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The Effects of Dexamethasone on the Histology and Histochemistry of Thyroid Gland in Female Rabbits

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Abstract

Background The adverse effects of corticosteroids are widely recognized but there are few qualitative data on which they adversely act on the tissue of thyroid gland, in this paper we scrutinize how these corticosteroids affect the thyroid tissues.

Objective To investigate the histological and histochemical changes, due to the effects of dexamethasone sodium phosphate, in the thyroid gland of female rabbits using a light microscope.

Methods Two groups, each one with seven female rabbits were used in this study. The control group received 0.9% saline solution intramuscularly and the treated group received a daily intramuscular injection of dexamethasone sodium phosphate (1.5 mg/kg b.w.) for 15 days, the thyroid glands obtained from these animals were dehydrated, cleared and embedded in paraffin and then sectioned and stained by Haematoxylline and Eosine and histochemically were stained by periodic acid- Schiff reagent, periodic acid- Schiff reagent with enzyme diastase, Toludine blue and Masson's trichrome.

Results Marked changes were observed in the thyroid glands treated with dexamethasone. Histolgically, these changes include a decrease in the height of the follicular cells to become low cuboidal and even squamous, and the follicles distended with colloid accumulation. These changes affected both central as well as peripheral follicles. Histochemically, the thyroid follicles showed a low positive reaction to glycoprotein which might indicate a decrease in the activity of the follicular cells of dexamethasone treated thyroid glands.

Conclusion Dexamethasone causes morphological changes in the thyroid gland consistent with a decrease in thyroid activity and is considered as side effects of this drug.

Key words Dexamethasone, histochemistry of thyroid gland, rabbits

Introduction

A host of pharmacological agents can influence the function and activity of thyroid gland, and among these agents are the glucocorticoids [5,9,24]. Michael *et al.* (1976) [22] observed that serum T_3 decrease significantly 24 hours following administration of dexamethasone in euthyroid subjects and there was little further reduction in serum T_3 with continued dexamethasone administration. However, T_3 briskly rose and it overshot within 48 hours after dexamethasone withdrawal.

In 1977, Westegren and coworkers [28] observed that dexamethasone may partially divert the deiodination of T_4 from the activating T_4 to T_3 to the inactivating T_4 to rT_3 (reverse T_3) pathway.

Maes *et al.* (1990) [19] found that dexamethasone has a pronounced suppressive effect on basal TSH and free T_3 levels. It has significant stimulating effect on rT_3 levels.

The effects of multiple injections of prednisone on thyroid morphology and on plasma T_4 and T_3 concentration in dogs were determined by Woltz *et al.* (1983) [29], plasma T_4 and T_3 concentration decreased significantly after repeated injections of prednisone. Histological examination of thyroid tissue revealed

accumulation of colloid droplets in follicular cell cytoplasm of dogs treated with prednisone. This indicates that prednisone may interfere with basal thyroid hormone secretion by inhibition of lysosomal hydrolysis of colloid in the thyroid follicular cell.

Chopra *et al.* (1975) [4] found that acute administration of glucocorticoids was associated with rapid and persistent decrease in serum concentration of T_3 and less marked decrease in T_4 in Gravis disease patients. However, in hypothyroid patients receiving treatment with synthetic T_4, cortisol causes decrease of T_3 but not accompanied by appreciable decrease in serum T_4 and thyroglobulin. These results suggested that corticosteroids may affect the peripheral conversion of T_4 to T_3. However, the conversion of T_4 to metabolically inactive rT_3 was enhanced.

Loebenstein *et al.* (1983) [16] found that administration of dexamethasone induced a fall in total T_3 and a rise in rT_3 concentration, while the withdrawal of glucocorticoid led to an increase in serum concentration of total T_3 and decrease of serum rT_3, basal plasma TSH concentration was unchanged by glucocorticoid withdrawal and it fell during subsequent dexamethasone therapy.

Methods

Healthy white New Zealand female rabbits weighing between 1000- 1250 grams were used and kept in separate plastic cages and fed *ad- libitum*. The animals were divided into two groups, seven animals in each. The first group was treated daily for 15 days with (1.5 mg/kg b.w.) intramuscular injection of dexamethasone sodium phosphate (ZMC import- export GmbH Germany) in the thigh muscle. The second group considered as a control animals, they received equal amounts of 0.9% saline solution as intramuscular injections.

Twenty- four hours after the last injection, the animals were anaesthetized with chloroform. After dissection of the neck, the two lobes of thyroid gland were removed from the side of superior part of trachea.

The glands were fixed in 10% formaline solution for 24 hrs., dehydrated, cleared, and embedded in paraffin and the blocks obtained were sectioned and stained by Haematoxylline and Eosine stain (H&E), Alcholic periodic acid-Schiff stain (PAS**)**, Alcholic periodic acid- Schiff stain with diastase digestion method (PAS-D**)**, Toluidine blue (TB) and Masson's acid Fuchin Aniline blue Trichrome stain (MT**)**.

Staining methods and techniques were done on the basis of Humason (1972) [12] and Luna (1968) [18].

Results

Histological Study:

Control Group:

The thyroid gland was encapsulated by a moderately thick layer of a well developed connective tissue. From this capsular connective tissue septa extended inward dividing the parenchyma of the gland into lobules which were incompletely separated from each other. So the thyroid gland was not truly lobulated. The interlobular stroma consists mainly of connective tissue, blood vessels, nerve fibers, and a group of cells which not surround follicular lumen (Figure 1a).

The thyroid gland is composed of an aggregation of spherical or oval cyst like follicles or acini of variable sizes, each follicle lined by a secretory epithelium composed of a single layer of cuboidal to low columnar cells with clear cytoplasm and distinct, mostly rounded nuclei, parafollicular cells sometimes were observed between the epithelial cells. The lumen of the thyroid follicles were filled with colloid material which in some follicles was vacuolated at the periphery (Figure 2a).

Generally, two types of follicles were found in the thyroid gland, the follicles located in the periphery of the gland had larger diameter than those in the central part. Furthermore, the peripheral follicles had a small cuboidal epithelial cells compared to those of the central follicles (Figure 3).

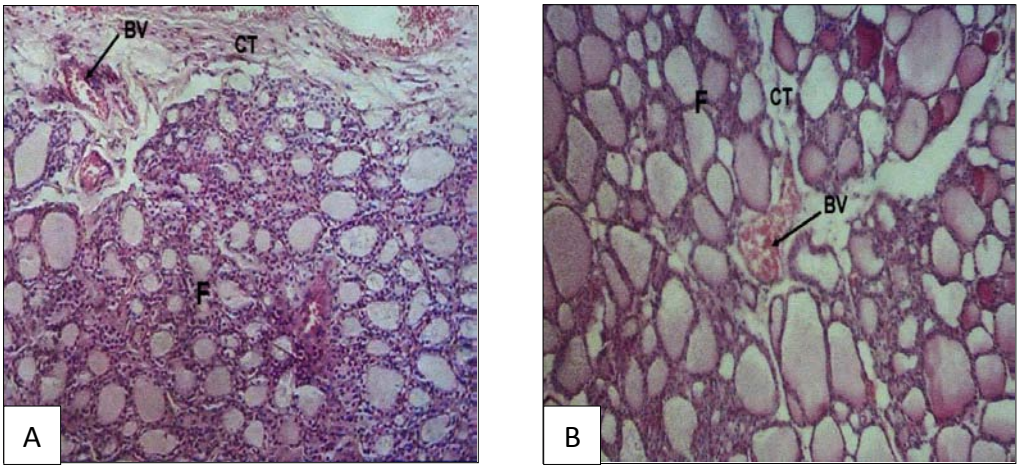

Figure 1. Light micrographs of the thyroid gland section in control (A) and treated (B) rabbits, showing the general structure of the gland. The connective tissue (CT) containing blood vessel (BV) and dividing the glandular tissue into incomplete lobules. H&E (100 X) for A & (150 X) for B.

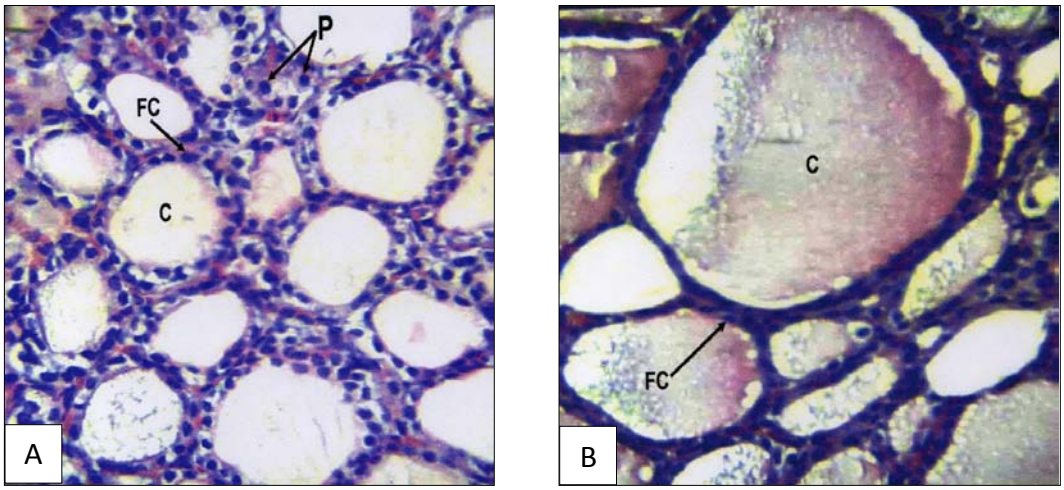

Figure 2. Light micrographs of the thyroid gland section in control (A) and treated (B) rabbits, showing the thyroid follicular cell (FC), colloid (C) and parafollicular cell (P). H&E (450 X) for A & (675 X) for B.

Treated Group:

The histological appearance of the thyroid gland in rabbits treated with dexamethasone sodium phosphate showed changes detected mainly in the follicular components. These changes differed from one animal to another and even from one lobule to another within the same animal. Generally the follicular epithelial cells became low cuboidal or even squamous, the nuclei were mostly flat and the cytoplasm was not distinct. The thyroid follicles were large in most parts of the glands, due to distention with colloid material (Figures 1 and 2b).

Dexamethasone treatment brought about almost same changes in both types of thyroid follicles, peripheral and central follicles (Figure 4).

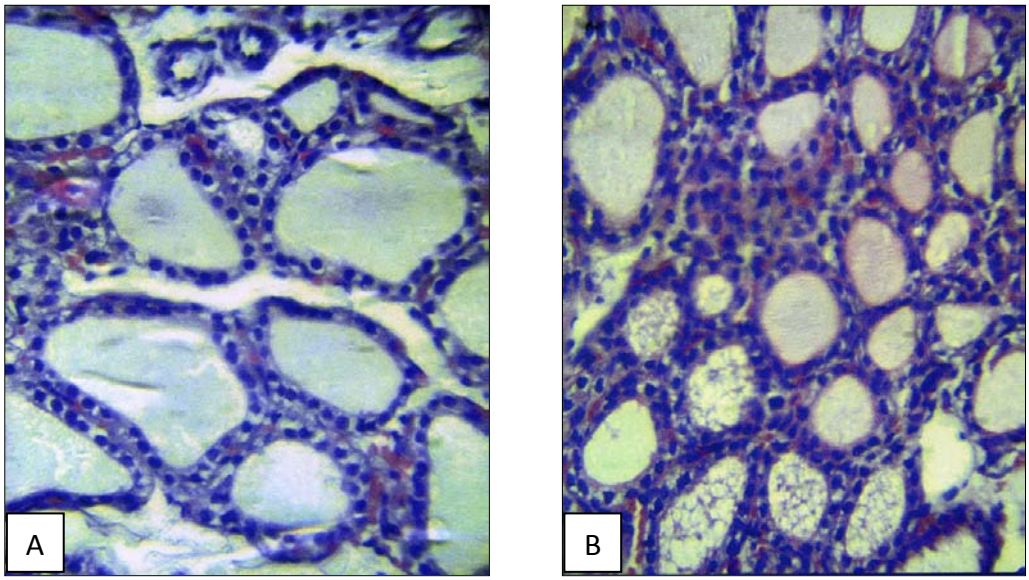

Figure 3. Light micrographs of the thyroid gland section from the peripheral (A) and central (B) areas in control rabbits. H&E (450 X).

Histochemical Study:

Control Group:

The intensity of the stains reaction in thyroid gland was summarized in table (1).

Table 1. The intensity of the stains reaction in the thyroid glands of control and treated rabbits.

Stains	*Control Group*	*Treated Group*
PAS	+++	++
PAS-D	+++	++
TB	-	-
MT	++	+

(+++) strong positive reaction
(++) moderate positive reaction
(-) negative reaction

The thyroid follicles stained strongly with PAS (Figure 5a) and PAS-D (Figure 6a) but they showed no metachromasia with TB (Figure 7a) which indicated that the secretory product of the thyroid follicle is mainly glycoprotein in nature.

Masson's trichrome staining (Figure 8a) showed a good amount of collagen fibers in the interfollicular stroma of thyroid gland.

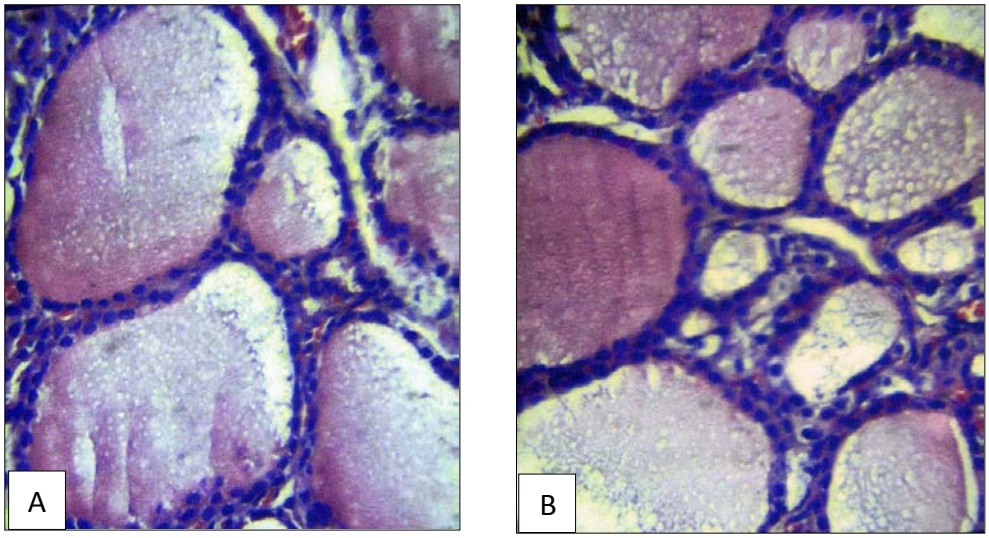

Figure 4. Light micrographs of the thyroid gland section from peripheral (A) and central (B) areas in treated rabbits. H&E (675 X).

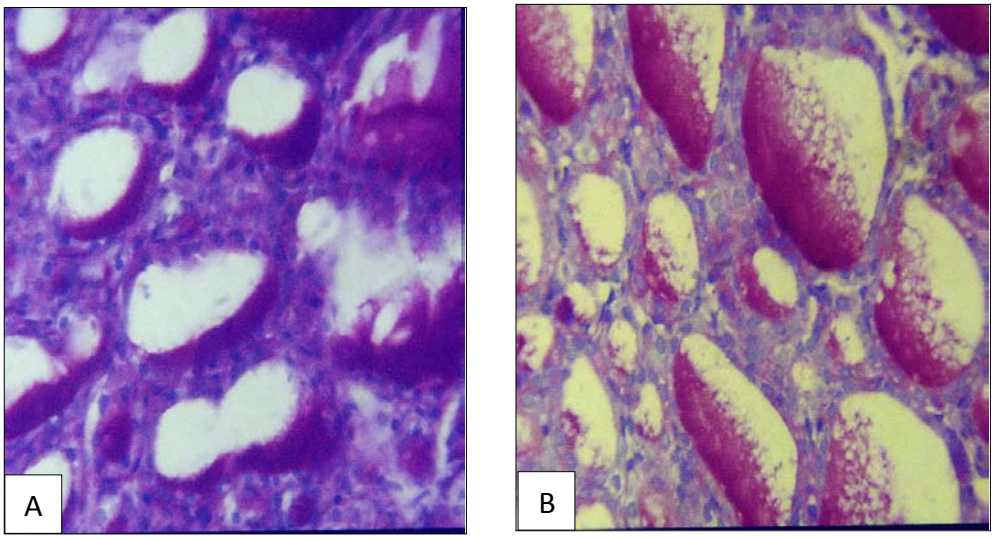

Figure 5. Photomicrographs of control (A) and treated (B) thyroid section stains with PAS. Shows a strongly positive staining of control group and moderately staining of treated group. (450 X).

Treated Group:

Intensity of the stains reaction in the thyroid glands of dexamethasone treated animal was summarized in table (1).

The thyroid follicles stained moderately with PAS (Figure 5b) and PAS-D (Figure 6b) while TB showed no metachromasia (Figure 7b). This indicates that the secretory product (glycoprotein) of the gland was reduced.

Masson's tri chrome (Figure 8b) staining showed that collagen fiber in the interfollicular stroma in the thyroid gland of treated animal is much less than that of control group.

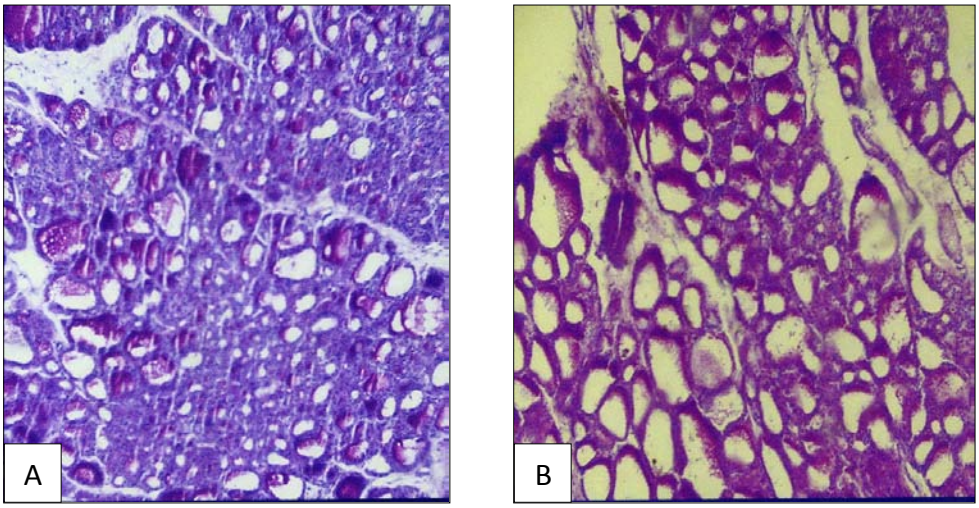

Figure 6. Photomicrographs of control (A) and treated (B) thyroid sections stains with PAS-D. Shows a strong positive staining of control group and moderate staining of treated group. (100 X).

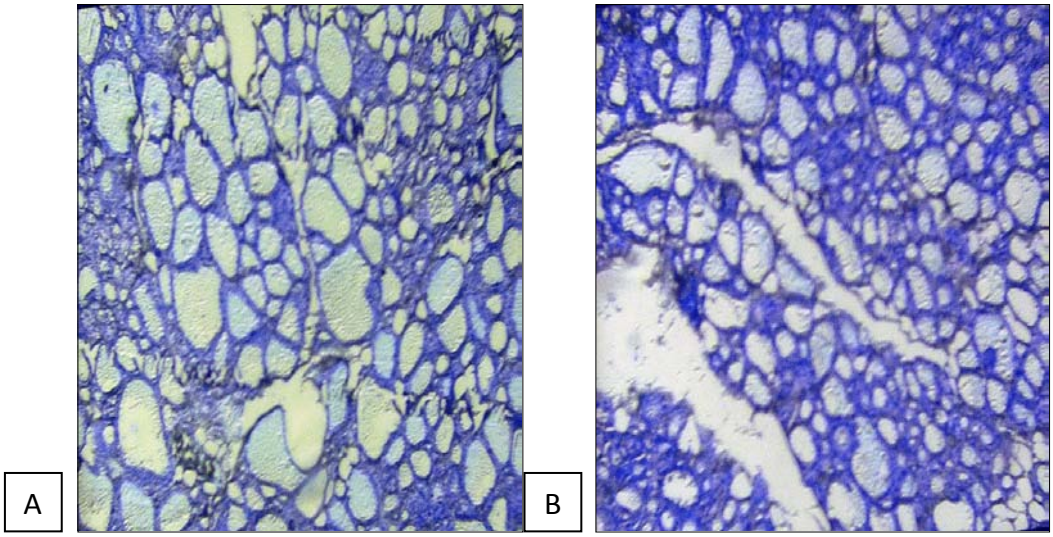

Figure 7. Photomicrographs of control (A) and treated (B) thyroid section stained with TB. Show no metachromasia of both groups. (100 X).

Discussion

Control Group:

The histological appearance of the control thyroid gland obtained in this study were similar to that described by other authors on the rat thyroid gland [20], on the mouse thyroid gland [7], and on dolphin thyroid gland [11]. Harach (1987) [10] described mixed follicles in human which are composed from squamous like epithelium and the lumen of these follicles contains an esonophilic and positive colloid like material which contains acid mucins and cell debris.

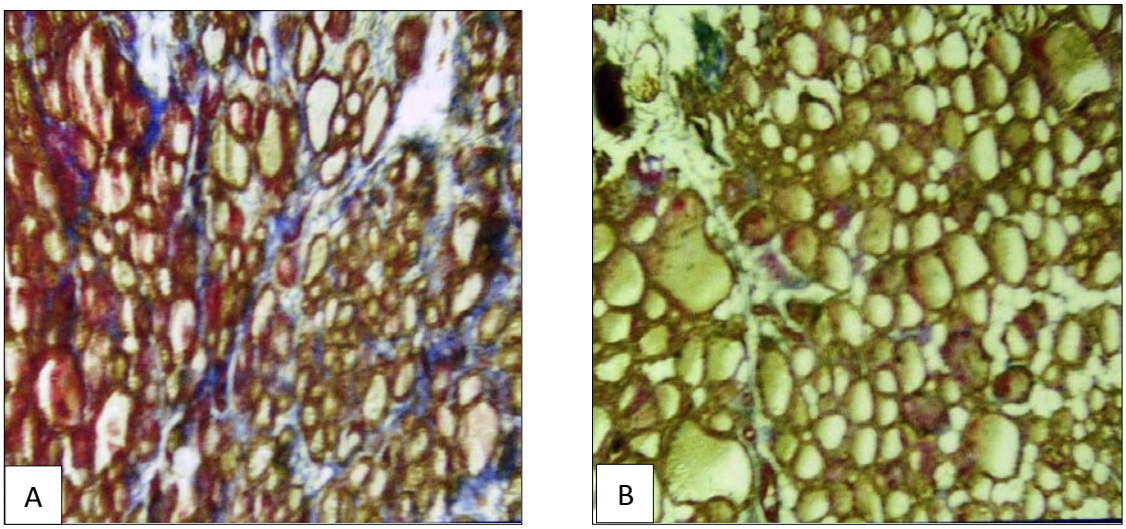

Figure 8. Photomicrographs of control (A) and treated (B) thyroid section stained with Masson's Trichrome. Showing a decrease amount of the stroma of treated group. (100 X).

In the present study, the parafollicular cell or C-cells which are large polygonal cells with clear cytoplasm were observed in a few number scattered between the follicular cell or in the interfollicular stroma. These cells are also described in dolphin thyroid gland [(11)], and on human thyroid gland [(3)].

Two types of follicles were found in rabbit thyroid gland, the follicles which are found in the periphery of the gland are larger than those found in the center. Furthermore, the peripheral follicles had a small cuboidal cells and large lumen as compared to the central follicles. These findings were in agreement with Maiti (1980) [(20)] who divided the follicle of rat thyroid gland into peripheral and central. Low (1988) [(17)] showed that in rabbit thyroid gland the larger follicles were found in the outer regions with low epithelial cells.

Our histochemical investigations showed that the colloid of the thyroid follicles react strongly with PAS and PAS-D. This finding suggested that the secretory products of follicular cells which are stored in the follicular lumen are mainly glycoprotein in nature. While staining with TB showed a negative reaction (no metachromasia) which indicated that the thyroid follicle might not contain mucopolysacchoride or the content was insufficient to produce a demonstrable staining with this technique. These findings were in agreement with Berndorfer *et al.* (1996) [(2)] who demonstrated that thyroglobuline was a complex molecule of glycoprotein extracellulary stored in the follicular lumen for future liberation of thyroid hormones. Similar observations were found in many other mammalian thyroid glands [(8,11,14)].

Our study also revealed that there was a good amount of collagen fibers in the connective tissue of the interfollicular space which reacts positively with masson's trichrome staining.

Treated Group:

This study showed that treatment of female rabbits with dexamethasone resulted in marked morphological changes in the structure of thyroid gland which might be due to the effect of dexamethasone on the thyroid function. Messer *et al.* (1995) [(21)] observed that the administration of dexamethasone in a large dose was associated with depression of many aspects of the thyroid function.

Although many previous studies indicated numerous dysfunction of the thyroid gland attributed to the treatment by dexamethasone

[1,9], few studies have been carried out to demonstrate histopathological changes in thyroid gland of the dexamethasone treated animals.

The response of thyroid follicles to dexamethasone treatments was not uniform and there were distinct differences in the response from rabbit to rabbit and even from lobule to lobule within a single gland. The thyroid follicles did not respond uniformly to dexamethasone treatments, as shown by the fact that there were areas of thyroid follicles which were indistinguishable from those of the control rabbits.

The general effects of glucocorticoids led to the shrinkage of thyroid follicular cells, the height of these cells decreased so they become low cuboidal or even squamous with flat nuclei. Consequently, it is difficult to point out the occasional presence of C-cell. The thyroid follicles in most cases were large due to distension with colloid. This histological finding may indicate a decrease in the activity of thyroid gland.

In contrast to this study, Kalamars *et al.* (1981) [13] observed that the height of follicular cells increased significantly while the nuclear volume decreased in the thyroid gland of rats that treated with hydrocortisone for one month. This finding might be explained by the observation of Tapioranta (1975) [27] who stated that the effect of glucocorticoid was a dose and time related. In contrast to our study, Sarria *et al.* (1994) [26] showed that adrenalectomy resulted in an increase in percentage and complexity of pituitary thyrotrophic cells which led to an increase in TSH secretion, so the thyroid gland showed some histological evidences of activation.

Assisting our histological study by histochemical investigation which shows an alteration in the intensity of staining of follicular component of thyroid gland in the rabbit treated with dexamethasone. There is a decrease in synthesis of thyroglobulin in follicular cells due to the action of dexamethasone; as a result, there is a decrease in the intensity of PAS and PAS-D. These histochemical results confirmed our histological results in that the secretory activity of follicular cell may be decreased by dexamethasone treatment.

In the present study, we found changes in the intensity of staining of connective tissue. There was a decrease in collagen fibers in the interfollicular spaces thus; there was a decrease in the intensity of connective tissue staining with MT. This finding was in agreement with the finding of Saitoe *et al,* 1997 [25] who stated that glucocorticoid was a fibroblast growth inhibitor factor.

Some compounds such as glucocorticoids have multiple effects and thus influence thyroid physiology at various levels. The physiological doses as well as pharmacological doses of glucocorticoids influenced the thyroid function; their effects were variable and multiple depending on the dose and on the endocrine status of the individual. The type of glucocorticoid and the rout of administration might also influence the magnitude of the effect [6,9,15,23].

From these results, we conclude that glucocorticoids may cause a decrease in the thyroid activity and that both structure and function of thyroid gland are affected by glucocorticoids.

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