous doe during the first five minutes of contact he would not mount at all. Thereafter all contacts were for five minutes per buck. Notes were made as to sexual interest (e.g. masturbating, riding one another), occurring among bucks penned together also.

I. Post-mortem Examination

Gross post-mortem examinations were made of several normal young goats which either died during surgery or were killed to obtain normal tissues prior to starting the experiment. Goats dying during the experiment were examined as soon as they were found dead. All control and experimental goats were killed between 14 and 18 months of age for gross and histopathologic examination. These goats were killed by electricity. Skin, horns, joints, skeletal muscles, all visceral organs and endocrine glands were carefully scrutinized for gross pathological changes.

The testes, epididymides, seminal vesicles, bulbourethral glands, adrenal glands, thyroid glands, and pituitary glands were removed and weighed. A careful search for accessory thyroid tissue was made and, whenever found, its location and weight were recorded.

Photographs comparing representative tissues from experimental and control goats were taken.

J. Tissue Procedures

1. Fixatives. Ten percent formol-saline or buffered formalin was used as the general fixative. Preliminary observations in goats and bulls revealed that testicular tissue fixed in formalin showed considerable loss of the spermatogenic elements in the seminiferous tubules. Zenker's fluid and Bouin's fluid were used to fix blocks of testicular tissue in an effort to overcome this deficiency of formalin.

Blocks of tissue approximately five millimeters thick were cut from the testes, epididymides, seminal vesicles, and bulbourethral glands, and a block was placed in each of the three fixatives. Adrenal glands, thyroid glands and pituitary glands were fixed in formalin and Bouin's fluid. Pancreatic tissue was fixed in Bouin's fluid. Routinely a block of tissue from the pillar between the dorsal and ventral sacs of the rumen was fixed in Zenker's fixative as was a portion of the abomasal wall. Blocks of skin from the neck, back and flank region were fixed in Zenker's fluid and formalin. Other tissues routinely fixed in formalin included lung, kidney, the femoro-tibial joint, and the tibio-tarsal joint. Tissues for frozen sectioning were preserved in formalin.

2. Staining procedures. Paraffin sections were prepared by dehydrating fixed tissues in gradually increasing concentrations of alcohol, clearing in xylene, and impregnating with paraffin on an Autotechnicon.

Sections were cut at six microns and stained with Harris' hematoxylin and eosin according to procedures outlined in the Armed Forces Institute of Pathology Manual of Histologic and Special Staining Techniques (1957).[1]

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[1] Manual of Histologic and Special Staining Techniques. 1957. Armed Forces Institute of Pathology, Washington, D. C.

Zenker-fixed testicular tissue sections were stained with Heidenhain's modification of Mallory's aniline blue stain to detect collagen in the interstitial tissue. Pituitary gland sections fixed in formalin were stained by the periodic acid - Schiff (PAS) technic using orange G as a counter stain as outlined by Jubb and McEntee (1955b).

IV. RESULTS

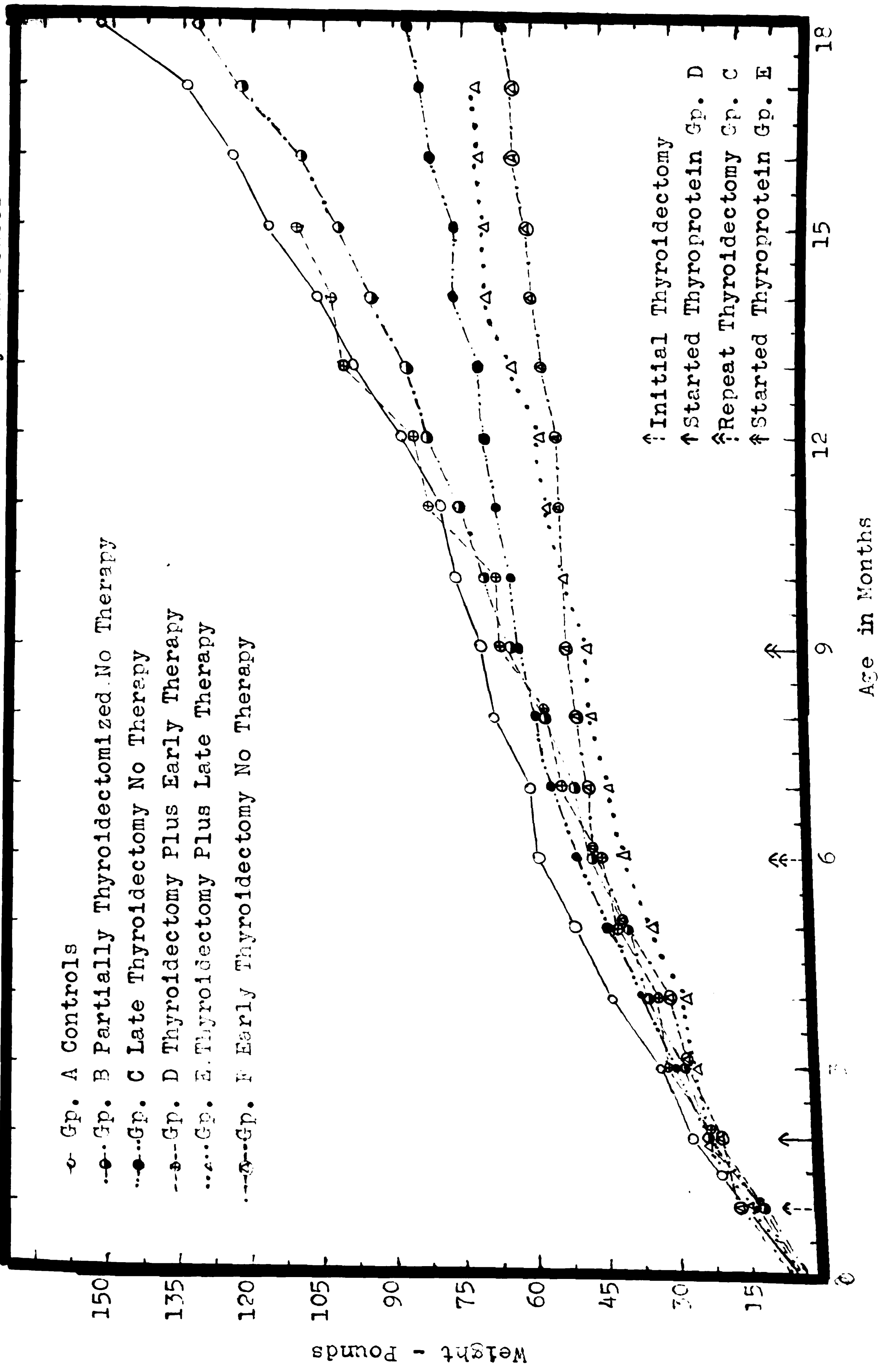
A. Clinical Observations

1. General. Completely thyroidectomized goats developed cretinoid symptoms starting about two weeks after surgery. First the hair coat began to have a harsh appearance. The subcutaneous tissues about the face and in the throat soon became puffy. The deficient groups E and F were reluctant to move about in the pen. During the development of cretinism there was no apparent lack of interest in food. As symptoms became more pronounced, particularly severe bloating, the completely thyroidectomized groups E and F ate very mincingly and were slow in coming to the feed rack. Goats in these two groups reclined a much greater part of the time than did those in the control group A.

2. Growth rates. Growth was extremely slow in thyroidectomized goats (group F). The growth curves for the 1958-59 groups of goats are depicted in Figure 1. The partially thyroidectomized goats in group C grew at rates comparable to the controls until complete thyroidectomy at about six months of age. Growth in this group then leveled off close to that of the group-F goats.

Table 2 shows the mean daily rates of gain for the combined groups for 1958 and 1959. Groups A, B, and D

Figure 1. Growth Weight Curves for Goats in Various Thyroid States



showed no significant differences in daily rate of gain throughout the experiment. Groups C, E, and F had significantly ($P < .01$) lower rates of gain after complete thyroidectomy than the controls. Treatment of group E with thyroprotein after prolonged cretinoid symptoms did not bring about a return of normal growth rate. A "Students" t test was used to test the significance of the differences between the mean rates of gain.

The relative size and appearance of representative goats from groups A, B, C, and F are shown in Figure 2. Goats representative of groups A, D, and F are shown in Figure 3.

TABLE 2. Rates of Gain in Control and Thyroidectomized Goats.

Group	No. of Goats	Rate of Gain lb. per da. Ave.		S.E.
A	8	.245	±	.0155
B	4	.226	±	.0187
C	4	(1) .242	±	.0115
		(2) .110**	±	.0291
D	2	.232	±	.0273
E	3	(3) .086**	±	.0057
		(4) .073*	±	.0592
F	3	.101**	±	.0157

(1) After partial thyroidectomy.
(2) After complete thyroidectomy.
(3) After complete thyroidectomy but before thyroid treatment.
(4) After complete thyroidectomy and thyroid treatment.
**$P < .01$
*$P < .05$

3. <u>Horn growth and skin appearance</u>. Horn growth was markedly stunted in the goats completely thyroidectomized before one month of age. Complete thyroidectomy after two months of age did not result in pronounced horn stunting although growth was less than in the controls. In the thyroprival goats the horns grew nearly straight up from the head while in the controls horns grew out in widely sweeping curves. Figure 4 shows the gross appearance of the horns of a goat in a prolonged thyroprival state. As mentioned previously some of the goats were polled and therefore a comparison of horn lengths in the various groups was impossible. The outer curvature of two representative control goats (nos. 135 and 137) measured 13 inches each while the horns from two representative completely thyroidectomized goats (nos. 144 and 158) measured 8.75 and 9.75 inches respectively. Iodinated protein supplementation after prolonged cretinism improved the texture of the outer horn lamina, but stimulated lengthening only slightly.

The skin of thyroidectomized goats underwent marked visible changes as the thyroid deficiency became more pronounced. One of the earliest symptoms of cretinism was a harsh dry appearing hair coat. Gradually alopecia developed until areas on the face and along the back were nearly void of hair. Marked "puffiness" about the face and neck accompanied the development of alopecia. Wrinkling of the skin became so pronounced that folds appeared on the neck of completely thyroidectomized goats. The skin about the face, flanks, and scrotum became hard and dry to the touch. Scurfing and peeling of keratinized epidermal layers occurred on the inner surfaces of the thighs, on the scrotum and in the axillary region.

The group-C late-thyroidectomized goats showed only harshness of hair coat after the thyroprival condition was established. Administering iodinated protein early in cretinism prevented skin changes. Treatment after pronounced alopecia occurred prevented further hair loss but did not stimulate complete replacement of hair.

4. Myxedema. Subcutaneous edema was absent in the control and partially thyroidectomized goats. Pronounced puffiness of the face and neck region suggesting edema was characteristic of the thyroidectomized goats in groups E and F. In group E, iodinated protein alleviated the edema within two weeks after starting replacement therapy. Group-C goats showed varying amounts of edema, principally about the face, although one (goat 158) showed marked wrinkling of the neck also. Group-D goats which received iodinated protein immediately following complete thyroidectomy did not develop edema.

5. Bloating. Between one and two months following complete thyroidectomy those goats in groups E and F became greatly bloated in addition to other cretinoid symptoms. This bloating caused a marked "pot belly" appearance and was accompanied by dyspnea. It was not typical of the

chronic bloat seen in cattle since it persisted continuously rather than appearing intermittently. Soon after administering iodinated protein the bloat symptoms disappeared. Temporary suspension of therapy was followed by recurrence of bloating.

6. Locomotor disturbances. Two to three months after complete thyroidectomy most of the goats were noted to be walking with a stiff-legged gait. Walking or running appeared to be painful. When they were led out of the pen for weighing or other purposes they balked and had to be dragged or carried. This reluctance was not noticed in the control or partially thyroidectomized groups. Extreme muscular weakness characterized by "knuckling" of the pasterns developed in one thyroidectomized goat (no. 169). Both the stiffness, which was noted consistently, and the muscular weakness in a single goat were alleviated by administering iodinated protein.

7. Dyspnea. A constant observation among the completely thyroidectomized goats was that of difficult breathing. Stertorous sounds were present in the trachea. Exertion and bloating aggravated the dyspneic symptoms. Auscultation revealed that ventral portions of the lungs in these goats were not receiving air. Though iodinated protein relieved the bloat in these hypothyroid goats, it did not bring about complete remission of difficult respiration.

8. Death prior to termination of experiment. In

the 1957-58 group of goats three died or were killed before termination of the experiment. One of these suffered unexplained damage to the testis and scrotum while nursing its dam in pasture. The other two died from the effects of prolonged hypothyroidism complicated by pneumonia. In the 1958-59 experiment one goat each from groups E (no. 681) and F (no. 159) died suddenly soon after they had developed pronounced hypothyroid symptoms. Cause of death in each case proved to be the lodging of a calculus in the urethra. The urinary bladder had ruptured in one of these goats (681).

9. Hematological and blood chemical findings. No significant differences were noted among the hematological values observed on monthly examination of blood from the control and experimental goats during 1958 and 1959. The average values for total hemoglobin, microhematocrit, total white blood cells, lymphocytes, neutrophils, monocytes, eosinophils, and basophils and circulating eosinophils are given in Table 3. From the table it appears that thyroidectomy results in a smaller number of circulating eosinophils. There were greater individual variations within groups than between the groups, however.

Aberrant erythrocyte morphology was present in all goats sampled at three months of age. Pointed cells with single elongated processes and poikilocytes were the most frequent abnormalities. Anisocytosis was also noted frequently. These abnormalities disappeared for the most part

TABLE 3. Average Hematological Values for Control and Thyroidectomized Goats.

Group	No. Goats	No. Determinations	Hemoglobin gm. per 100cc.	Microhematocrit %	White blood cells per cu. mm.	Lymphocytes %	Neutrophils %	Monocytes %	Eosinophils %	Basophils %	Circulating Eosin. per cu. mm.
A	4	50	12.3	32.4	9,023	70.4	28.0	.54	.59	.28	50.3
B	2	25	12.2	32.5	8,755	72.3	26.2	.93	.38	.28	62.5
C	2	25	12.5	33.4	6,603	71.4	25.9	.69	.92	.51	33.9
D	2	24	11.7	32.7	11,121	74.9	23.8	.58	.67	.29	82.7
E	2	25	12.3	32.9	8,186	70.2	28.8	.37	.33	.40	32.2
F	2	25	12.4	34.3	9,359	78.8	20.5	.21	.25	.12	22.1

by six months of age except in the completely thyroidectomized untreated group-F goats and in the late-treated group-E goats. Although the proportion of abnormal erythrocytes declined from 90 percent to approximately 10 percent by six months of age, five to 10 percent of pointed cells persisted intermittently in three of four goats in groups E and F. The significance of these observations was not determined.

Biochemical determinations for blood sugar were made monthly from three months of age until the end of the 1958-59 experiment. Serum calcium, phosphorus, sodium and potassium were determined during the latter part of the 1958-59 experiment. In part, the latter determinations were made in an effort to establish whether imbalances existed which could explain the occurrence of the urethral calculi in two thyroidectomized goats which died earlier in the experiment. Table 4 includes the values for blood sugar, serum calcium, phosphorus, sodium and potassium for the control and experimental groups in the 1958-59 goats. Very little difference existed among the groups with respect to these constituents, with the possible exception of serum calcium and phosphorus. Here a narrowing Ca to P ratio for the thyroidectomized goats was observed. The trend toward lower blood sugar values noted in the thyroidectomized groups was offset by marked variation within individuals.

TABLE 4. Blood Sugar, Serum Calcium, Phosphorus, Potassium and Sodium in Control, Partially Thyroidectomized and Thyroidectomized Male Goats.

Group	Goats	Blood Sugar		Serum Ca		Serum P		Ca/P	Serum K		Serum Na
	No.	Det'ns	Mg% Av.	Det'ns	mg% Av.	Det'ns	mg% Av.	ratio /1	Det'ns	milli-equival. per liter	milli-equival. per liter
A	4	42	50.3	15	11.2	7	7.6	1.4	11	4.7	149
B	2	21	47.8	7	11.2	3	7.9	1.4	5	5.5	149
C	2	21	46.2	8	10.6	4	8.7	1.2	6	5.7	156
D	2	20	46.0	7	10.2	3	11.4	.92	5	5.7	150
E	2	21	43.4	8	10.2	4	10.5	.97	6	5.3	151
F	2	19	46.3	7	10.1	4	9.9	1.02	6	5.4	150

B. Semen Data

1. Volume and motility. Semen samples collected by electrical ejaculation were examined for volume and percent of sperm which were motile immediately following collection. The observations made on 163 ejaculates are presented in Table 5. The average volume and range in volume are given for each group of goats as outlined under procedures. The volume differences between the control group A and thyroidectomized group-F goats were not significant. Group E produced an average volume similar to the group-F goats. Only one goat appeared in group E because the second goat in this group failed to produce sperm. Clear seminal fluid was obtained each time that collection was attempted. Volume varied greatly between ejaculates among goats, and within individuals. This variability appears similar to that reported for ejaculate volume in bulls (Dziuk, 1954) when semen was collected by electrical ejaculation.

The average sperm motility for group-A goats was considerably higher than for the group-F thyroidectomized goats, 57.8 and 40.3 percent respectively. Treatment of cretinoid male goats in group E with iodinated casein failed to increase the average motility above that of the untreated group-F goats. Late thyroidectomy decreased the number of motile sperm 10.1 percent below that of the controls.

2. Concentration and total motile spermatozoa. Table 5 also shows the mean sperm concentration in billions

TABLE 5. Summary of Semen Volume, Percent Motile Sperm, Sperm Concentration, and Total Motile Sperm per Ejaculate in Intact and Partially and Completely Thyroidectomized Goats.

Group	No. Goats	No. Ejaculates	Volume cc. Av. (Range)	Percent of Motile Sperm Av. (Range)	Concentration Billions Per cc. Av. (Range)	Total Motile Sperm Per Ejac. Billions Av. ± S.E.
A	8	61	1.42	57.8	2.63	1.71 ± .26
			(.2 - 4.5)	(10 - 85)	(.26 - 5.4)	
B	4	31	1.59	53.2	2.24	2.15 ± .33
			(.3 - 3.5)	(5 - 85)	(.48 - 4.8)	
C	4	28	1.30	47.7	2.31	1.40 ± .29
			(.4 - 2.9)	(15 - 70)	(.32 - 5.9)	
D	1	8	1.8	52.5	2.02	2.39 ± .84
			(.5 - 3.5)	(35 - 75)	(.28 - 4.02)	
E	3	25	1.1	40.2	1.63	.89** ± .21
			(.3 - 3.0)	(10 - 75)	(.01 - 3.28)	
F	3	18	1.1	40.3	2.11	.59** ± .12
			(.1 - 2.7)	(0 - 65)	(.63 - 5.53)	

**P < .01

per cubic centimeter and total number of motile sperm per ejaculate in billions. No statistically significant differences in concentration existed among any of the groups of goats although the lowest average concentration occurred in group-E cretinoid goats which received belated thyroxine treatment. A "Students" _t_ test for significance revealed that the .89 billion sperm per ejaculate for group E and the .59 billion sperm for group F were significantly lower ($P < .01$) than the average number of motile sperm in group-A goats. Differences for the other groups were non-significantly different from the control.

An analysis of variance among the groups on the number of total motile sperm cells per ejaculate is shown in Table 6.

TABLE 6. A Summary of the Analysis of Variance for Total Motile Sperm per Ejaculate in Control and Thyroidectomized Goats.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio
Groups of goats	4	42.02	10.51	3.46 ($.05 > P > .01$)
Goats within groups	17	51.73	3.04	1.72 ($P \cong .05$)
Ejaculates within goats	141	250.05	1.77	

Group D is not included in this analysis since only one goat in this group produced semen and no group variance occurred. The "F" ratio of 3.46 among all groups of goats lies between the one and five percent level of probability according to Snedecor's (1934) tables of "F" ratios. That considerable variation in total sperm per ejaculate existed, is evidenced by the 1.72 "F" value for goats within groups. This value was significant at the five percent level of probability.

3. Abnormal spermatozoa were counted for each ejaculate collected. The summary of the percent of abnormal sperm cells is presented in Table 7. A significantly greater average number of morphologically abnormal sperm cells were found in groups C, E, and F goats than in group-A goats.

TABLE 7. Summary of Percent Abnormal Spermatozoa Produced by Control and Thyroidectomized Goats.

Group	Goats	Ejaculates	Abnormal Spermatozoa %	S.E. %
A	7	60	16.0	±1.2
B	4	30	19.1	±2.7
C	4	27	23.9*	±3.0
D	1	6	21.9	±5.6
E	3	23	22.5*	±2.8
F	3	18	22.6*	±2.9

*P =< .05
S.E. = Standard Error

A protoplasmic extrusion frequently projected out from the galea capitus of the sperm in smears examined from both the control and thyroidectomized goats. This did not appear to be associated with immaturity of the sperm cells since the remaining portion of the head, midpiece, and tail usually showed no abnormality when the droplet was present.

Ejaculates from the completely thyroidectomized goats showed damaged midpieces, with the head separated from the tail, more frequently than did ejaculates from the controls. Bent and tightly coiled tails were the other frequently seen types of abnormality. Only a small percentage of ejaculates showed protoplasmic droplets along the midpiece or tail section. The latter abnormalities were about equally distributed among the control and thyroidectomized groups.

C. Libido Measurements

Sexual desire varied considerably among the control and partially thyroidectomized goats. The results of the libido trials are shown in Table 8. Two of the control goats (174 and 689) failed to mount an estrous doe on repeated opportunities. They seemed to fear the presence of the doe in the pen. On the other hand, placing these bucks back in the pen with other bucks often resulted in immediate attempts to mount them. Relatively normal libido was exhibited by group-B males and by two males in group C. The latter showed sexual interest even after exhibiting early myxedema and rough hair coat.

The two bucks to which thyroprotein was given soon after thyroidectomy failed to show active sexual interest in the estrous does throughout the experiment. They did, however, attempt to mount other bucks penned with them regularly and frequently were noted to be masturbating.

TABLE 8. Summary of Libido Trials Among Control and Thyroidectomized Goats.

Group	Goats	Trials	Failure to Mount
A	7	41	21
B	3	22	6
C	3	17	5
D	2	15	15
E	3	12	9
F	3	21	20

One of the three goats (173), thyroidectomized, then allowed to develop pronounced symptoms of hypothyroidism, and then treated with thyroprotein, showed normal sexual interest. The other two (169 and 144) would not mount an estrous doe but were noted to obtain erection in her presence. None of these bucks showed any sexual interest prior to the thyroid replacement therapy.

None of the thyroidectomized bucks showed sexual interest during the libido trials except on one occasion when goat 168 mounted but did not obtain erection.

It appears that partial thyroidectomy does not essentially affect the ability of male goats to exhibit normal sexual desire. On the other hand, replacement therapy with thyroprotein in completely thyroidectomized goats did not effect resumption of normal libido. Either the level of therapy was not optimum for these goats or variations in response to the stimulus of an estrous doe did not result in exhibition of normal sexual desire. The latter seems possible since two of the control goats showed similar behavior.

No measurable differences in libido occurred among the various times of the year when trials were made.

D. Post-mortem Findings

1. Gross pathology

a. Reproductive organs. Immediately following death and examination for external pathological changes the testes were removed from the scrotum. Each was checked for the presence of possible adhesions which might have resulted from earlier testicular biopsy. As shown in Table 9, 10 of 24 goats which had been biopsied showed minor adhesions between the parietal and visceral surfaces of the tunica vaginalis surrounding the testis.

In three of the goats, (99, 166, 756) enlarged epididymides were observed. Upon incision, a caseous core was found in the center of each enlargement. Goat 756 had not produced semen during the experiment. Although the semen passage from the testis might have been impaired by the epididymal lesion noted in goat 756, no anatomical abnormality of the other testis which should have impaired sperm movement could be found on gross examination.

The point of entry of the biopsy needle was identified in 11 of 25 goats by calcified streaks along the path

TABLE 9. Tabulation of Occurrence of Testicular Adhesions, Testis Calcification, Accessory Thyroid Tissue, and Joint Lesions in Control and Thyroidectomized Goats on Gross Post-mortem Examination.

Group	Goat	Testicular Adhesions	Calcification in Testis	Accessory Thyroid Tissue	Tibio-Tarsal Joint Erosions
A	99	+	+	+	-
	886	+	+	-	-
	135	+	-	-	-
	163	-	-	-	-
	166	+	+(2)	-	-
	174	-(1)	-(1)	+	-
	689	-	-	-	-
B	778	-	-	+1.5gm.	-
	137	-	-	+ .7gm.	+(3)
	164	-	+	+2.0gm.	-
	165	+	-	+1.4gm.	-
C	158	-	-	-	+(4)
	899	-	+	-	+(4)
	161	-	-	+.1gm.	+(3)
	167	-	-	-	-
D	149	+	+	+.1gm.	+(3)
	756	-	+(2)	-	-
E	144	+	+	-	+(6)
	169	-	+	-	+(3)
	173	+	+	-	-

TABLE 9 (Continued)

Group	Goat	Testicular Adhesions	Calcification in Testis	Accessory Thyroid Tissue	Tibio-Tarsal Joint Erosions
F	132*	+	-	-	+(4)
	168	-	-	-	+
	613	+	+	+.3gm.(5)	+(3)
	681*	-(1)	-(1)	-	+(4)
	159*	-(1)	-(1)	-	+(4)
	758*	-(1)	-(1)	-	+

*Died before end of experiment.
(1)No biopsy
(2)Granuloma present
(3)Slight
(4)Marked
(5)Non-functional
(6)New cartilage

of the biopsy. These streaks extended into the testicular parenchyma for a distance of one or two cm. Small flecks of calcium were noted occasionally scattered through the testis. Grossly no changes in the tissue adjacent to the calcified areas was evident. Calcification did not occur more frequently in one experimental group than another, with the exception that all group-D goats showed calcified deposits.

Testicular weights for the various groups of goats are presented in Table 10. The accessory glands were not weighed in all goats completing the experiment in 1958. When it was discovered that some bulbourethral glands exhibited cystic changes it was decided to weigh all the accessory gland structures in the remainder of the goats.

TABLE 10. Mean Weights of Reproductive Organs in Control, Partially Thyroidectomized and Thyroidectomized Goats.

Goats Group	No.	Mean Body Wt. Kg.	Testis gm.	Eipididymis (1) gm.	Bulbo-urethral Glands (1) gm.	Seminal Vesicles (1) gm.
A	7	61.7	265.7	44.6	3.75	15.98
B	4	55.0	264.6	44.7	3.35	8.00
C	3	39.7	190.3	41.1	3.55	6.80
D	2	50.1	178.7	48.6	2.45	8.30
E	3	32.2	153.9	32.2	2.65	9.45
F	3	26.7	112.8	31.1	2.55	8.10

(1) Weights represent four goats in "A" group, two goats in each of the remaining groups.

Although the mean testicular weight for group-A goats (265.7 gm.) was much greater than in the athyroid group-F goats (112.8 gm.), the significance of the difference disappears when these weights are correlated with body weight. Table 11 contains the mean ratios of tissue weights to body weight for the various experimental and control groups.

The ratio of epididymis, bulbourethral gland, and seminal vesicle weight to body weight was non-significantly higher for goats in group-F than those in group A.

Cystic bulbourethral glands were seen in three thyroidectomized and two control goats. The center of the gland in each case was filled with gelatinous fluid. In one control goat (163) concretions approximately two centimeters in diameter were seen within the fluid.

TABLE 11. Ratio of Mean Reproductive Organ Weight to Body Weight in Control and Thyroidectomized Goats.

Goats Group	No.	Testis $X10^{-4}:1$	Epididymis (1) $X10^{-4}:1$	Bulbourethral Gland (1) $X10^{-4}:1$	Seminal Vesicles (1) $X10^{-4}:1$
A	7	43.1	6.7	.57	2.41
B	4	48.1	7.2	.56	1.29
C	3	47.9	9.3	.81	1.55
D	2	35.6	10.6	.69	1.66
E	3	47.8	10.8	.88	2.68
F	3	42.2	10.6	.87	3.23

(1) Weights represent four goats in group-A, two goats in each of the remaining groups.

No gross pathological changes were seen in the seminal vesicles, ampullae or vas deferentia of the control or thyroidectomized goats.

b. <u>Skin and subcutaneous tissue</u>. In the completely thyroidectomized goats which were unsupplemented with iodinated protein the skin about the face and neck was consistently wrinkled, dry, scaly, and showed marked alopecia. Peeling of the outer keratinized layers was observed around the scrotum and inner surface of the thighs. The skin did not appear greatly thickened nor crusted as is characteristic of hyperkeratosis and parakeratosis. A comparison of the gross appearance from a control and thyroidectomized goat is seen in Figure 5. Group-C goats in which thyroidectomy was completed between six and eight months of age did not show the marked alopecia of groups E and F. Group-E goats which had been fed iodinated protein after cretinism developed showed little scaling and scurfing but did have marked alopecia. In groups B and D, partially thyroidectomised and thyroprotein supplemented, respectively, the skin appeared, grossly, identical to that of the controls.

In the subcutaneous tissue of thyroidectomized goats in groups C and F varying amounts of a mucoid type of edema were seen. This myxedematous condition was most pronounced in the tissue beneath the skin of the neck and in the axillary and inguinal regions.

c. <u>Accessory thyroid tissues</u>. Careful examination for tissue containing thyroid follicles was made at autopsy. In Table 12 there is a summary of the findings as to the relative amount of accessory thyroid tissue in control and incompletely thyroidectomized goats. From 7.1 to 33 percent of the injected dose was detected by counting the I^{131} present in the thyroid region of control goats. Surgically thyroidectomized goats had from 0 to 16 percent of the injected dose present in the thyroid or neck region. All goats showing .5 percent or less uptake of injected I^{131} proceeded to show cretinoid symptoms without further treatment. On the other hand when as little as 1.7 percent of the injected I^{131} was detected in the thyroid region the goats gained normally and required treatment with heavy doses of I^{131} or repeat surgery to destroy or remove the accessory tissue. Ten of nineteen goats showed accessory tissue causing .5 percent or more uptake of the injected I^{131}. Results following repeat surgery or a thyroid destroying dose of I^{131} are presented in Table 13. Although some tissue remained after repeat thyroidectomy it was insufficient to prevent development of cretinism or myxedema. Similarly, the thyroid destroying dose of I^{131} caused regression of any remaining thyroid tissue.

Accessory tissue was found at autopsy in one control goat, four partially thyroidectomized and three supposedly completely thyroidectomized goats. One-tenth-gram amounts of tissue were present in group-C goat 161 and group-D goat 149. Even though these goats underwent repeat surgical

TABLE 12. Showing Comparison of I^{131} Counts per Minute in Control, Partially Thyroidectomized and Completely Thyroidectomized Goats.

Goat No.	Group	Net C.P.M.	Percent of Injected Dose	Percent Control Count	Location
847	A	11,320	7.1	-	Sub-Laryngeal
886	A	12,690	8.4	-	" "
135	A	57,970	37.6	-	" "
163	A	23,240	18.3	-	" "
166	A	17,180	13.5	-	" "
174	A	21,080	16.6	-	" "
689	A	43,380	33.3	-	" "
778	B	- 400 *	0		Thoracic Inlet
137	B	6,200	4.9	23.0	Lower cervical
164	B	20,280	16.0	77.0	" "
165	B	3,580	3.0	14.4	Laryngeal
158	C	3,300	2.1	12.1	"
899	C	2,920	1.9	10.7	"
161	C	13,400	10.7	51.2	Thoracic Inlet
167	C	2,200	1.7	8.8	Laryngeal
149	D	4,640	3.7	18.0	Mid-cervical
756	D	300	.2	1.1	Lower cervical
144	E	- 540 *	0	0	-
169	E	-1,180 *	0	0	-
173	E	10,180	8.1	38.8	Laryngeal and lower cervical

TABLE 12 (Continued)

Goat No.	Group	Net C.P.M.	Percent of Injected Dose	Percent Control Count	Location
132	F	890	.5	3.3	Intermandibular
168	F	500	.4	1.9	Laryngeal
613	F	7,260	5.8	27.8	Laryngeal and lower cervical
159	F	- 200*	0	0	-
681	F	-2,080*	0	0	-
758	F	-1,800*	0	0	-

*Indicates thyroid count less than body background count.

thyroidectomy for removal of accessory tissue, small fragments still remained. One group-F goat (613) had a piece of thyroid tissue still remaining in the ventral neck region after radioactive iodine destruction. The cellular appearance of this will be discussed under histopathological results. Figure 6 depicts the location of an accessory thyroid gland found on autopsy.

TABLE 13. Radio-active Iodine (I^{131}) in Thyroid Region Following Surgery or I^{131} Thyroid Gland Destruction.

Goat no.	Net Counts per minute	Percent Injected Dose	Outcome
158	360	0	Cretinoid
899	no count		Cretinoid
167	2250	.3	Myxedema
161	4650	.8	Myxedema
149	90	-	Treated
173	790	.1	Cretinoid
132	no count		Cretinoid
613	1550	.3	Cretinoid

d. Lungs. Some atelectasis was observed in the thyroidectomized goats. This was notably present in the ventral portions of the diaphragmatic lobes. Several of the lungs observed in the 1958 group of goats had small nodules palpable on the dorso-lateral surface of the diaphragmatic lobes. No gross changes were evident in the trachea and larynx which might have suggested the cause of the marked stertorous breathing noted in the thyroidectomized goats. One of the goats dying before the end of the experiment (goat 159, group F) had extensive pneumonia and pleural adhesions.

e. Urogenital system. Three of the thyroidectomized goats had calculi in the renal pelvis and urinary bladder. Two of these (681 and 758) died as a result of calculi lodging in the urethra. The hilus of the kidney in these two goats was moderately distended. The calculi removed from the kidney hilus of goat 173 in group E were soluble in dilute hydrochloric acid but insoluble in acetone.

f. Rumen and abomasum. Since bloating had been pronounced in group-F thyroidectomized goats the rumen was examined carefully for possible differences from the controls. Although the papillae of the rumen wall were smaller and the wall thinner than in the controls no gross pathological changes were seen.

Small grayish white nodules were present on the surface of the abomasal wall in several of the group-F goats. These may have been the site of entry of parasitic larvae although none were seen in the abomasal contents.

g. Locomotor system. Joint lesions, manifested as shallow erosions of the articular cartilages, were found in the athyroid untreated goats but not in the control nor partially thyroidectomized goats. A tabulation of their occurrence is included in Table nine. In one goat (159) hemorrhage was present in the joint cavity. Erosion of the medial condyle of the femur in the stifle joint was noted in one goat (899). Lesions were seen in the joints of the anterior limb in only one thyroidectomized goat. The erosions were prone to occur at points where the cartilaginous surfaces suffered the most contact.

In goats which were made cretinoid and then treated with iodinated protein some healing of the eroded areas was noted. A thin layer of cartilage appeared to overlie the erosions.

Grayish white streaks were found in the muscles of the posterior limb of athyroid but treated goat 169. Changes were most pronounced in the gastrocnemius muscle. Muscular changes of this nature were not detected in any other goats.

h. Circulatory changes. Two thyroidectomized goats showed marked roughening of the aortal walls just dorsal to the heart. These roughened areas did not appear to be calcified on gross examination. In goat 169 from athyroid group E the heart muscle was extremely pale.

i. Endocrine glands. Weights of the adrenal, thyroid, and pituitary glands are presented in Table 14 along with the ratios of average glandular weights to the average body weights for each experimental group. The average weight of the pituitary glands of goats in group F was .31 gm. or less than half that of the controls (.69 gm.). The ratio of gland weight to body weight was not significantly different, however. Differences at the five percent level of significance existed between the ratios in control group A and groups B, C, and D.

TABLE 14. Mean Weights of Endocrine Glands and Ratio of Endocrine Gland Weight to Body Weight in Control and Thyroidectomized Goats.

Goats Group	No.	Body Kg	Pituitary gm.	Adrenal gm.	Thyroid gm.	Pituitary Gl:Body $X10^{-4}$:1	Adrenal Gl:Body $X10^{-4}$:1
A	7	61.7	.69(1)	3.08	4.2	.110(1)	.501
B	4	55.0	.97	2.68	1.4	.170*	.498
C	3	39.7	.83	1.70	.03	.208*	.430
D	2	50.1	.85	1.6	--	.171*	.522
E	3	32.2	.38	2.1	--	.120	.644
F	3	26.7	.31	2.1	--	.129	.927

(1) Average of six goats.
* Significant at 5% level.

Adrenal gland weight to body weight ratios were not significantly different among groups.

No gross abnormalities in either the adrenal or pituitary gland were seen. The pituitary fossa appeared slightly compressed vertically in the thyroidectomized goats in groups E and F.

2. Histopathology

a. Reproductive system.

(1). Testis. The principal changes detected in the reproductive system occurred in the testis of thyroidectomized goats. Cellular changes occurred in both the intra- and inter-tubular tissues.

To some extent the cellular picture was affected by the fixative used to preserve the testis. Figures 8, 9 and 10 show the differences encountered. Formalin fixing preserved the interstitial structures better than Zenker's fluid or Bouin's fluid but did not preserve the cells within seminiferous tubules as well. Bouin's fluid caused more separation of tubules from the intertubular reticulum but preserved the cells within the seminiferous tubules well. Zenker's fluid gave results intermediate between the other two and permitted more intense staining of nuclei with hematoxylin.

In hematoxylin and eosin stained sections the number of seminiferous tubules showing active spermatogenesis with developing spermatozoa was smaller in athyroid than in the control goats. Most of the control goats showed 60 percent or more of the tubules to contain spermatozoa. In the athyroid untreated goats less than 50 percent of the tubules contained developing spermatozoa.

A pronounced hyalinizing of the secondary spermatocytes and spermatids occurred in the thyroidectomized goats.

Compared to the controls the athyroid goats had a greater number of spermatogonial cells and primary spermatocytes along the basement membrane which contained vacuolated cytoplasm and pyknotic nuclei. See Figures 11 and 12.

When formalin-fixed, testicular tissue was stained with Sudan IV there was a much greater concentration of sudanophilic material in the seminiferous tubules of the athyroid group. Large sudanophilic droplets were found along the basement membrane as well as in the lumen. The sudanophilic substance present in the tubules of control goats appeared as finely dispersed droplets while in the thyroidectomized goats it appeared more coarsely globular. Sudan IV-stained sections of testes from a control and thyroidectomized goat are shown in Figures 13 and 14.

Multinucleate cells were more numerous in the lumina of the seminiferous tubules of athyroid goats than in the control, (Figure 15). Goats in group E, treated with iodinated protein after cretinism had developed, showed testicular changes similar to the completely athyroid goats.

Histologically, necrosis and calcification occurred along the path of the biopsy needle in testes which had been biopsied. Adjacent to the area of calcification there was complete absence of spermatocytes, spermatids and developing spermatozoa. The epithelium of the seminiferous tubules had become cuboidal. The width of the area varied considerably, ranging from approximately 100 microns to three millimeters.

The Leydig cells in control goats contained large oval nuclei surrounded by abundant cytoplasm which contained finely dispersed granules. In thyroidectomized goats the Leydig cells were scarcer and frequently had pyknotic nuclei and vacuolated, scanty cytoplasm (Figures 16 and 17).

Heidenhain's modification of Mallory's aniline blue stain of testicular tissue showed a slight increase in interstitial connective tissue in the thyroidectomized goats. This stain also served well in identifying the Leydig cells in the interstitial tissue. Sections stained with aniline blue are shown in Figures 18 and 19. Sudan IV staining of the interstitial tissue revealed a faint reddish orange tinge in the cytoplasm of the Leydig cells which was absent in most of these cells in the thyroidectomized goats.

Sertoli cell changes, if any, could not be adequately assessed. Developing spermatozoa in athyroid goats migrated toward the Sertoli cells much as they did in the controls. The increased number of sudanophilic cells noted in the athyroid goats suggests either some change in activity of the cells of Sertoli type or that fatty changes were occurring in the spermatogonia.

Those goats in groups B and D had testicular histology similar to that found in the control goats. The degenerative changes in group-C goats were not as pronounced as those in groups E and F.

(2). <u>Testicular biopsies</u>. Testicular biopsies were made at approximately three, six, and twelve months of age in goats from all groups. Not more than two biopsies per goat were made. Sufficient tissue for histologic examination was obtained in 21 of 26 biopsy attempts.

At three months of age neither the athyroid nor the control goats showed spermatogenesis. A few of the spermatogonial cells had begun to divide in both groups. There were greater numbers of Leydig cells showing prominent cytoplasmic granules (Figure 20) in the control than in the athyroid goats, (Figure 21).

At six months of age testicular biopsy specimens from goats in the control group-A, showed large numbers of primary and secondary spermatocytes and spermatids which had migrated to the Sertoli cells (Figure 22). Testicular tissue was not obtained in two biopsy specimens from athyroid goats at six months of age. However, a biopsy specimen obtained from a Group-F goat at nine months showed only limited spermatogenesis. Vacuolation of spermatocytes and absence of granulation in the cytoplasm of the Leydig cells was also noted (Figure 23). Leydig cell activity and spermatogenesis continued to be more prominent in biopsy specimens taken at twelve months of age from the control goats.

(3). Epididymis. There was decreased epithelial height, more hyalinization, fewer secretory granules, fewer stereo-cilia and fewer sperm in the proximal portions of the epididymis in the thyroidectomized goats than in the controls. Goats in groups A, B, and D showed tall columnar epithelium with numerous secretory granules at the tips of the cells in the head of the epididymis. The tail portion of the epididymis was distended with spermatozoa. Treatment of cretinoid goats with iodinated protein did not improve this condition. Sections of epididymides in control and thyroidectomized goats are depicted in Figures 24 and 25. A sperm granuloma resulting from biopsy trauma of the epididymis is seen in Figure 26.

(4). Accessory glands. The seminal vesicles did not show marked histologic variation between the control and thyroidectomized goats. There was less secretory fluid in the lumina of the seminal vesicular glands of athyroid goats.

Bulbourethral glands did not show consistent histologic variations between the control and experimental groups. What appeared to be cystic glands on gross examination was merely distention of the central ducts with secretion.

b. Endocrine glands other than gonads

(1). Thyroid gland. The thyroid glands removed from the young experimental goats showed follicles of moderate size which contained colloid. Two thyroid accessory glands removed from goats previously partially thyroidectomized showed marked hypertrophy of the acinar epithelium and absence of colloid (Figure 27).

At autopsy the goats having accessory thyroid glands showed acinar epithelial hypertrophy roughly inversely proportional to the amount of tissue present. The epithelial cells were irregularly tall columnar cells varying from 15 to 30 microns in height. The epithelium projected to the center of many of the acini where little or no colloid existed. Very little hyperplasia was noted in the accessory thyroid tissues.

Goat 613 from group F which had been radiothyroidectomized had degenerative thyroid tissue interspersed in a lymph nodule. The epithelial cells were atrophic and most of the follicles were filled with erythrocytes. No colloid material was seen within the acini.

(2) Parathyroid glands. Although careful examination was made for the parathyroid glands they were recovered in only two athyroid goats at autopsy. No histopathologic changes were detected in these tissues.

(3). Pancreas. Islet cell nuclei showed more early pkynosis in the thyroidectomized than in the control goats. This observation is of doubtful significance in relation to other more profound changes occurring in the athyroid goats.

(4). Pituitary. The anatomical structure of the goat pituitary is similar to that described for the bovine (Jubb and McEntee, 1955). Basophilic cells comprise most of the cells seen in the medullary portion of the anterior pituitary gland. In hematoxylin-eosin stained sections the basophilic cytoplasm of cells in the central area take on a faintly bluish hue. Acidophilic cells are found largely at the periphery of the pars distalis. Occasional acidophils occupy the central zone. The beta basophilic cells lie chiefly adjacent to the relatively large sinusoids in the central pars distalis. However, they are also scattered among the acidophils in the peripheral portion. Some delta basophilic cells are found in the central portion of the pars distalis but most are found close to the vascular net among the acidophils. Both the acidophils and basophils tended to be arranged in clumps which are closely invested with capillaries and small vessels.

Sections made from Bouin-fixed portions of the glands preserved the architecture well but tended to give poorer differentiation of the acidophils from the basophils with hematoxylin and eosin stain. Bouin-fixed sections did not take the PAS stain sufficiently well to differentiate cell types. Periodic acid-Schiff-positive material stained a bright rose purple in the formalin-fixed sections.

Maximum numbers of PAS-stained cells occurred in the control goats. Large polyhedral cells which correlate with beta basophilic cells described by Jubb and McEntee (1955) occupied the anterior portion of the pars distalis nearest the pituitary stalk. In the completely thyroidectomized goats these cells are degranulated and largely vacuolated, leaving only a spider web-like cytoplasm and sometimes a poorly stained nucleus. Occasional beta cells contained some PAS-positive material which was only faintly granulated. In the control goats, PAS-positive beta and delta cells showed granules ranging from faint through marked granule formation to intensely stained cytoplasm in which the granules were obliterated.

Table 15 shows, subjectively scored, the number of vacuolated beta basophils, delta basophils and acidopils This scoring was made on PAS-stained sections. Not all of the pituitary glands were scored because formalin-fixed sections of the median portion of the gland were not available.

Examination of the table reveals that vacuolation and degranulation is much more pronounced in the thyroidectomized than in the control or partially thyroidectomized goats.

More vacuolation and degranulation was apparent in the delta basophilic cells in the thyroid-deficient goats than in the controls. In the thyroidectomized goats which were treated with iodinated protein, granulation and vacuolation were variable. Figures 28 and 29 compare the histologic structure of the pituitary in a control and thyroidectomized goat.

TABLE 15. Summary of Vacuolation, Cell Degranulation, and Type of Granulation Present in Pituitary Glands in Various Thyroid States.

Group	Goat	Vacuolation[1]			Approx. no. Cells Degranulated[2]			Type of Granulation[3]		
		B	D	A	B	D	A	B	D	A
A	886	0	0	0	1	0	0	4	3	3
	174	1	0	0	1	1	1	3	3	3
	163	2	1	1	2	2	1	2	2	2
	689	0	1	1	1	2	1	3	2	3
	166	1	0	0	1	2	3	3	2	2
B	778	0	0	0	1	0	0	4	3	3
	137	2	1	1	3	1	1	2	3	4
C	158	3	2	1	4	3	1	0	1	3
	167	3	2	1	3	2	1	1	3	3
D	756	3	2	1	3	2	1	1	2	3
	681	2	1	0	3	2	2	2	2	2
E	144	2	1	1	2	3	2	2	2	3
	169	3	0	0	3	1	1	1	3	3
F	758	3	2	1	3	3	2	1	1	2
	613	3	2	2	3	3	2	1	1	2

[1]Vacuolation: 3 - marked; 2 - moderate; 1 - slight; 0 - None.

[2]Proportion of cells degranulated: 4 - 100%; 3 - 67-100% 2 - 34-66%; 1 - 0-33%.

[3]Type of granulation: 4:intense stain, granules not visible; 3:Pronounced stain, granules prominent; 2:moderate stain, granules faint; 1:faint stain, no granules

B=Beta Basophils; D=Delta Basophils; A=Acidophils

(5). <u>Adrenal glands</u>. Histologic examination of the adrenal glands revealed significant variations between the euthyroid and completely thyroidectomized goats. Hematoxylin and eosin stained sections revealed a marked difference in the relative thickness of the cortical zone between athyroid and control goats. The average cortex depth measured 2.4 mm. in three group-A goats and 1.4 mm. in four goats from groups E and F. The medullary diameter in three group-A goats averaged 1.9 mm. while in goats from groups E and F it was 2.8 mm. These differences in width were not suspected until microscopic examination was made. Figures 30 and 31 compare differences in depth of cortex and diameter of the medulla.

The hematoxylin and eosin sections revealed that there is little vacuolation of the cytoplasm of the glomerulosa. The glomerulosal layer also extended deeper into the cortex in the control goats than in the athyroid group.

Sudan IV staining of the adrenal gland revealed much more lipid material in the zona glomerulosa cells in the thyroidectomized than in control goats. The zona fasciculata also showed greater sudanophilia. Partially thyroidectomized group-B and iodinated protein supplemented group-D athyroid goats did not show the greater sudanophilia in the adrenal cortex. Treatment of athyroid goats after cretinism developed did not effect a return to the histological state shown in the control goats (Figures 32 and 33).

c. Extra-endocrine Tissues

(1). Skin. The skin of the athyroid goats consistently showed hyperkeratinization. The epidermis was thrown up in folds which accounted for the rough appearance of the skin grossly. The Malpighian layer of cells was thinner in the thyroid deficient than in the control goats. In one goat (613) only one or two layers of the stratum spinosum cells remained.

In the hair follicles excessive keratinization of the Malpighian layer of the external root sheath and the cuticle of the hair shaft occurred in the thyroidectomized goats. In many of the follicles almost complete degeneration of the hair follicle by hyperkeratinization was noted (Figures 34 and 35).

The sebaceous glands in some of the deficient goats had pyknotic cell nuclei. Increased vacuolation of the cytoplasm in these cells was observed. In some areas keratinized epithelium appeared to completely close the excretory ducts leading from the sebaceous glands into the hair follicle.

The papillae of the dermal layer had largely disappeared in the athyroid goats. Edema was prevalent in the connective tissue of the derma and hypoderma. The bluish cast elicited by this edema in hematoxylin-eosin-stained sections suggested that it was mucin. Smith and Jones (1957) describe a similar appearance for the mucoid degeneration seen in myxedematous tissue.

The cytoplasm of the smooth muscle cells in the erector pili muscles was much more vacuolated in the athyroid than in the control goats. These cells were differentiated from connective tissues and possible nerve trunks by applying Heidenhain's aniline blue stain and Weil's myelin stain to triplicate sections.

(2) Rumen and abomasum. The keratinized epithelium was not as thick in the thyroidectomized goats as in the controls. In some of the deficient goats ballooning degeneration of the Malpighian layer was noted. The papillae were less well developed in the athyroid than in the control goats.

The smooth muscle cells in many areas of the rumen wall were vacuolated and their nuclei pyknotic in the thyroid-deprived goats.

(3) Kidney. The kidneys of the thyroidectomized unsupplemented goats showed pyknosis and coagulation necrosis of many of the epithelial cells in the proximal and distal convoluted segments of the nephrons. Hyaline casts were also present in the tubules. Some early interstitial fibrous tissue was observed to be formed about the glomerulus. Figure 36 shows the kidney from an athyroid goat which was typical of the changes seen.

(4). Musculo-skeletal tissues. One of the thyroidectomized goats (169) showed marked degeneration of the skeletal muscles of the posterior limb, particularly the gastrocnemius muscle. Microscopically the muscle fibers appeared first to become hyalinized exhibiting a bright homogeneous red color in eosin and hematoxylin stained sections. Where the cross striations were absent, the sarcolemma appeared to have disintegrated leaving bundles of hyaline fibrils. Some of the nuclei were pyknotic while others remained vesicular. The nuclei tended to accumulate along the junction between degenerating and healthy tissue. No inflammatory cells were present and fat cells had extensively replaced the necrosed muscle tissue.

In the heart the changes were somewhat different. The hyalinization was not as pronounced. Following necrosis the cells appeared to have fragmented into long fibrils. Lacuna-like spaces formed about the nuclei which gave the muscle the appearance of hyaline cartilage. There was little replacement of necrosed muscle with fat. No calcification was evident in either the heart or skeletal muscle.

Figures 37 and 38 show skeletal and heart muscle changes.

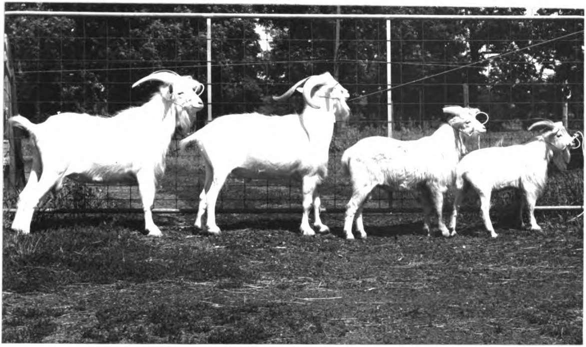


Fig. 2. Appearance of goats at conclusion of experiment. From left to right goats are from control group A, partially thyroidectomized group B, late thyroidectomized group C, early thyroidectomized group F.

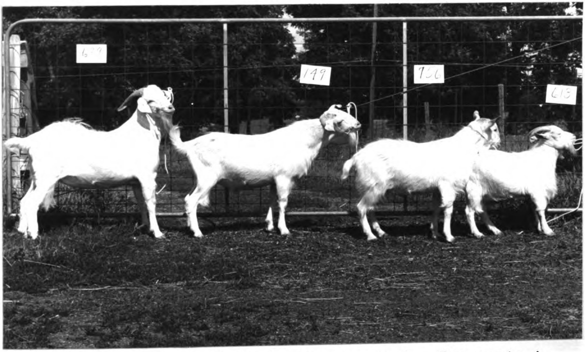


Fig. 3. Goat on left is from group A. Two goats in center were thyroidectomized early (group D) but received .04 gm. iodinated protein per ten pounds body weight. Goat on extreme right is from group F.

Fig. 4. Goat from group E showing arrested horn growth. Extreme cretinoid symptoms were present before iodinated protein therapy was instituted.

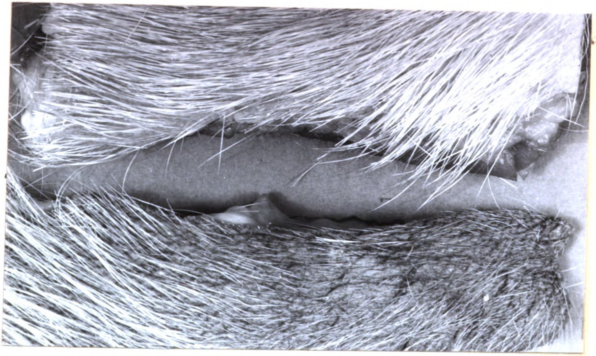


Fig. 5. Strips of back skin removed from control and athyroid goats at necropsy. Top: control goat--note absence of alopecia. Lower: skin from athyroid goat. Much loss of hair and hyperkeratosis.

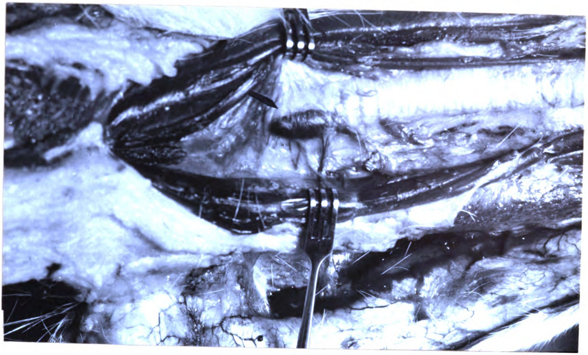


Fig. 6. Accessory thyroid tissue in group-B goat located near anterior end of the sternum on the ventral surface of the trachea.

Fig. 7. Condylar surfaces of the bones of the tibiotarsal joint. Group-A goat on the left and group-F goat on the right. Arrows indicate eroded areas of cartilaginous surface.

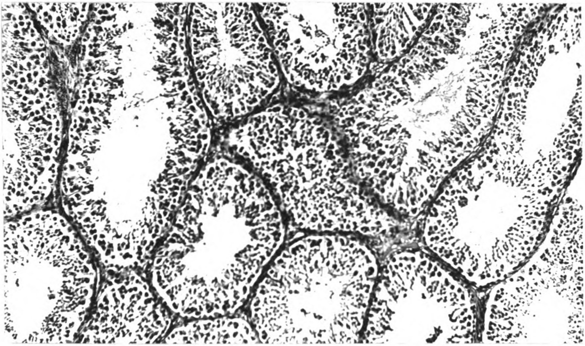


Fig. 8. Testis section showing seminiferous tubules from control goat. Tissue was formalin fixed. Note good retention of interstitial tissue but loss of cellular elements from lumen of tubules. H & E x 110.

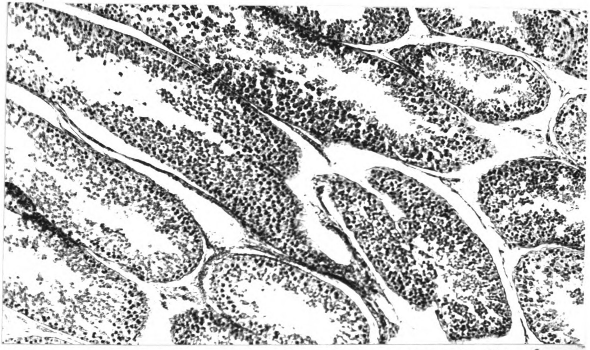


Fig. 9. Testis section from same goat as in fig. 8 Zenker-fixed. Some separation of tubules but cell nuclei stained more intensely. H & E x 110.

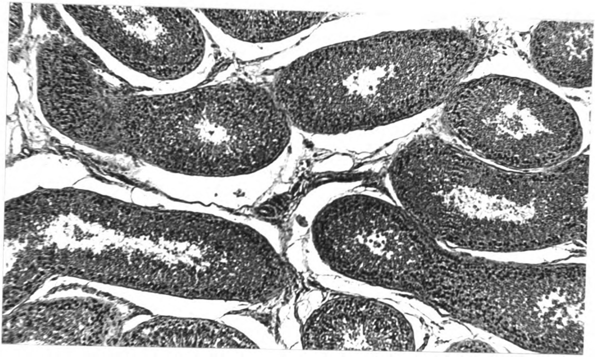


Fig. 10. Testis section from same goat as in fig. 8. Bouin-fixed seminiferous tubules are widely separated, but there is good retention of intratubular cells. HE x 110.

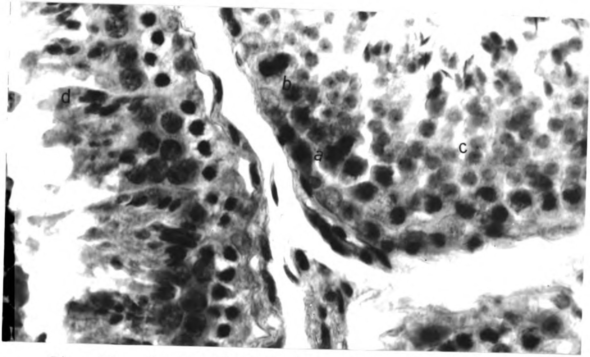


Fig. 11. Testis from control goat. Note absence of vacuoles among primary and secondary spermatocytes (a & b) and spermatids (c). Maturing sperm cells regularly lined up at tips of Sertoli cells (d). Zenker-fixed. H & E x 575.

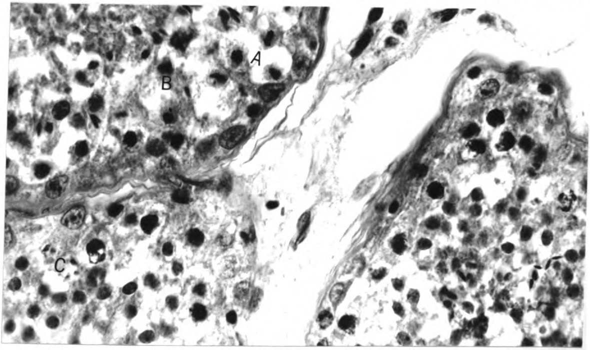


Fig. 12. Testis from group-F goat. Spermatogenic cells vacuolated (A). There is disruption of the spermatogenetic wave in the epithelium with pyknosis (B) and karyorrhexis of nuclei (C) in many spermatocytes. Zenker-fixed. H & E x 600.

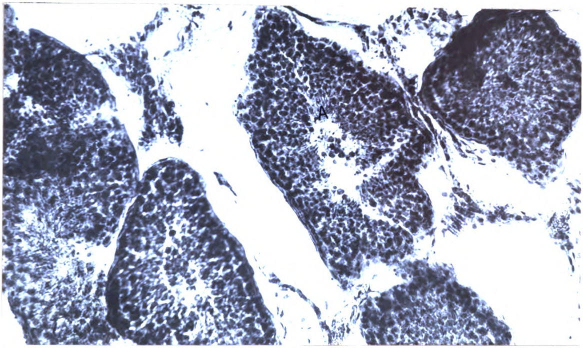


Fig. 13. Testis from control goat. Sudan IV stained section counterstained with Harris' hematoxylin. Spermatogenic cell nuclei stain intensely with counter stain. Only cells and cell debris near lumen (A) are Sudanophilic. x 150.

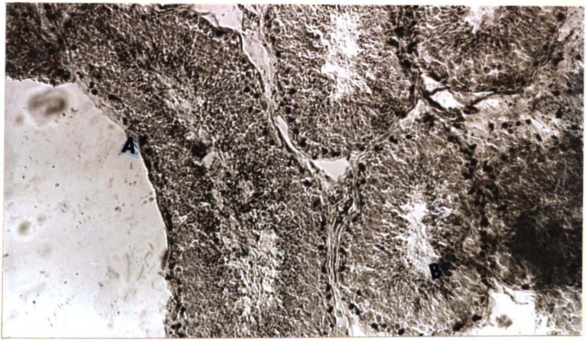


Fig. 14. Testis from athyroid goat. Sudan IV stained section counterstained with Harris' hematoxylin. Note relative absence of nuclear stain, compared to fig. 13. Sudanophilic cells along basement membrane (A) and in lumen of tubule (B). x 150.

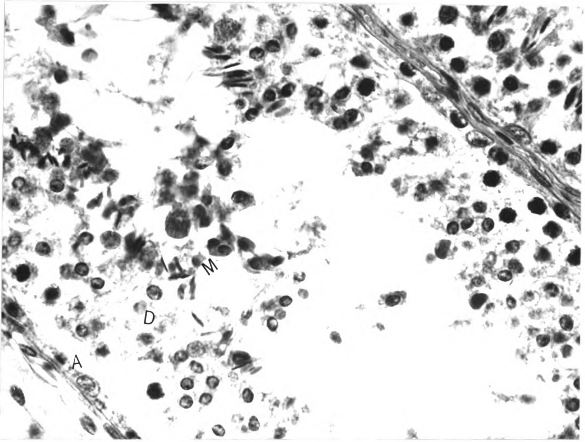


Fig. 15. Seminiferous tubule from athyroid goat showing atrophy of germinal epithelium (A) degenerating spermatids (D) and multinucleate cells in lumen (M). H & E x 590.

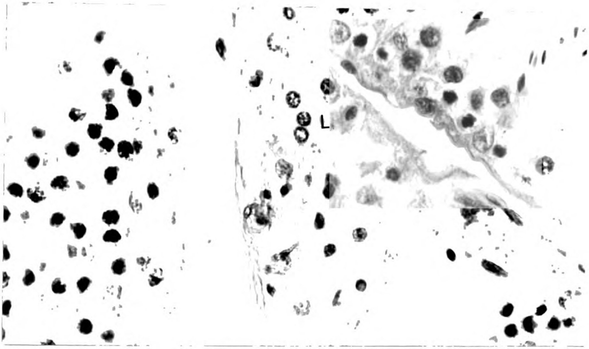


Fig. 16. Section showing Leydig cells in testicular interstitial tissue from group-A goat. Nuclei are rounded and abundant cytoplasm shows slight granulation (L). Zenker-fixed. H & E x 590.

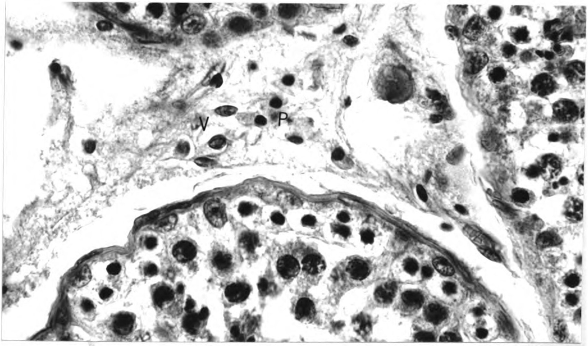


Fig. 17. Section showing Leydig cells in testicular interstitial tissue from group-F goat. Pyknotic nuclei are evident (P), vacuolation is present in cytoplasm (V), granulation of the cytoplasm is faint. Zenker-fixed. H & E stain x 600.

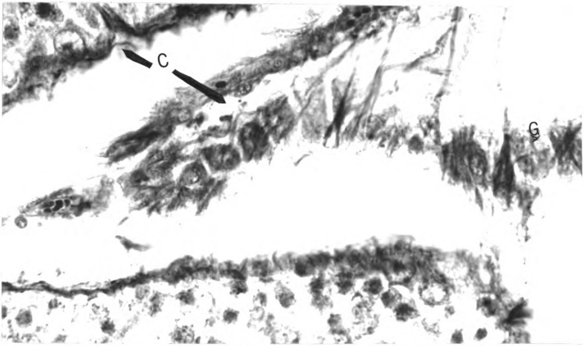


Fig. 18. Heidenhain's aniline blue stain of testis from control group-A goat for collagenous fibers (C) in basement membrane and interstitial tissue. Also note prominent granules in cytoplasm of Leydig cells (G). x 625.

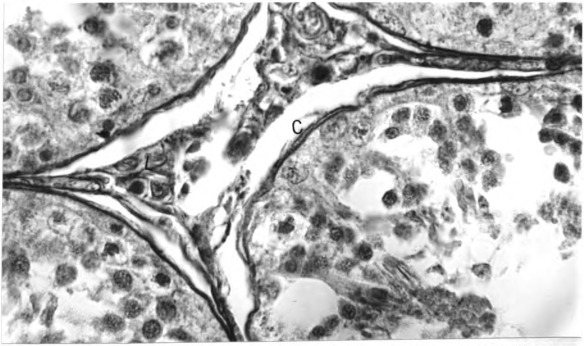


Fig. 19. Heidenhain's aniline blue stain of testis from athyroid group-F goat. Collagen increased in basement membrane of seminiferous tubules (C). Leydig cells (L) are small and have little cytoplasm. x 625.

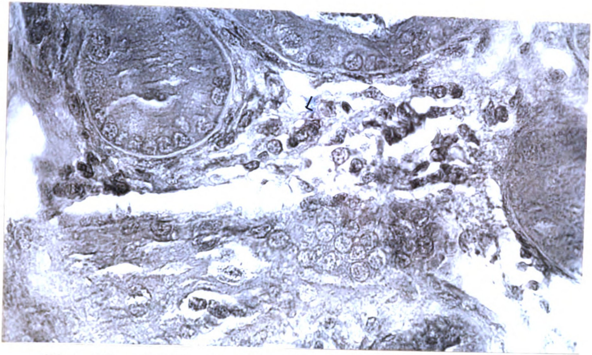


Fig. 20. Testicular biopsy section from control goat at three months of age. Leydig cells prominent (L). Spermatogonia inactive except for occasional primary spermatocyte. H & E x 625.

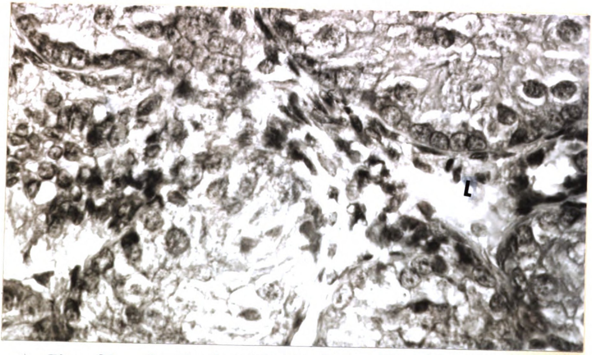


Fig. 21. Testicular biopsy from athyroid group-F goat at three months of age. Interstitial cell nuclei are small and cytoplasm is scanty (L). Occasional spermatogonia have divided and moved toward lumen of seminiferous tubules. H & E x 675.

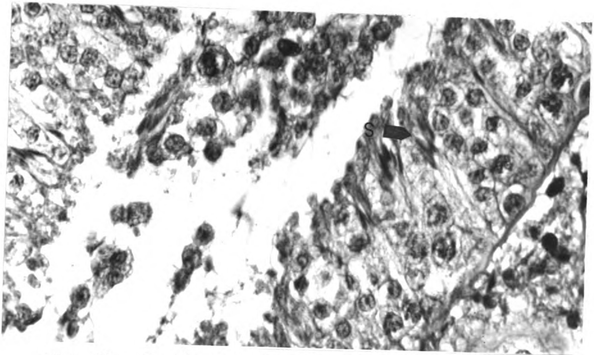


Fig. 22. Testicular biopsy section from group-A goat at six months. Active spermatocytogenesis present, with developing spermatids lined up (S) at tips of Sertoli cells. H & E x 675.

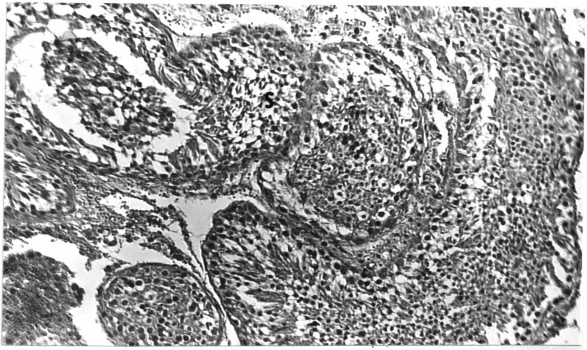


Fig. 23. Testicular biopsy section from group-F goat at age nine months. Limited spermatogenesis is evident. There is much vacuolation of primary and secondary spermatocytes and degeneration of spermatids (S). H & E x 160.

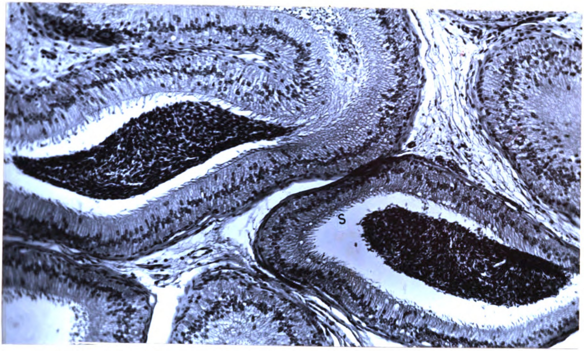


Fig. 24. Section from head of epididymis of control goat showing prominent stereocilia (S), secretion droplets, relative absence of vacuoles in epithelium and many speratozoa in the lumen. H & E x 110.

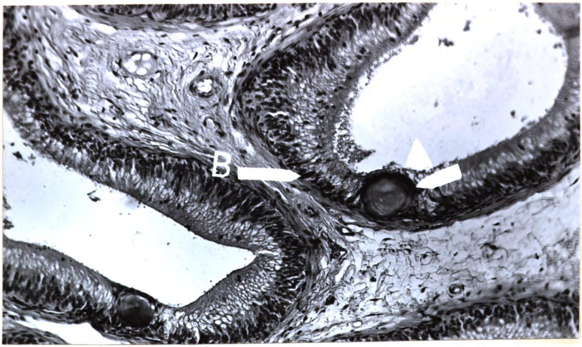


Fig. 25. Section from head of epididymis group-F goat. Circular body at (A), epithelium much vacuolated (B). Few stereocilia, spermatozoa, and secretion granules in the lumina of the epididymal ducts. H & E x 160.

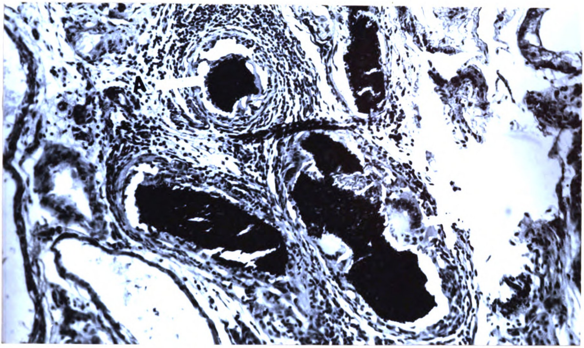


Fig. 26. Granulomatous reaction in testis following biopsy with Vim-Silverman biopsy needle. Calcification of spermatozoa has occurred at (A), giant cell formation is shown at (B). Seminiferous tubules immediately adjacent show degeneration and atrophy. H & E x 135.

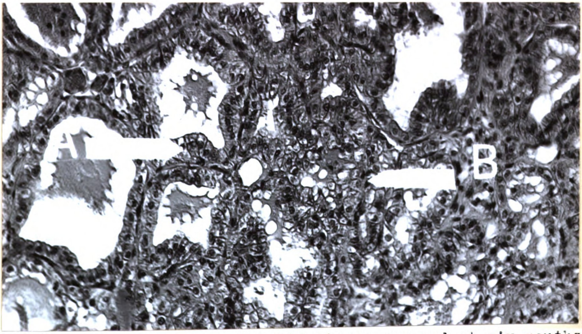


Fig. 27. Accessory thyroid tissue removed at six months from group-C goat originally thyroidectomized at one month of age. Note hypertrophy of acinar epithelium at (A) and hyperplasia at (B). H & E x 375.

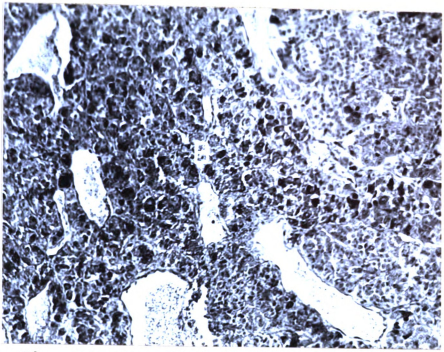


Fig. 28. Anterior pituitary gland from group-A goat. Central area consisting primarily of basophilic cells to the left of (B). Acidophilic cells in outer zone at (A). PAS - Orange G stain x 230.

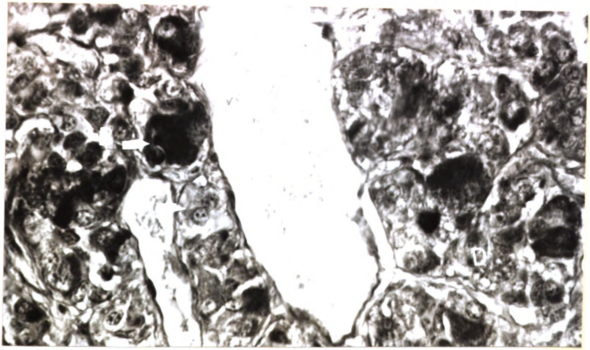


Fig. 28 a. Higher power magnification of fig. 28. Beta basophilic cell showing intense PAS positive mucoprotein (B). Acidophilic cell (A). Smaller delta basophilic cells showing moderate granule fromation (D). PAS - Orange G x 650.

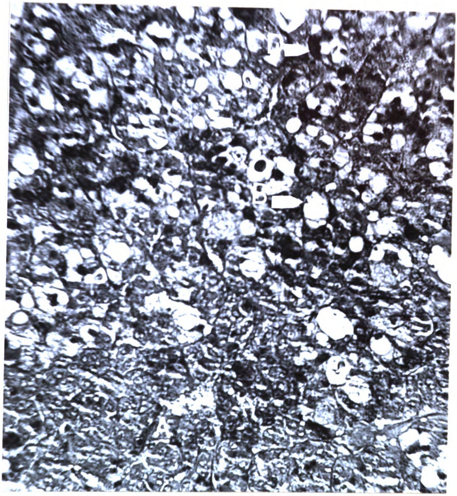


Fig. 29. Anterior pituitary gland from group-F goat. Acidophilic zone (A), vacuolated beta cell at (B), delta cell containing PAS positive cell at (D). Other delta cells to left of (D) are vacuolated. x 230.

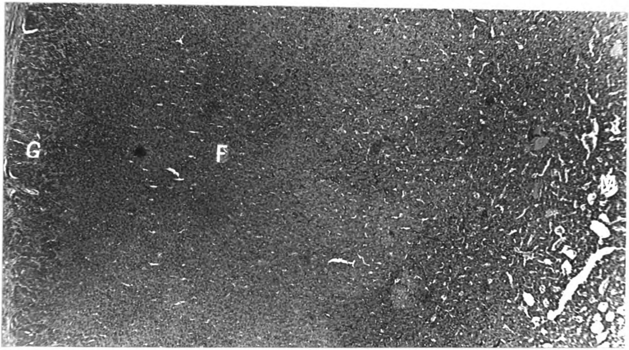


Fig. 30. Adrenal gland from group-A goat. (G) Zona glomerulosa, (F) Zona fasciculata, (M) Edge of medullary zone. H & E x 45.

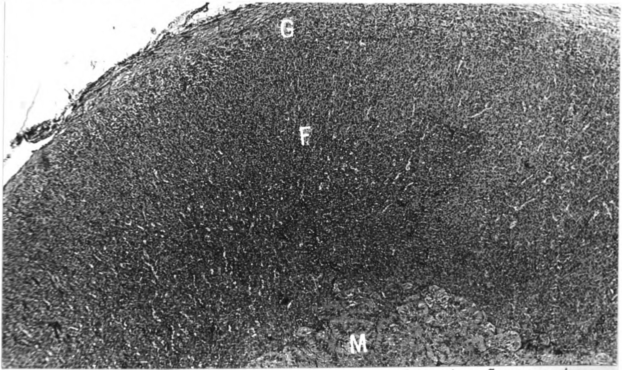


Fig. 31. Adrenal gland from group-F goat. Compare to fig. 30. Cortical zones (G) and (F) much shallower than in control goats. H & E x 45.

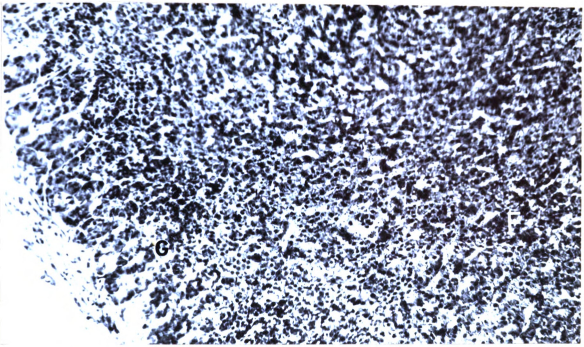


Fig. 32. Adrenal gland from group-A goat stained with Sudan IV. Sudanophilic substance equally distributed in zona glomerulosa (G) and zona fasciculata (F). Sudan IV x 150.

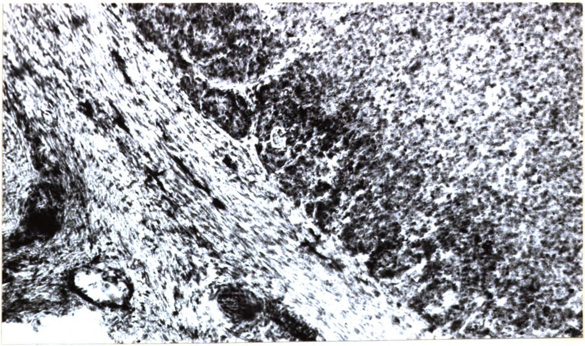


Fig. 33. Adrenal gland from group-F goat stained with Sudan IV. Intense sudanophilic staining in zona glomerulosa (G). Decreased stain intensity in fascicular zone (F). x 150.

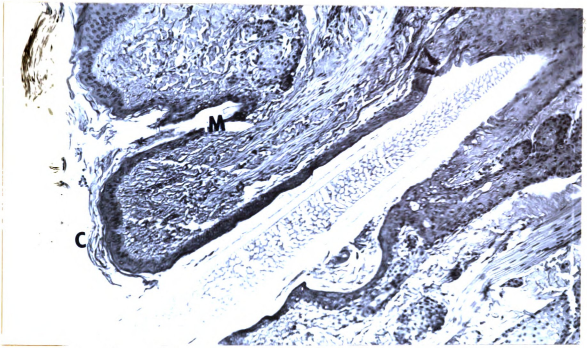


Fig. 34. Skin from back of group-A control goat. Excessive keratinization of epidermis is absent (C). Smooth muscle cells (M) show little vacuolation. H & E x 130.

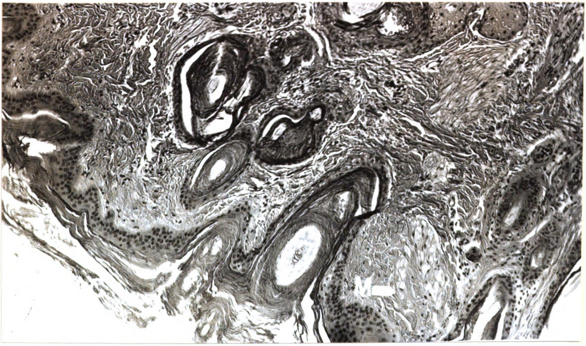


Fig. 35. Skin from back of group-F athyroid goat. Showing hyperkeratosis of stratum corneum (C), degeneration of hair shaft (S), and vacuolation of smooth muscle (M). H & E x 115.

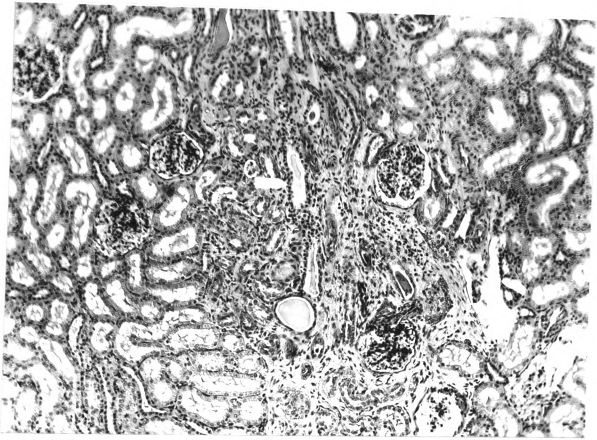


Fig. 36. Section from kidney of group-E goat which had calculi in renal pelvis. Cast within tubule at (C). Fibrous tissue proliferation (F) present around glomerulus. H & E x 115.

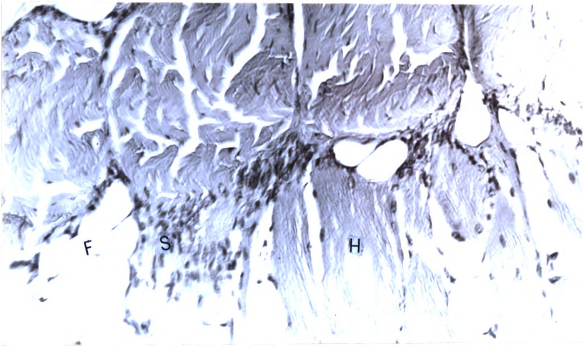


Fig. 37. Gastrocnemius muscle from group-E goat showing hyalinization (H), muscle nuclei (S) and fatty tissue (F) which has replaced destroyed muscle fibers. H & E x 160.

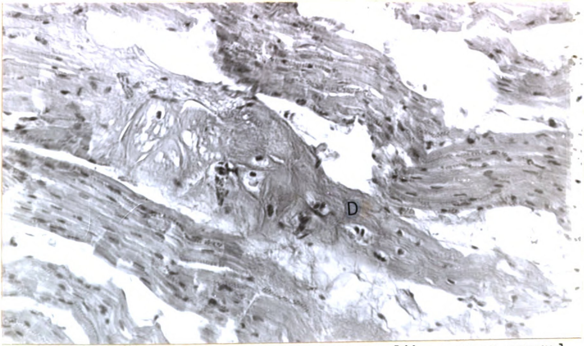


Fig. 38. Mycardium showing lacunae like spaces around Purkinje fiber nuclei (D) and fragmentation of cardiac muscle fibers. H & E x 160.

V. DISCUSSION

A. Relation of Clinical Symptoms to Thyroid Deficiency

The onset of symptoms about two weeks following complete thyroidectomy correlates closely with the observations of Reineke et al. (1941) and Simpson (1924a). The latter author reported that rate of gain began to decline on the nineteenth day after thyroid removal. The failure of many thyroidectomized sheep to show cretinism, was reported by Marston and Pierce (1932). At autopsy varying amounts of accessory thyroid tissue were found. The data from the experiment reported here indicate that accessory thyroid tissue is frequently present in goats. This may be found in varying locations extending from the larynx to the thoracic cavity.

The thyroid uptake data suggest that a large part of the normal thyroid gland can be removed without clinical impairment of body functions. Radioactive I^{131} uptake measurement provides a convenient method of determining the presence of thyroid tissue soon after surgical ablation. Although a reasonable attempt was made to standardize the counting procedure for each goat some variations existed which could account for differences in percent uptake of the injected dose. Nevertheless goats showing less than .5 percent of the injected dose present in the thyroid region went on to become cretins. Those showing more than 1.7 percent of the injected dose showed no cretinoid symptoms. Low counts in the thyroid region may be obtained in the complete absence of thyroid tissue. Bustad et al. (1957), Fletcher et al. (1956), Flexner et al. (1942), Logothetopoulos and Myant (1956), Taurog et al. (1959) have shown that salivary tissue can concentrate iodine.

The symptoms of athyroidism in I^{131} treated goats were slower in developing than when all tissue was removed by surgery. Bustad et al. (1957) report that destruction of the iodine concentrating power required one month and nine months in ewes fed 1800 and 240 microcuries I^{131} per day respectively. It appears that eight millicuries of I^{131} given subcutaneously was a reasonable dose to destroy the remaining accessory thyroid tissue.

The lethargy, stiffness, bloating, alopecia, skin dryness, myxedema and reduced horn growth noted in the athyroid goats in this experiment agree with similar observations made in goats by Simpson (1927 a and b), and Liddel and Simpson (1926). In sheep similar symptoms were reported after thyroidectomy by Marston and Pierce (1932) and after radiothyroidectomy by Bustad et al. (1957).

Replacement therapy with iodinated casein when symptoms of cretinism first appeared, at a level suggested by the work of Flamboe and Reineke (1959), gave essentially the same growth rates and other clinical features shown by goats with intact thyroids. Delaying therapy until cretinism was well established resulted in only partially alleviating the symptoms.

The cessation of libido in thyroidectomized goats was similar to that reported by Maqsood (1951b) in rams during the summer season. Libido was restored in thyroidectomised goats when thyroxine was replaced as soon as symptoms of cretinism appeared. However, replacement therapy failed when cretinism was prolonged. This might explain why some aged animals with a history of prolonged infertility fail to respond to thyroid therapy.

B. Semen Production and Sperm Morphology

Several factors enter into the semen data which bear discussing. First, no attempt was made to limit feed intake of controls to that of the thyroprival goats. It was felt that to do so would not be fair to the control goats whose energy requirements would be considerably greater than that of the athyroid goats. Secondly, the necessity for using electroejaculation to obtain semen creates quite different results than those obtained by collection in an artificial vagina. Thirdly, the collection frequency was less than would have been optimum. This may have resulted in a difference in sperm concentration, motility, and volume between the thyroidectomized and control goats, because of the lack of masturbation in the former and its frequency in the latter. Fourthly, the output of semen could have been affected by the testicular biopsies performed. However it was felt that, since testicular biopsy was performed in both control and thyroidectomized goats, similar relative differences should occur between groups.

So far as could be determined this is the first experiment in which semen was collected by electroejaculation from young completely thyroidectomized goats. The data indicate that, though spermatozoan production is not completely inhibited, significantly fewer motile spermatozoa per ejaculate were obtained from them than from the control goats. The decreased spermatogenesis encountered in athyroid goats confirms the findings of Maqsood (1950b, 1951a) that thyroid inhibition by thiouracil exerts similar effects in young rabbits and rams.

The average semen volume for the control goats was higher (1.4cc. vs. .65 cc.), the concentration lower (2.63 vs. 2.72 billion per cc.) and the total spermatozoa per ejaculate higher (3.180 vs. 1.658 billion) than values for goats reported by Eaton and Simmons (1952).

That no detectable seasonal variation occurred in semen production or in numbers of abnormal spermatozoa was attributed to the fact that the goats were kept inside a building which greatly modified extremes of outside environmental temperatures during the summer and winter months.

The percent of abnormal spermatozoa seen in the control goats was considerably higher than that reported in goats by Eaton and Simmons (1952) (16.0% vs. 8.46%). It is speculated that this difference might be explained by the effect which testicular biopsy had on spermatids which were in the process of development at the time of tissue removal. The protoplasmic extrusion seen on heads of spermatozoa from both the control and athyroid goats constituted approximately one half of the total abnormalities seen. It is possible that this may be a normal structure which would not be seen by ordinary light microscopy.

C. Pathological Changes

The general pathological changes noted in the completely thyroidectomized goats agree closely with those noted by Goldberg (1927).

Hematology and blood chemistry were also similar except that Goldberg (1927) noted a larger drop in blood sugar in the athyroid goats than was obtained in this experiment. Reference to the erythrocytes with pointed processes in young goats was not found in the literature. These abnormal erythrocytes persisted longer in young athyroid goats than in the controls. Goldberg and Simpson (1925) noted that thyroidectomized sheep and goats showed greater than normal numbers of megakaryocytes, formative erythroblasts and granular leucocytes. Whether those two observations are correlated remains to be determined.

Bustad _et al_. (1957) report that serum calcium in lambs born to ewes fed 240 μc I^{131} per day was lower and the serum inorganic phosphate higher than in the control lambs. In the present experiment the athyroid goats had lower serum calcium and higher serum phosphorus than the controls. Whether parathyroid gland function was impaired is a matter for some conjecture. Marston and Pierce (1932) stated that sheep have two pairs of parathyroids, one embedded in thymic tissue dorsal to the thyroid gland and the other intimately bound to the thyroid gland. Simpson (1912) reported that acute fatal tetany followed the removal of the thyroid and parathyroid gland in lambs five to seven weeks old. In the experiment reported here no tetany was seen among the athyroid goats. It was assumed that parathyroid tissue remained in these goats even though it was found in only two of those which were completely thyroidectomized.

Urinary calculi were not mentioned by Goldberg (1927) as one of the findings in thyroidectomized sheep and goats, though renal pathology was noted. The acid solubility of the calculi found in the present experiment indicates they were not steroid in nature. In view of the increase in inorganic phosphorus in the serum it is speculated that increased urinary phosphorus may have followed, by itself or in combination with other factors causing conditions favorable to the formation of phosphatic calculi. Further experimental work will be necessary to elucidate the role of hypothyroidism in urinary calculus formation.

Joint erosions, particularly in the tibio-tarsal joint have not been reported before in thyroidectomized goats in so far as could be ascertained. Silberberg and Silberberg (1955) reported that aging mice of certain hypothyroid strains develop lesions of the articular cartilage similar to those seen in the thyroidectomized goats in this experiment. Ulceration of the articular cartilage in mice however was followed by ossification of the underlying bone, marginal outgrowth of cartilage and bone in the joint cavity, and fibrosis and ingrowth of the synovial membrane into the epiphyseal marrow. The latter changes were not characteristic of the arthritic ulceration seen in goats although the lesions may not have persisted long enough to permit development of the chronic form.

Lung changes were difficult to correlate with the hypothyroid condition. Although the atelectasis was more extensive in the athyroid group it also occurred in some of the controls. The marked stertorous breathing noted during life, however, was confined to the athyroid groups of goats. Bustad _et al_. (1957) reported that ewes whose thyroids had been destroyed by I^{131} experienced increased sensitivity to cold and were subject to respiratory infection.

The adrenal gland changes in goats confirm the observations of Deane and Greep (1947) concerning the increased Sudan IV-staining and shrinkage in the cortex. Decrease in size of the adrenal gland in proportion to body weight in the thyroidectomized goats was not noted, however. No mention was made in the literature reviewed about the relative increase in diameter of the medulla accompanying the decrease in depth of the cortex. Whether this medullary increase is some form of compensatory enlargement remains a matter for conjecture and further investigation.

The pituitary gland changes in thyroidectomized goats agree closely with those observed by Jubb and McEntee (1955b) in the thyroidectomized bull and with those of Halmi (1950), and Purves and Griesbach (1951b) in hypothyroid rats. The large beta basophilic cells in the central part of the pars distalis which stain PAS positive become degranulated and vacuolated following thyroidectomy. A subjective decrease in the small delta cells suggested but did not establish that the gonadotroph cells described by Jubb and McEntee (1955b) in bulls were decreased in the group-F goats subjected to prolonged athyroidism.

Although the testes and accessory glands were smaller in thyroidectomized goats from groups E and F than in the controls, the size in relation to body weight was the same as for the controls. This is somewhat contradictory to the evidence presented by Maqsood and Reineke (1950) in mice, Scow and Simpson (1945) in rats, Maqsood (1950b) in rabbits, and Greenwood and Chu (1939) in cockerels that thyroidectomy or thiouracil caused reduction in testicular size. The failure of thyroidectomy to cause decrease in relative testicular size in goats many have resulted from removal too late to prevent testicular development.

The histological changes in the testis (atrophic and degenerative changes in the seminiferous tubules and the interstitial tissue) are similar in thyroidectomized goats to those reported in thyroid-inhibited mice by Maqsood and Reineke (1950), in rats by Scow and Simpson (1945), in rabbits and rams by Maqsood (1950b).

The observation that the degenerative changes occur principally in secondary spermatocytes and spermatids agrees with the findings of Knudsen (1954). He observed that in bulls, whose testes were subjected to thermal insulation for short or extended periods, primary spermatocytes failed to show normal mitotic activity.

Sudan IV-staining of testicular sections revealed increased sudanophilic substances in cells along the basement membrane and in cells in lumina of the seminiferous tubules. Whether this increase in sudanophilic substance was due to accumulated neutral fat or a possible increase in ketosteroid materials was not determined. Deane and Greep (1947) by special techniques showed that increased sudanophilia in the glomerulosa of the adrenal gland was due to an increase in ketosteroids. Since the Sertoli cells are believed to produce estrogenic substances in certain pathologic states such as Sertoli-cell tumors (Smith and Jones 1957) it seems that the increased sudanophilia might be due to ketosteroids.

Interstitial tissue changes approximated those reported by several authors studying the effect of thiouracil feeding on spermatogenesis in rams, rabbits, and mice (Maqsood and Reineke, 1950; Maqsood 1951a, 1951b). Thyroidectomy of rams resulted in atrophy of testicular interstitial tissues in studies reported by Berliner and Warbritton (1937). In the present study there were fewer Leydig cells in testes from thyroidectomized goats and a greater proportion showed nuclear pyknosis, and cytoplasmic atrophy. The aniline blue stain (Heidenhain's modification) differentiated the Leydig cells well from the collagenous interstitial fibers. In the control goats the majority of the Leydig cells stood out as large ovoid cells with large round nuclei and a distinct granular cytoplasm. Sudan IV stained the cytoplasm of these cells very faintly. In the athyroid goats the cytoplasmic granules were much diminished or absent.

Pronounced changes in the epithelium of the epididymis suggest diminished function in this portion of the reproductive organs. Few changes in the epididymis have been reported following thyroid inhibition. Maqsood and Reineke (1950) reported a decrease in seminal vesicular weight in young mice fed thiouracil.

From these studies it is concluded that total thyroidectomy is necessary to produce the typical effects of decreased reproductive function. Thyroidectomy apparently has its most profound effect on libido. However, since libido is dependent on neurological as well as endocrine factors the extent to which other conditions such as arthritis and its accompanying pain affected this function could not be accurately determined.

D. Testicular Biopsy as a Tool for Measuring Testicular Function

Testicular biopsy was successful in the majority of the goats in which it was tried. It is doubtful that indiscriminate repeated biopsy with the Vim-Silverman needle should be performed since some permanent damage to testicular tissue occurs. Barker (1949) reported that, of three methods of testicular biopsy, the Vim-Silverman biopsy needle caused the least damage.

The technique of Barker (1949) using the dorso-lateral portion of the testis of goats proved effective in obtaining specimens with the Vim-Silverman needle. It was determined that the onset of spermatogenesis was delayed in the thyroidectomized goats. The results of the biopsies indicate that care must be exercised in young goats not to puncture the head of the epididymis since escape of spermatozoa will result in the formation of a sperm granuloma.

VI. SUMMARY AND CONCLUSIONS

The clinical and pathological effects of complete and partial thyroidectomy on reproductive function in young male goats were studied. Because of the complete lack of libido in athyroid goats electroejaculation was employed to collect semen from all goats. It was further observed that compared to the controls the athyroid goats:

1. Produced significantly fewer motile spermatozoa per ejaculate (.59 billion vs. 1.71 billion.)
2. Produced significantly more abnormal spermatozoa.
3. Had lower absolute mean testicular weights, but equivalent ratio of testicular weight to body weight.
4. Showed more cytoplasmic degranulation and atrophy and more pyknosis of Leydig cells.
5. Had more vacuolation and hyaline-like change in secondary spermatocytes and spermatids.
6. Exhibited greater sudanophilia among cells in the seminiferous tubules, particularly in the lumen and along the basement membrane.
7. Showed more vacuolation, fewer stereocilia and the formation of laminated bodies resembling corpora amylacea in the epithelium of the head of the epididymis.

8. Had more pronounced degranulation and vacuolation of the large beta basophils in the anterior lobe of the pituitary gland.
9. Had a shallower adrenal cortex and a greater medullary diameter in the adrenal gland.
10. Showed hyperkeratosis of the epidermis and hair follicles and vacuolar degeneration of smooth muscle in the dermis.
11. Had consistently appearing shallow erosions of articular cartilages of the tibiotarsal joints while the controls had none.

It was concluded that athyroidism more profoundly affected libido than it did sperm production even though the latter was significantly reduced.

Reproductive and other functions were not impaired when male goats were only partially thyroidectomized. Likewise administering iodinated protein soon after complete thyroidectomy permitted normal growth and semen production. Replacement therapy after pronounced cretinoid symptoms developed did not restore normal growth nor reproductive function but did alleviate other symptoms of cretinism. This may explain in part the failure of bulls and other male animals experiencing chronically reduced reproductive function to respond to thyroid or other therapy. When complete thyroidectomy was delayed till about six months of age there was a non-significant decrease in sperm production and some loss in libido. Had this latter group of goats been kept longer it is probable that reproductive function would have steadily declined.

It was concluded that surgical thyroidectomy must be followed by I^{131} uptake studies to determine whether accessory thyroid tissue remains in goats used for athyroid experimentation. Approximately eight millicuries of I^{131} destroyed the remaining tissue without causing other undue local lesions in incompletely thyroidectomized goats.

Testicular biopsy provided an adequate means of assessing testicular histology but resulted in some local necrosis and calcification along the path of needle entry. It was found that extreme care should be taken to prevent puncture of the epididymis, or when it is punctured, the escaping sperm invariably cause formation of a granuloma.

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