

Ultrastructural Changes in Thyroid Gland from Fetus and Maternal Rats *Rattus Norvegicus* with Induced Hypothyroidism

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Summary

The study evaluated the effect of hypothyroidism on experimental rats (*Rattus norvegicus*). All animals were housed, breeding and adapted, and then only (50) virgin females were chosen.

The animals subdivided into two groups, the first group was regarded as control, while the second group were treated with propylthiouracil (ptu) as a drug with a dose (0.05%) to induce hypothyroidism, then the females left mating and studied the changes after (14.5,16.5,18.5,20.5and 21.5) days post gestation.

In this study, the results of ultra-structural changes in the thyroid gland from maternal rats and fetus with induced hypothyroidism and their fetus at each period of gestation were clarified with transmission electron microscopy; these changes included alteration in apical surface and cytoplasm of follicular cells that lining the thyroid follicles by Changes in shapes of microvilli. Like blebs and protrusions, also dilated rough endoplasmic reticulum, increased with lysosomes, and the empty vacuoles appeared more than the dense vesicles. In addition to that, there was an increase in mitochondria and golgi apparatus cisternae, the

nucleus of follicular cell showed changes in their chromatin and irregular outlines and more cellular debris. Also, the pictures referred to the deposition of crystals from iodine salts in most follicular cells and the connective tissue showed thickening that separated thyroid follicles from rat fetus with induced hypothyroidism at (18.5) days post gestation.

Biochemical results revealed to a significant increase in the mean concentration of (TSH) in all pregnant rats with hypothyroidism compared with control females, also there were a decreased in both (T3 and T4) hormones in the serum of pregnant rats in the hypothyroidism group compared to the control group, during each period of gestation (14.5,16.5,18.5,20.5 and 21.5) days.

Levels of some oxidative enzymes (GSH and MDA) were also determined in this study; there was a significant increase in their concentration in serum of pregnant rats related to the hypothyroidism group compared with their concentration in the control group.

Keywords: Thyroid Gland, Fetus and Maternal Rats, *Rattus Norvegicus*, Induced Hypothyroidism.

1. Introduction

The thyroid gland plays a vital role in the overall body function during all stages of life, although it was relatively small, but it produces hormones that regulate the body metabolism, in addition to the important effect on the other hormones' action (Delbert, 2009).

The thyroid gland produces the hormones (T4), (T3) and calcitonin, more than (80%) of (T4) was converted to (T3) by peripheral organs such as the liver, kidney and spleen, and (T3) is about ten times more active than (T4) (David, 2005). Thyroid stimulating hormone (TSH) stimulates the thyroid gland to secrete the hormones (T4 and T3).

Thyroid diseases have been known to affect the female reproductive system, who thus have trouble in conceiving or have more miscarriage, also thyroid

dysfunction has a high prevalence during pregnancy, affecting up to 5% of all pregnant women (Haddow, et al, 1999; Abalorich, et al., 2003).

Hypothyroidism is almost caused by a disease within the thyroid gland leads to decrease in the production of thyroid hormones, and the most disorder may be the (TSH) dependent hypothyroidism which causes abnormalities during fetal development or early infancy growth, and infants not treated within the first three months suffer irreversible mental retardation (Boelaert et al., 2005).

Enzymatic antioxidants defenses include superoxide dismutase (SOD) and glutathione (GSH), there is a balance between the activities of these enzymes and the intercellular levels of these antioxidants (Ahmed, 2002).

Hypothyroidism was found to be associated with marked oxidative stress, one of the earliest manifestations of which was a decline in the level of glutathione (Rahman, et al., 2001).

Many studies suggested that the increased activity of (SOL) and (GSH) caused in thyroid tissue (Dasgupta et al., 2005; Ademoglu et al, 2006)

Since the fetus completely depends on maternal thyroid hormones that supplied during the early period of gestation so the present study was planned to evaluate the ultra-structural changes with rat maternal thyroid gland with induced hypothyroidism and their fetus during different period of gestation and changes with thyroid hormones (TSH, T3 and T4) in relation to alteration in oxidative enzymes (GSHandMAD).

2. Materials and Methods

2.1 Experimental Animals

In this study (50) females wistar albino (*Rattus norvegicus*) were used aged (8-10) weeks and weighting about (200-250) gm. All animals were kept in an animal house under controlled conditions, for mating only the virgin females were choosed and placed as (2:1) ratio of females to males, when a vaginal plug was observed then this day consider as day zero of gestation go.

2.2 Induction of Hypothyroidism

Fifty pregnant rats were isolated and divided into two groups the experimental and control group of (10) rats for each period (14.5, 16.5, 18.5, 20.5 and 21.5) days of gestation. Hypothyroidism was induced by adding 0.05% of propyl thiouracil (ptu) to drinking water given to pregnant rats starting from (8.5) day of gestation and continue stillbirth (Gravina et al., 2007). while euthyroid rats regarded as control were received only tap water during the same period of gestation.

2.3 Samples Collection

Pregnant rats from each group (control and hypothyroidism) were killed after anaesthetized on each period of gestation with overdose of chloroform, then directly the blood (5ml) was collected from it by heart puncture into sterilized tube without anticoagulant to separate the sera and then keeping in freezer to determine some biochemical parameters like thyroid hormones (TSH, T3 and T4) and oxidative enzymes (GSH, MAD). Also, thyroid tissue related to maternal rats and their fetus were obtained and very thin pieces prepared for electron microscopy examination.

2.4 Biochemical Study

2.4.1 Measuremen of (TSH, T3 and T4)

The serum thyroid hormones were measured on the basis of the instruction of kits for each that is Cusabio Eliza Kit from Cusa Biotec Hco. This assay employs the quantitative sandwich enzyme immunoassay technique. The concentration of (TSH, T3 and T4) was expressed according to the standard curve which prepared from standard solution dilution and then the optical density (OD) were read, the concentration expressed as ($\mu\text{lu}/\text{ml}$, ng/ml , $\mu\text{g}/\text{dl}$) respectively.

2.4.2 Estimation of Rat (GSH and MAD) Enzymes

Briefly as above the blood from each pregnant rats at each period of gestation were used and within serum separate tube (SST) allow samples to clott for (30)

min. before centrifugation for (15) min. at (3000) rpm then we removed serum, to determine of each kit was followed, this assay employs the competitive inhibition enzymes immunoassay technique.

Absorbance at (450) nm was read with Eliza reader within (5) min and the concentration of (GSH and MAD) enzymes level was estimated for all samples compared to control, the normal values was (16-400) $\mu\text{g/ml}$ for (GSH) enzyme and (31.2-2000) pmol/ml for (MAD) enzyme.

2.5 Ultra Structural Study by Transmission Electron Microscopy Exam

Specimens from each maternal thyroid gland and very small pieces of fetus thyroid gland were excised on each period of gestation (14.5, 16.5, 18.5, 20.5 and 21.5) days, then prepared for electron microscopy study, so the samples were processed as follow, the fixation with two steps, the primary fixation with De-Castro fixative (Al-Malaak, 1992) which composed from two solutions mixed just before used, the samples fixed for (2hrs) at (4C°).

The post fixation established with osmium tetroxide (OSM4) for (2hrs), and then the samples were passed through series of alcohol concentration 50%, 70%, 90% and absolute alcohol for (20 min) on each concentration. Then the samples embedded in pure araldite with appropriate steps, Then the samples embedded in pure araldite with appropriate steps, then the blockes were providing for cutting, this sections (50-80) nm were cut and attach on EM grided for used to examination to examination by (TEM). Staining with uranyl acetate and lead citrate, then examining and photographed (Al-Malaak, 1992).

NOTE: The step starts from dehydration completed in Bendahaari University Malaya, Electron microscopy unit, Faculty of Medicine.

2.6 Statistical analysis

The results were analyzed with Anova test by univariate analysis of variance, the data were expressed as(mean+-SD), also (LSD) was used to test different between groups, $p < 0.05$ was considered significant.

3. Results

3.1 Biochemical Study

3.1.1 Effect of Hypothyroidism on Thyroid Hormones (TSH, T3 and T4) of Pregnant Rats

The results referred to an increase in (TSH) while a decrease in (T3 and T4) concentration in blood serum of hypothyroidism pregnant rats at $p < 0.05$ compared to the control group (table 1, 2, and 3), on each period of gestation (14.5, 16.5, 18.5, 20.5 and 21.5) days, the results expressed as (mean \pm -SD).

3.1.2 Estimation of (GSH and MAD) Oxidative Enzymes Concentration of Pregnant Rats (Treated and Control)

The data were determined the concentration of GSH in all pregnant rats with hypothyroidism and results of statistical analysis referred to significant increased at ($p < 0.05$) in all hypothyroidism rats compared to control on each periods of gestation compared to control group (table 4).

Also, the concentration of (MDA) was determined and showed increased significantly in all pregnant rats with hypothyroidism at ($p < 0.05$) compared to control group, the results clarified gradual increased with (MAD) concentration start from (14.5) days post gestation till (21.5) days in all rats with hypothyroidism (table 5).

Table (1): Effect of Hypothyroidism on Thyroid Hormones TSH compared to control group.
Values expressed as (mean \pm SD) ($p < 0.05$)

Period of pregnancy day	14.5	16.5	18.5	20.5	21.5	total	LSD
Control	1.4	0.84	0.71	13.14	4.02	3.97 \pm -6.20	*
Treatment	1.14	0.84	0.71	13.14	4.02	7.73 \pm -6.11	*

*: Referred to significant difference, the number of animals in each group: 5 rats.

Table (2): Effect of Hypothyroidism on Thyroid Hormones T3 compared to control group.
Values expressed as (mean±SD) ($p \leq 0.05$)

Period of pregnancy day	14.5	16.5	18.5	20.5	21.5	total	LSD
Control	0.19	0.42	0.48	0.37	0.54	0.40±0.18	NS
Treatment	0.28	0.42	0.24	0.28	0.29	0.30±0.22	NS

a: Mean nonsignificant, the number of animals in each group: 5 rats.

Table (3): Effect of Hypothyroidism on Thyroid Hormones T4 compared to control group.
Values expressed as (mean±SD) ($p \leq 0.05$).

Period of pregnancy day	14.5	16.5	18.5	20.5	21.5	total	LSD
Control	4.01	2.45	1.89	1.24	2.29	2.37±1.16	*
Treatment	1.42	1.71	1.42	1.05	2.46	0.61±0.80	*

*: Referred to significant difference, the number of animals in each group: 5 rats.

Table (4): Effect of Hypothyroidism on Thyroid Hormones GSH compared to control group.
Values expressed as (mean±SD) ($p \leq 0.05$).

Period of pregnancy day	14.5	16.5	18.5	20.5	21.5	Total
Control	28.42±3.79	21.26±5.03	0.36±0.11	27.92±9.79	9.32±1431	17.45±13.50*
Treatment	30.4±1.90	27.18±3.18	25.78±8.89	26.72±5.64	20.94±23.44	26.20±11.05*

*: Referred to significant difference, the number of animals in each group: 5 rats.

Table 5: Effect of hypothyroidism on thyroid hormones MDA compared to control group.
Values expressed as (mean±SD) ($p \leq 0.05$)

Period of pregnancy day	14.5	16.5	18.5	20.5	21.5	Total
Control	827.4±424.04	1109±805.75	1288.80±74.241	12656.60±602.46	12672.20±516.10	1151.60±605.48a
Treatment	12698±587.63	13066±442.06	977.8±268.09	1135.4±414.23	1373.8±589.85	1212.68±457.55a

a: Mean non-significant, the number of animals in each group: 5 rats.

3.2 Transmission electron microscopy study.

3.2.1 Thyroid gland of hypothyroidism maternal rats.

Results of (TEM) on specimens of rats with hypothyroidism showed the thyroid follicles were composed of cuboidal follicular epithelium cells with its organelles, pyknotic nucleus in the center of cells at the base of cells there was rich rough endoplasmic reticulum with asecretory granules of an adjacent follicle, the apical surface of follicle cell showed large numbers of small short projection (microvilli), the (c-cell) may be clear or inside the basal lamina of follicular cells, also the number of mitochondria, the basement membrane delineated the follicles, dense secretory granules appeared in the cytoplasm of each cell, and the lumen of follicle cell with colloid material the photograph clarified numerous capillaries with erythrocytes (fig 1 and 2).

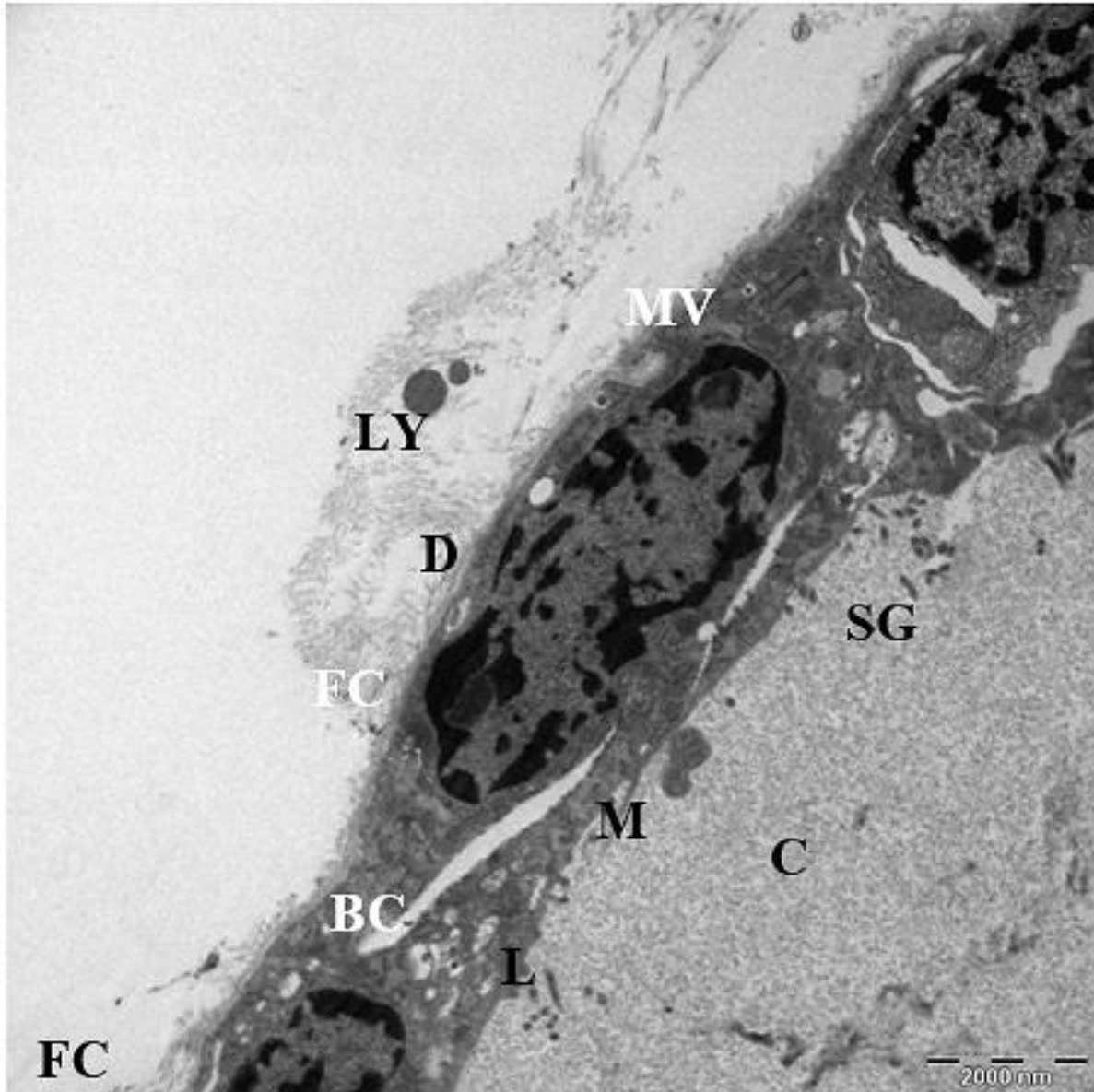


Fig (1): photograph of T.E.M show follicular cells (FC) with noticed colloid material (C), almost blood capillary (BC), packed microvillus (mv), few number of mitochondria (M) large number of lysosome (L), and few number of secretion granules (SG) in treated phase in pregnant rat of (20.5) day of gestation, 2000nm

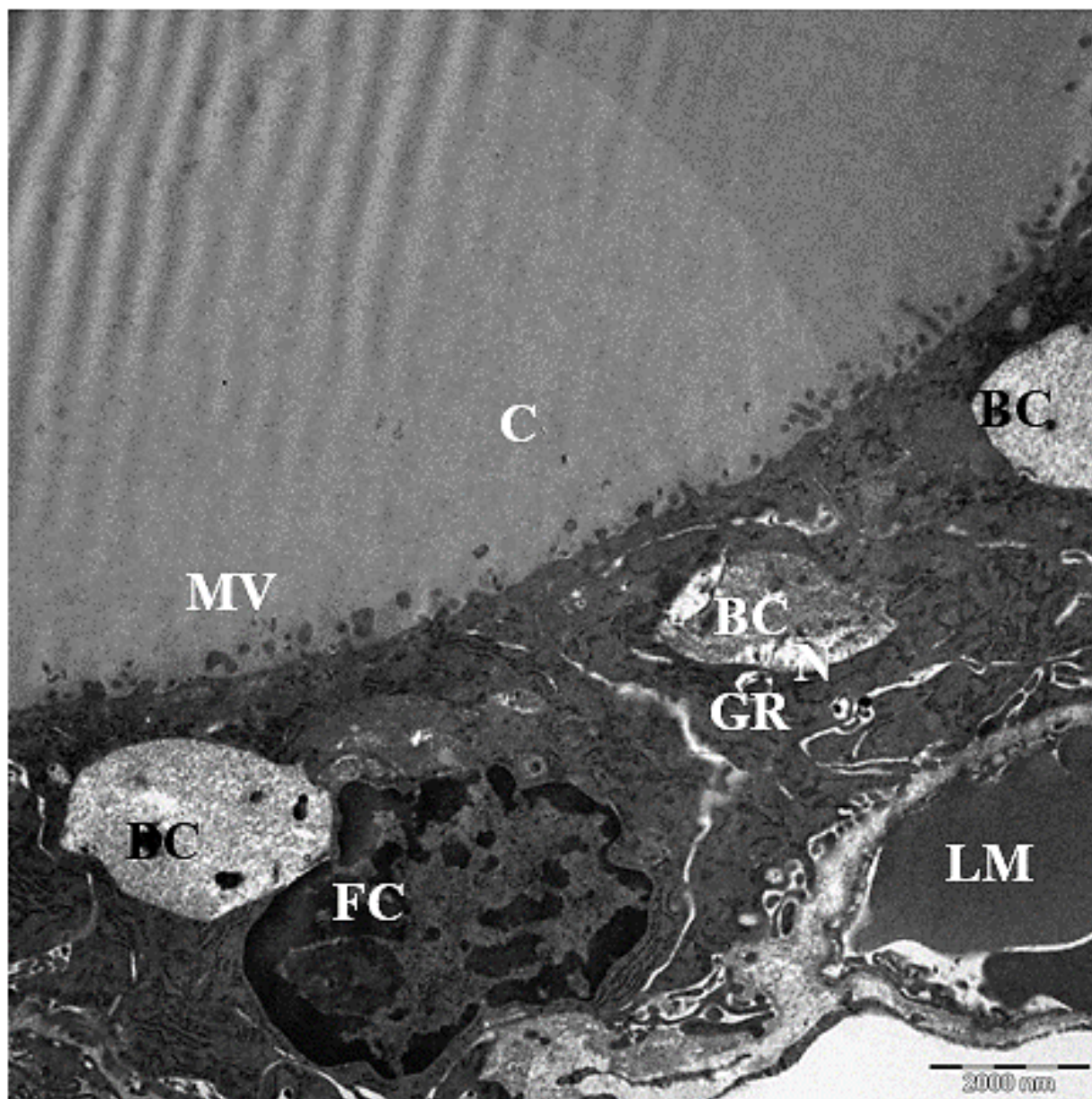


Fig (2): section of T.E.M on thyroid gland from pregnant rat treated with (ptu) showed large follicular cell (FC) apical microvillus (MV) facing lumen (LM) lymphatic (c) and alote of dilated rough endoplasmic reticulum (RER) few golgi apparatus (GR), dilated blood capillary (BC), 2000nm

Also, sections on the thyroid gland from pregnant rats at (20.5) day post gestation showed cells ultra-structural changes on follicular that appeared with

irregular microvilli at apical surface, dilated rough endoplasmic reticulum with developed cisternae, euchromatic nuclei, and thickening with basement membrane but less number of golgi apparatus with few secretory granules (fig 3 and 4).

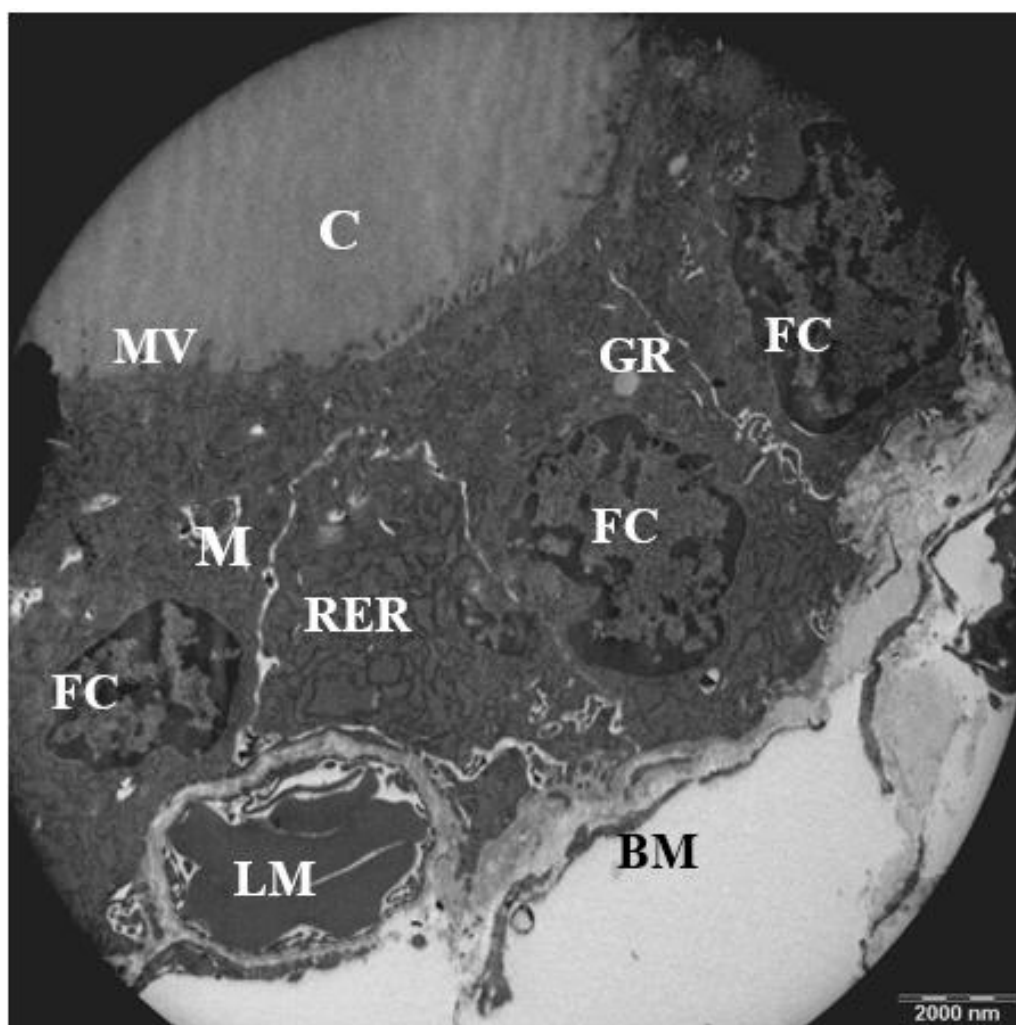


Fig (3): section of T.E.M show follicular cell (FC), colloid material (C), irregular microvillus (MC), less number of mitochondria (M), irregular dilated of rough endoplasmic reticulum (RER), undeveloped golgi apparatus (GR), treated with ptu of pregnant rat at (20.5) day of gestation, thickening basement membrane (Bm), lymphate vessels (LM) 2000nm

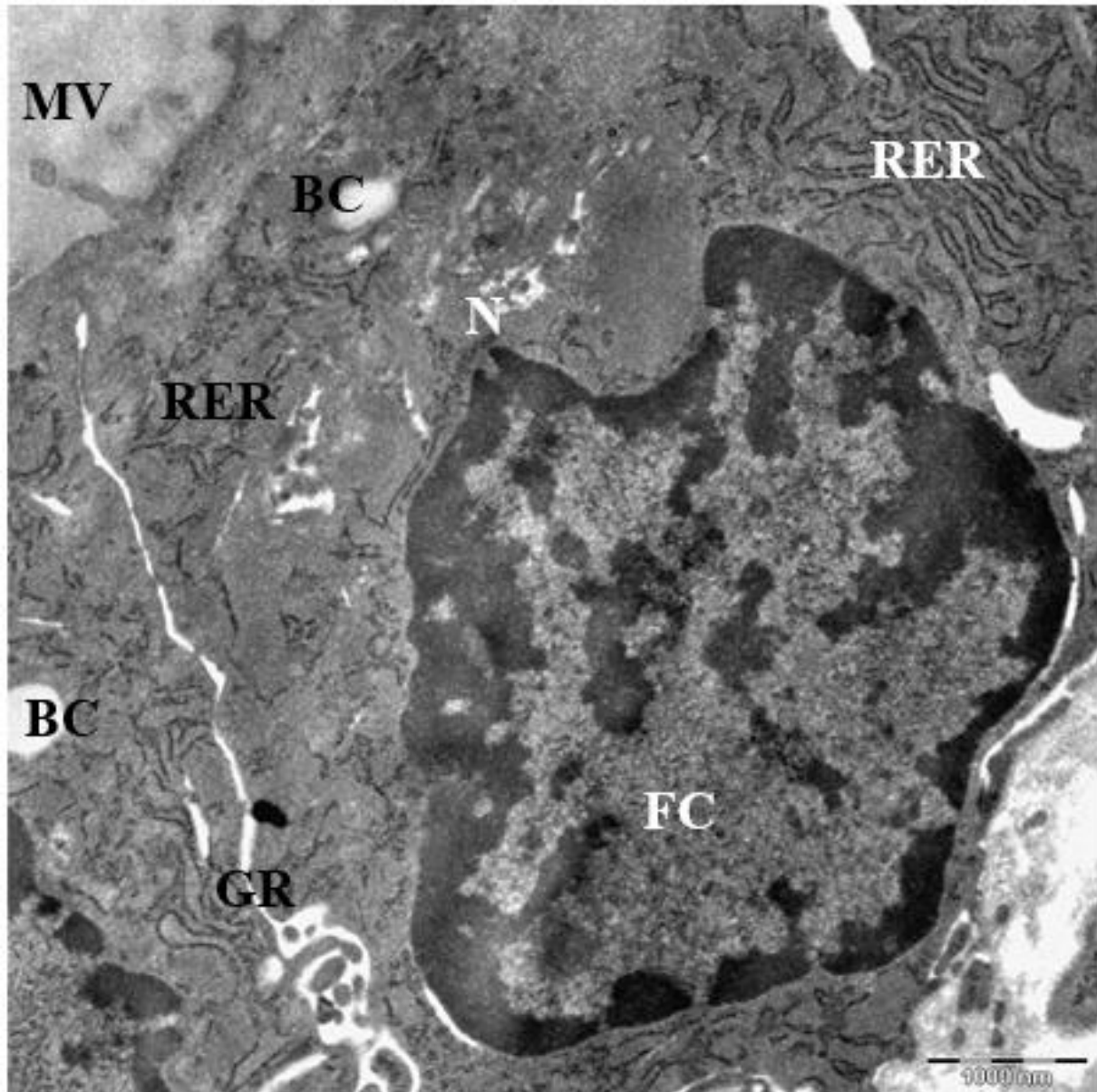


Fig (4): Section in rat thyroid gland treated with (ptu) showed follicular cell (FC) with much dilated rough endoplasmic reticulum (RER), few number of Golgi apparatus (GR) with undeveloped, bleb microvillus (MV) and nucleus (N) was euchromatic with irregular outline, number of blood capillary (BC), 1000nm

Electron micrograph on thyroid gland of fetus from the hypothyroidism rats showed degenerated cytoplasm of follicular cells with nuclei(rounded) and

dispersed chromatin, large number of mitochondria and lymph vessels were noticed (fig 5).

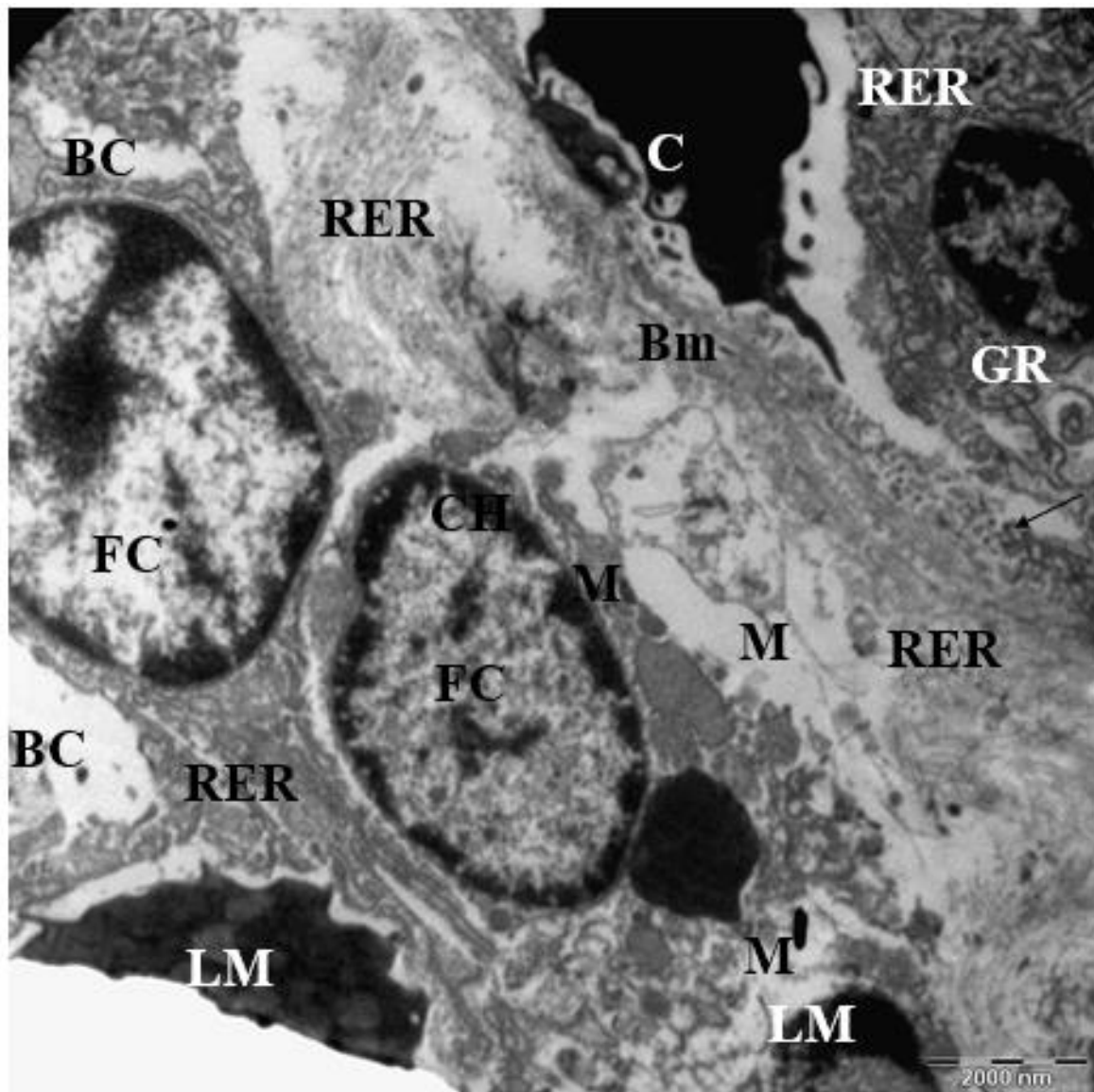


Fig (5): Electron micrograph on fetus thyroid gland from hypothyroidism group showed at the base of follicular cell (fc) appeared with rounded nuclei and dispersed chromatin (CH), thickening basement membrane (Bm), degenerative product (C), mediated (RER), well developed Golgi apparatus (GR), with secretory granules (→), less number of with dilated blood capillary (BC) and lymphotic vessels (LM) mitochondria (M), 2000 nm

The results clarified changes on the thyroid gland from fetus on (18.5) day post gestation like irregular, sparse microvilli on follicular cells apex and congested blood capillary, well developed rough endoplasmic reticulum and developed golgi apparatus closed to packed secretory granules (fig 6).

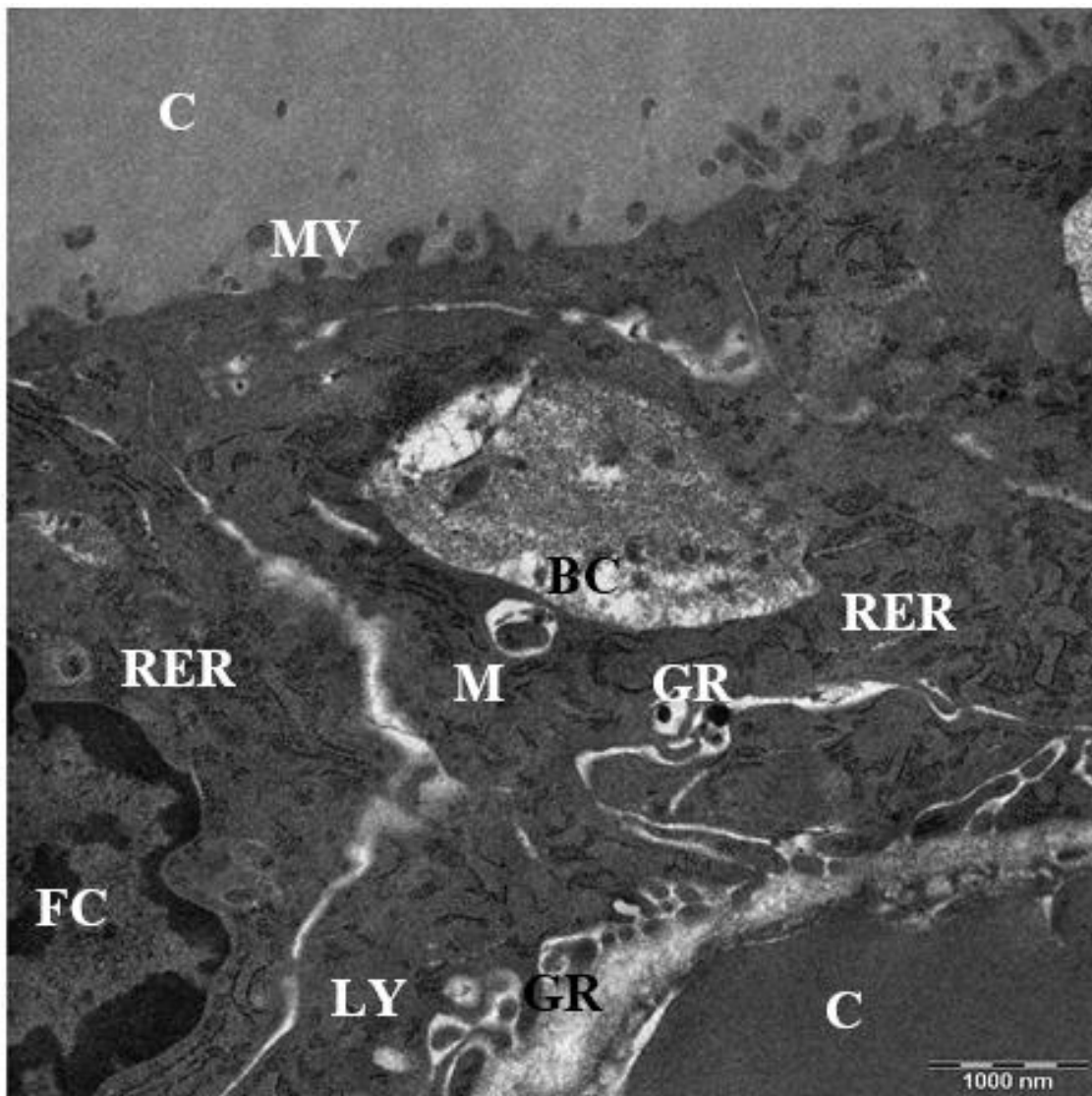


Fig (6): Photograph of T.E.M show colloid material (c), spares, irrargular microvillus (MV) and cisternae of well-developed of Golgi apparatus (GR) cisternae, section in fetus thyroid gland, irregular dilated of rough endoplasmic reticulum (RER), follicular thyroid cell (FC), abundant lysosomes (LY), 1000nm

Also, photograph referred to sections from fetus thyroid gland treated with propylthiouracyl (ptu) at (18.5) day of gestation appeared that the follicular cells full with deposite of iodine salt crystals and thick connective tissue septa separated between these follicular cells, and the parafollicular cells at the base was noticed (fig7).

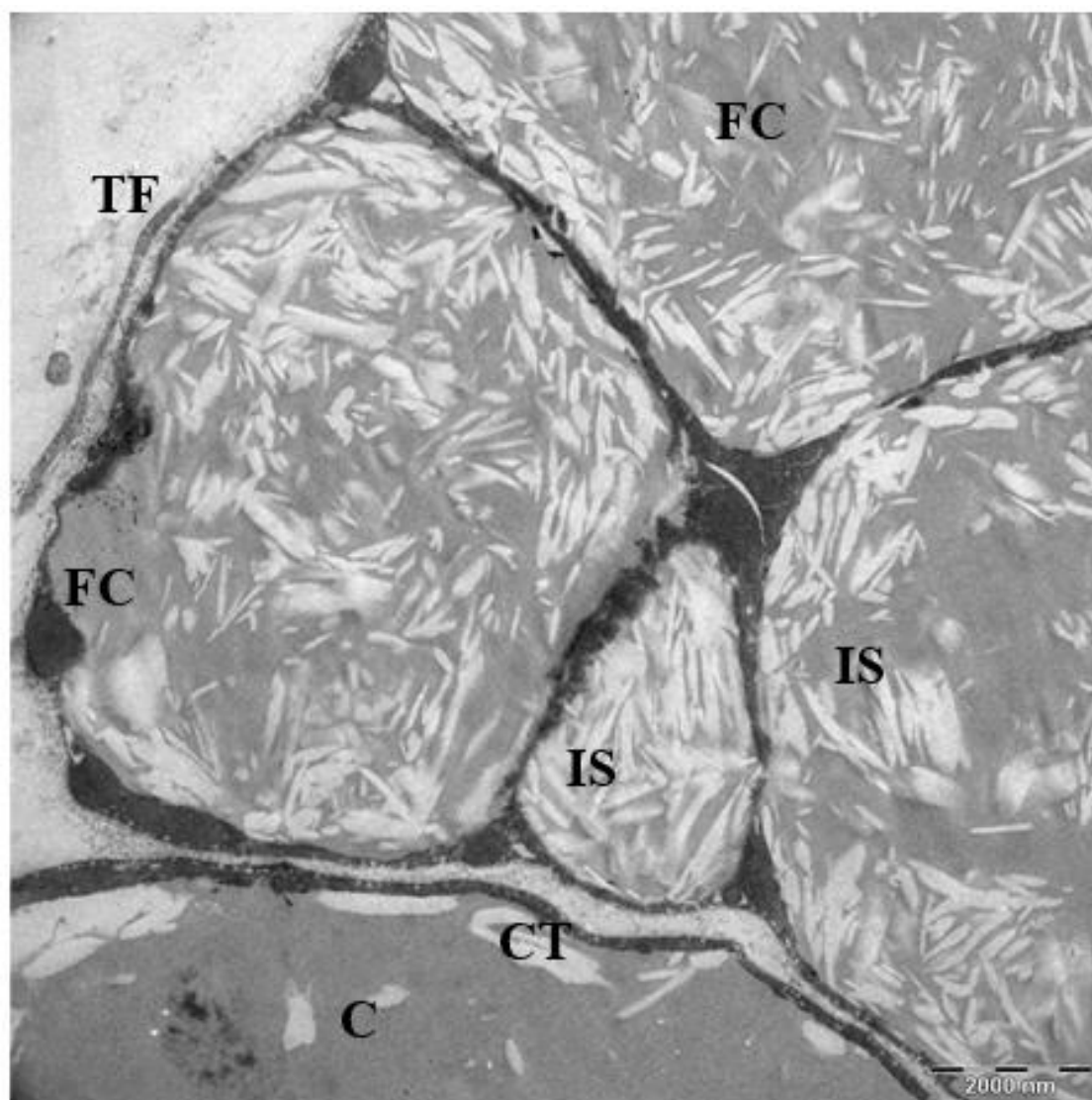


Fig (7): Electron micrograph of thyroid gland from rat embryo at (14.5, 16.5, 18.5, 20.5 and 21.5) day of gestation showed thyroid follicles (TF), lining with follicular cells (FC), crystals of iodine salt (IS) and septa of thick connective tissue (CT), 2000 nm

Discussion

The biochemical results referred to an increase in (TSH) and decreased in (T3 and T4) hormones with significant differences at ($P < 0.05$) in all hypothyroidism pregnant rats treated with (PTU) compared to the control group, this may be related to the important roles of these hormones as markers to thyroid gland function, this agreed with Kelly (2000) who commonly used these hormones as reliable indicators of thyroid function in humans and experimental model.

The thyroid gland is affected by (PTU) which may be caused inhibition in peroxidase and de-iodinase key enzymes involved in thyroid hormones biosynthesis, this led to decreased levels of circulating hormones that caused increased secretion of (TSH) by providing a growth stimulus to the thyroid (Udgata & Naik, 2007).

The recent results showed a significant decrease in (T3 and T4) in all pregnant rats with hypothyroidism from (10) day post gestation and continuing up to the delivery period, this may be suggested to the effect of (PTU) which caused changes with thyroid hormones appeared clearly at all gestation period, this result agreed with Buimer et al., (2008). Who discussed these results previously.

Ertek et al., (2010). investigated a relatively large number of samples and found a linear relationship between (PTU) and (TSH) in women with a different type of thyroid disorders such as thyroid autoantibody levels in healthy subjects, patients with autoimmune thyroid disease (AITD) and women with nodular goiter evaluated.

Also, an increase in oxidative enzymes (GSH and MAD) was recorded in this study in serum of all pregnant rats with hypothyroidism compared to control rats at different periods of gestation, this may be related to the effect of (PTU) which caused changes with thyroid gland structure, damaged of most follicular cells and disturbances in enzymes secretion this also discussed by other studies referred to decline in the level of (GSH) and this found to be associated with oxidative stress (Rahman et al., 2001).

The present study focuses on the ultrastructure changes in the thyroid gland related to hypothyroid rats and results showed most follicles small, irregular, vacuolated cytoplasm of follicular cells, the nucleus with irregular outlines, dilated cisternae of rough endoplasmic reticulum, less developed Golgi apparatus and increased with basement membrane thickness, these results may be caused by the (PTU) which disturb the normal structure, cytoskeleton of cells, damaged on plasma membrane, and this agreed with Kelly (2000); Soltaninejad et al. (2003) who suggested that many changes take place like dense colloid material deposited within cells resulted from colloid endocytosis and inhibition of phagocytosis/pinocytosis of the colloid (thyroglobulin) caused it to accumulate in the follicular lumen and this lead to diminishing the height of the follicular epithelium.

Dilation of (RER) in follicular cells of hypothyroidism thyroid gland illustrate the role of (RER) in damaged cells caused by (PTU) or may be reflect the metabolic activity needed in these cells, this confirmed by Jarrar (2001) who showed that the dilation caused since the injured cells need for oxidative enzymes which are required for detoxification.

Results of the present study revealed that (PTU) was a significant harmful effect of the structure and function of the thyroid gland, and these effects clarified the pathogenesis of (PTU) was multifactorial, interrupting protein synthesis (Erteak et al., 2010).

Electron microscopy also showed that thyroid cells were separated by two layers of basement membrane from capillaries which showed as dilated congested blood capillaries, this may be attributed to the high level of (TSH) hormone which induced follicular cells hypertrophy and increased vascularity.

The dilated of (RER) and nucleus irregularity which also recorded in this study discussed by El-Rouby (2010) who clarified that dilation of (RER) compressed the follicular cells nuclei causing its indentation and irregularity.

Photograph on section from fetus thyroid gland treated with (PTU) showed most follicular cells lining with flatted cells and this was caused by the present of colloid material inside it which caused convert of cuboidal cells to squamous, also an increase in lysosomes number, increased blood supply and this may be regarded to the functional state and reflect changes occurs because of (PTU) during pregnant period and the effect of mothers with hypothyroid on its fetus.

An increase in lysosomal number reflects the increase in the synthesis of hydrolytic and detoxifying enzymes secondary to the degenerative and apoptotic seen in many cells (Jarrar, 2001)

The figure referred to an accumulation of salts and iodine crystals within follicular cells cytoplasm this may be considered as a result of (PTU) which disturbs the enzymes that inhibit transformation rate from T4 to T3.

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