

## ***Development of the thyroid gland of New-Zealand white rabbit***

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The development of thyroid glands of New-Zeland rabbits was studied in 28 fetuses (10-30 days) and 28 rabbits (3 days-5 years). The thyroid premordia appeared at the 12<sup>th</sup> day of gestation as 2 masses of cords and clumps of cells. The gland assumed the bilobed form with a narrow isthmus in between at the 18<sup>th</sup> day. Small primitive follicles with narrow empty lumina appeared in 14-day-old fetuses. True follicles were recorded in 20-22 days old fetuses while colloid was seen in the lumina of the central follicles at the 24<sup>th</sup> day. Light (C- or Parafollicular) cells were seen for the first time at the 12<sup>th</sup> day. With development of the follicles, C-cells appeared between the follicular cells and some of them come in contact with colloid. The ultimobranchial body (UBB) was observed in 20 days old fetuses close to the thyroid anlage and entered into its tissue at the 22<sup>nd</sup> day. Later on, it gave thyroid-like follicles or ultimobranchial cysts. With the electron microscope (EM) follicular cells of early-developing fetuses were not yet differentiated. They contained few ill-developed rough endoplasmic reticulum (rER) and mitochondria. The organelles became well developed and the cells started to secrete colloid in full term fetuses. The maximum rate of secretory activity was achieved in the glands of adult rabbits. Changes in the thyroid glands during the postnatal life up to 5 years of age (age of senility) were followed up and described.

The thyroid gland is considered one of the most important endocrine glands since its hormones regulate the metabolic activities of all body cells including its own cells. Also, the gland plays an important role in the prenatal development of mammals (Gorbman and Evans, 1943 and Hill, 2003). Owing to these functions and others, a lot of studies had been done on the thyroid gland of different species of mammals (Amin, 1963; Fahmy and Moustafa, 1965; El-Gharbawy, 1986, Naser El-din *et al.*, 1988; Okada *et al.*, 1990; Elwan, 1994 and El-bargessy, 2001). Despite of this fact, rabbit thyroid gland received little attention. Therefore, the present investigation aimed to throw a spot of light on the development and structure of the thyroid gland in one of the economic breeds of rabbits; New-Zealand white rabbits.

### **Materials and methods**

The thyroid glands of 28 rabbit fetuses (10-30 days old) and of 28 male and female rabbits (3 days-5 years old) were fixed in 10% buffered neutral formalin and in Bouin's fluid. Early developing fetuses (upto16 days) were taken intact into fixatives. In other fetuses, the neck regions containing the thyroid glands were cut and fixed. After birth, the glands were dissected and fixed.

Serial paraffin sections of the thyroid glands

were cut and stained with H&E, Crossmon's trichrome, Gomori's reticulin and Weigert's elastic stain methods. The methods were done as described by Bancroft and Stevens (1982).

For transmission electrom microscope (TEM) examination, the thyroid glands of 3 fetuses (20, 25 and 29 days), one new born (7days), 3 adults (2, 4 and 6 months) and 2 senile rabbits (3 and 5 years) were fixed in 4 % gluteraldehyde and post-fixed in 1 % osmium tetroxide. Epoxy-resin embedded sections were then cut. One micron thick sections were stained with toulidine blue and ultra thin sections were stained with uranyl acetate and lead acetate (Hayat 1989).

### **Results**

**Prenatal development.** The thyroid gland was organized at the 12<sup>th</sup> day of gestation. The glands of 12-18 days old fetuses were found to be quite similar in structure. The gland's premordia appeared as 2 oval masses of cells lying close to the tracheal analage (Fig. 1). The two lobes were connected together by a transverse band of the same tissue (Fig. 2). Both were formed of irregular cords and clusters of darkly stained cells with central spherical nuclei (Fig. 3). The cell aggregates and were supported by a delicate mesenchymal tissue. Some of the cells arranged themselves to form small vesicles with narrow

empty lumina (Fig. 4). Development of the thyroid follicles began at the periphery of the gland at the 18<sup>th</sup> day of gestation. Occasionally, large pale cells with large central euchromatic nuclei were seen lodging themselves peripheral to some of the follicular cells (Fig. 5). As a general rule, many of the gland cells were observed at different stages of mitotic division.

Between 20-30 days of the prenatal life, significant changes could be observed in the developing glands. The thyroid follicles were progressively increased in number. Development of the follicles progressed centripetally. Also, the follicle became larger in size (Fig. 6) and was lined with cuboidal or columnar cells together with few parafollicular cells in between (Fig. 7). The latter were scattered singly or were aggregated as little groups of 2-3 cells. A very thin basement membrane surrounded the follicular and parafollicular cells together were found.

The follicular cells in 20-30 days old fetuses were considered to be inactive since their cytoplasm contained few ill developed rER, few ribosomes, small Golgi apparatus as well as few rounded mitochondria (Fig. 8).

Between the 24<sup>th</sup> and 26<sup>th</sup> days, the cells attained a secretory activity and a colloidal material appeared within some of the follicles (Fig. 9). The surrounding cells contained euchromatic nuclei and numerous well-developed rER, mitochondria and Golgi apparatus. Furthermore, short microvilli were seen projecting toward the colloid (Fig. 10).

The connective tissue framework of the gland became pronounced. It was represented by a delicate fibrous capsule around each lobe of the gland and around the isthmus by a reticular net existing between the follicles (Fig. 11).

Close to the thyroid premordia of 20 day-old fetuses, aggregates in the form of cords and clumps of cells forming the premordia of the ultimobranchial body (Fig. 12). The cells contained central spherical nuclei surrounded by a darkly stained cytoplasm. Between the above-mentioned cells, numerous lightly stained cells were frequently seen. Occasionally, follicles were observed to develop from ultimobranchial body cells (Fig. 13).

**Postnatal development.** The glands of newly born rabbits didn't vary greatly from those of full term fetuses. A fibrous capsule enveloped the gland's tissue and the follicles were surrounded by a vascular connective tissue mainly reticular

fibers (Fig. 14). The structure of the thyroid follicle was also unchanged. They became larger in size. The presence of colloid became a common finding in many of follicles (Fig. 15). Furthermore, some vacuoles appeared within the colloid (Fig. 16). As development proceeds (up to 30 days of age), the capsule became thicker, the follicles were larger in size and more colloid was stored. The number of intracolloid vacuoles was also increased. Fig. (17) showed that the follicular cells contained numerous electron dense vesicles as well as lysosomes. In addition, adjacent cells were seen connected together by desmosomes.

The ultimobranchial body became smaller in size, embedded in the thyroid tissue and cystic structures were developed from its cells. The cysts were lined with simple cuboidal epithelium together with some light cells in between. Frequently the lumina of the cysts were irregular.

Adult rabbits at 2-6 months of age showed that their glands became well developed and attained their maximum activity.

Large follicles containing variable amounts of colloid was the predominant picture. The surrounding cells were usually cuboidal (Fig. 18) and their cytoplasm contained well-developed rER, well-developed Golgi saccules, numerous lysosomes as well as multiple electron dense secretory vesicle (Fig. 19). Fig. (20) showed that the vascularity of the gland was at its maximum. Colloid within follicle was found taken by the follicular cells. It appeared as minute droplets, scattered in the apical part of the cytoplasm (Fig. 21). Many empty vacuoles appear in the stored colloid. Parafollicular cells were still few in number but they became much larger in size. Some of these enlarged cells came in contact with the colloid (Fig. 22).

Senile rabbits (3-5 years old) showed some changes that could be attributed to the advance of age. The follicles were more or less irregular in shape, smaller in size and contained remnants of shrunken colloid. The follicular cells were usually flattened with darkly stained cytoplasm and flattened pyknotic nuclei. The parafollicular cells also showed variable degrees of degeneration (Fig. 23) but not as marked as in the follicular cells. Frequently, the thyroid follicles and some of the cysts that developed from the ultimobranchial body showed some degenerative changes. The cells were smaller in size, shrunken with dark cytoplasm and pyknotic

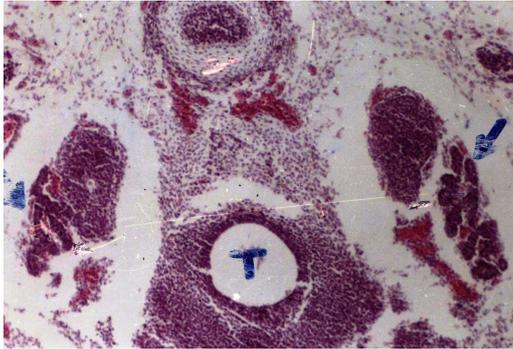


Fig. (1): Neck region of 12 day-old rabbit fetus showing the 2 oval masses of thyroid anlage (arrows) lying on the sides of the developing trachea (T). H&E, x100.

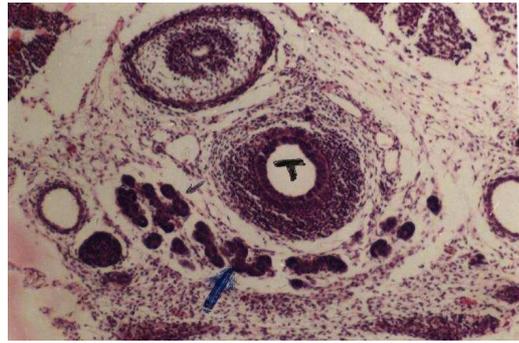


Fig. (2): Neck region of 18 day-old rabbit fetus showing a narrow cellular isthmus (arrow) in front of the developing trachea (T). H&E, x100

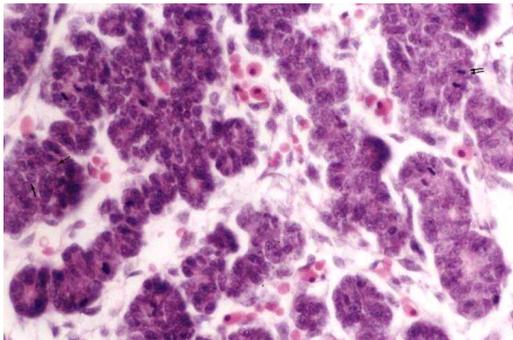


Fig. (3): Neck region of 12 days-old rabbit fetus showing that the thyroid premordia is formed of cords and clumps of cells supported by a delicate mesenchymal tissue. H&E, x400.

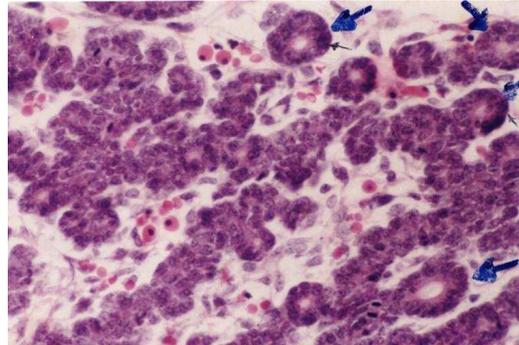


Fig. (4): Neck region of 14 days-old rabbit fetus showing the developing of some primitive follicles (arrows) at the periphery of the thyroid anlage. H&E, x400

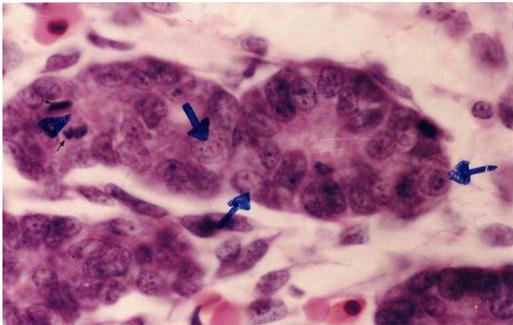


Fig. (5): Neck region of 14 days-old rabbit fetus showing 2 clusters of the thyroid premordia. Note the presence of light parafollicular cells (arrows) and one of the mitotically dividing follicular cells (arrow head). H&E, x1000.

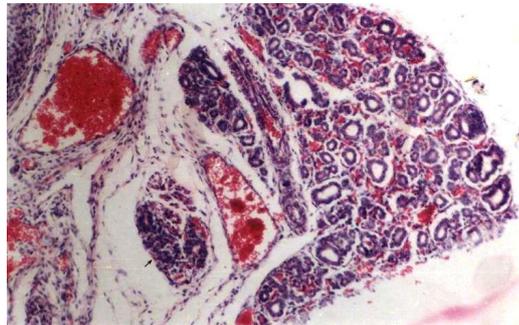


Fig. (6): Neck region of 20 days-old rabbit fetus showing progressively increased number of developing thyroid follicles. H&E, x100.

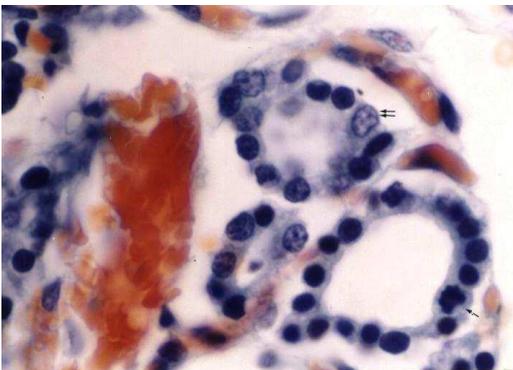


Fig. (7): Thyroid follicles of 25 days-old fetus showing few parafollicular cells (arrows) between the parafollicular cells. (Crossman's trichrome stain). x100.

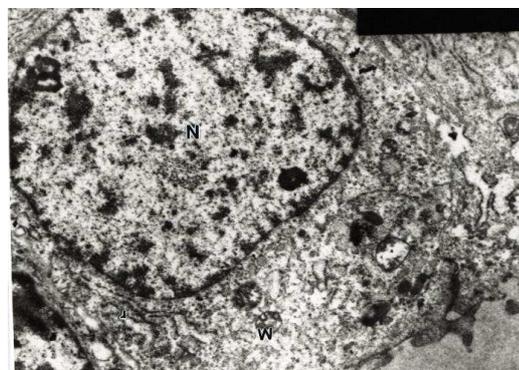


Fig. (8): Follicular cells of 20 days-old fetus showing few ill developed rER (R), nucleus (N) and few mitochondria (M). x7600.

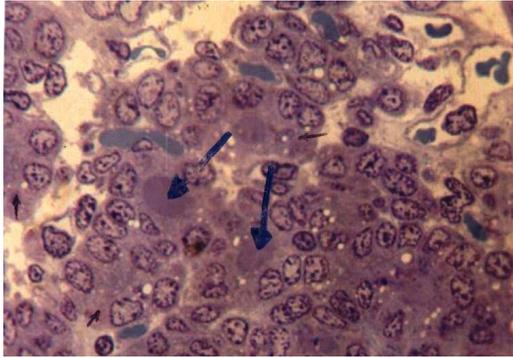


Fig. (9): Thyroid gland of 24 days-old fetus showing a colloid secretion within some of the follicles (arrows) toluidine blue, x1000.

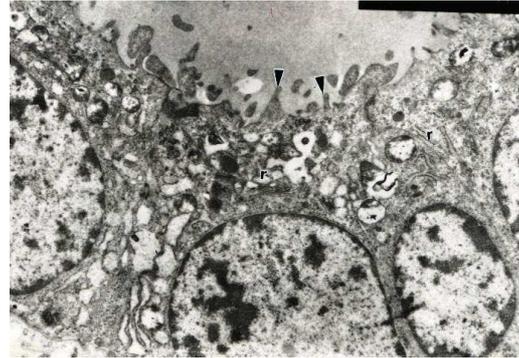


Fig. (10): Follicular cell of 25 days-old fetus showing numerous well-developed rER (r). microvilli were seen projecting toward the colloid (arrows). x5000.

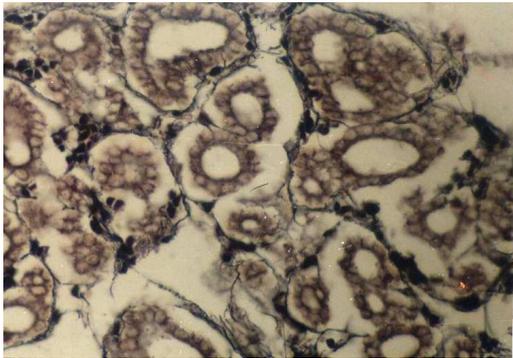


Fig. (11): Thyroid gland of 29 days-old fetus showing the reticular network between the follicle. Gomori's reticulin method, x400.

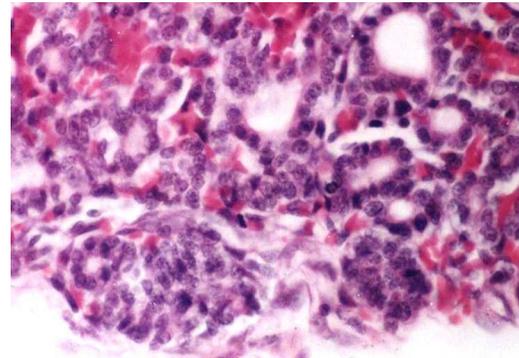


Fig. (12): Neck region of 22 days-old rabbit fetus showing that the ultimobranchial body (U) lying close to thyroid primordia (T). H&E. x 400.

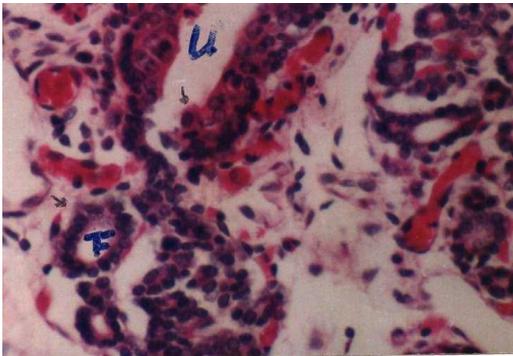


Fig. (13): Neck region of 30 days-old rabbit fetus showing development of some of the follicles (F) from the ultimobranchial body (u) H&E, x400.

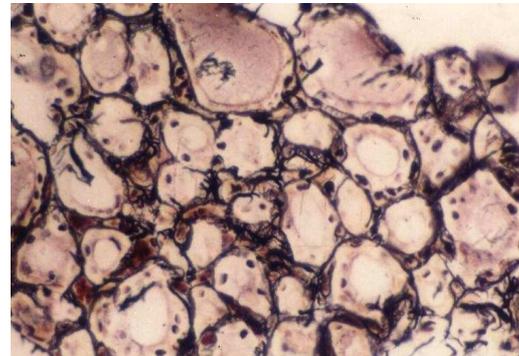


Fig. (14): Thyroid gland of one week-old rabbit showing the interstitial reticular stroma. Gomori's reticulin method, x400.

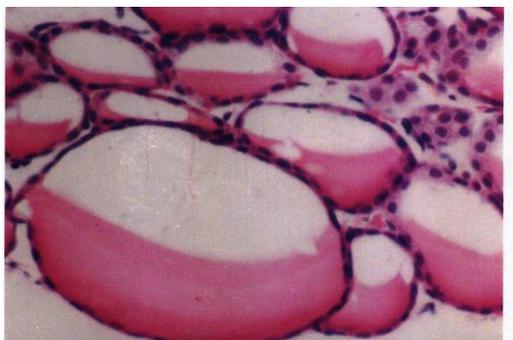


Fig. (15): Thyroid gland of 7 days-old rabbit showing that the follicles were larger and stored some colloid. H&E, x400

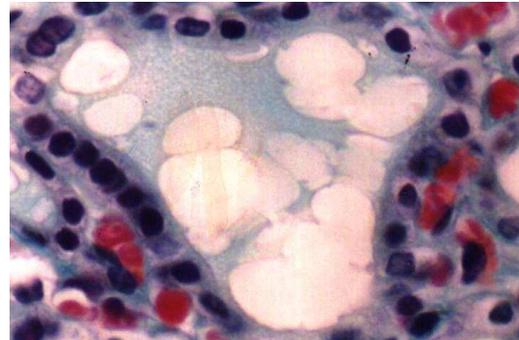


Fig. (16): Thyroid follicle of 2 weeks-old rabbit showing the presence of numerous vacuoles in the stored colloid. Crossman's trichrome stain. x1000.

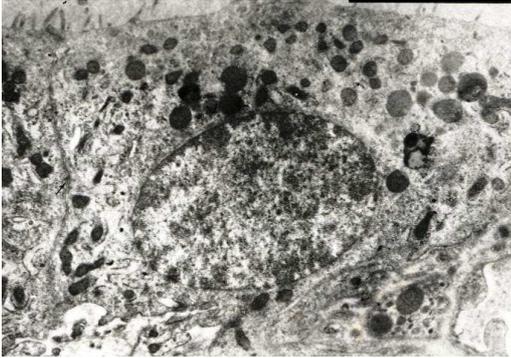


Fig. (17): Follicular cells of 7days-old rabbit showing numerous electron dense vesicle (V) and lysosome (L). Note the desmosome (D) (arrow) that connects the cell to the adjacent one. x7600.

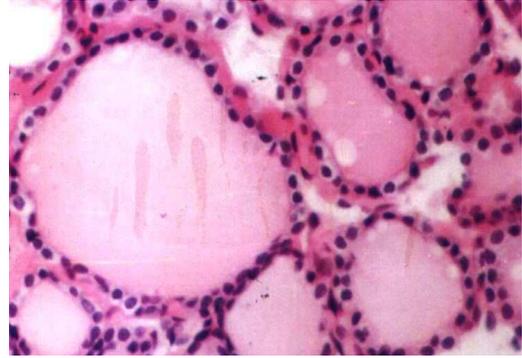


Fig. (18): Thyroid gland of one month-aged rabbit showing the well developed follicles. They were lined with simple cuboidal

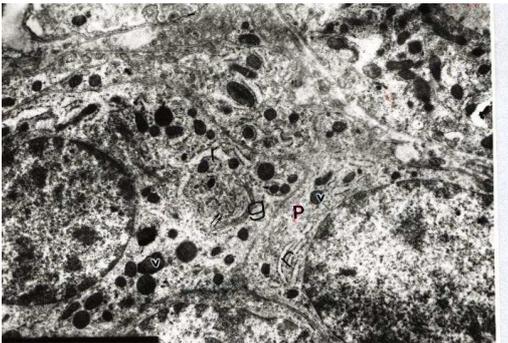


Fig. (19): Follicular cells of 4 months-old rabbit showing well developed rER (R), Golgi apparatus (G), lysosomes (L), and electron dense secretory vesicles (V) x5000.

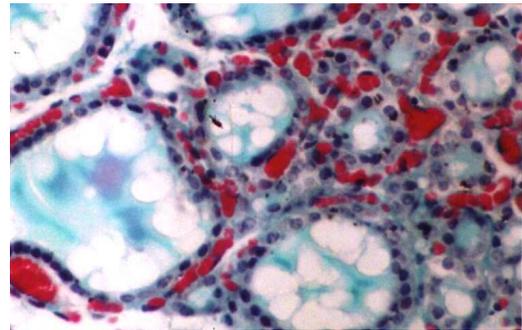


Fig. (20): Thyroid gland of 6 months-aged rabbit showing a vascular stroma between the well-developed active follicles. Crossman's trichrome, x100.

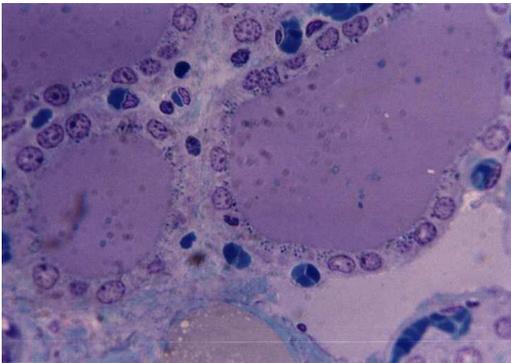


Fig. (21): Thyroid follicles of 6 months-aged rabbit showing the presence of minute colloid droplets in the apical cytoplasm of many follicular cells. Toludin blue, x400.

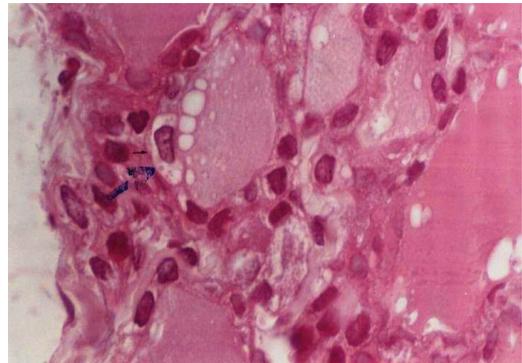


Fig. (22): Thyroid gland of 6 months-old rabbit showing enlarged parafollicular cells that come in contact with the colloid (arrows). H&E, x1000.

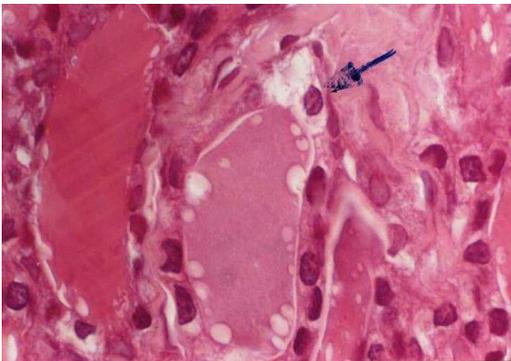


Fig. (23): Thyroid gland of a senile rabbit (5 years) showing hypertrophy and vacuolation of some of the parafollicular cells (arrow). H&E, x1000.

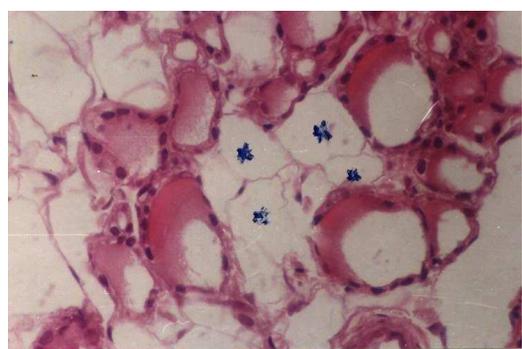


Fig. (24): Thyroid gland of a senile rabbit (5 years) showing shrinkage of the follicle and appearance of some adipocytes in the interstitial stroma H&E, x400.

nuclei. Sometimes these cystic structures contained aggregates of cell debris co-existed with many macrophages. Shrinkage of the thyroid follicles and decreased amount of colloid gave the chance to the interstitial stroma to increase in amount and some adipocytes to appear (Fig. 24).

### Discussion

It is generally accepted that the thyroid gland was developed as ventral out pocketing from the foregut between the first and second branchial pouches. Its premordia was recorded in 9 days-old opossum fetuses (Taniguchi *et al.*, 1990) and in 10-17 days old rabbits (Waterman and Gorbman, 1956 and El-Shammaa, 1996). In the present investigation, the gland's anlage was demonstrated at the 12<sup>th</sup> day of the rabbit's foetal life as 2 masses of thick irregular cords and as clumps of cells close to the tracheal premordia.

Rabbits seemed to be similar to other laboratory animals (Conde *et al.*, 1992 and El-Shammaa, 1996) and to domestic animals (Jordon *et al.*, 1973 and Elwan, 1994) in that the thyroid glands are formed of follicles. Regarding their origin, 3 possible sites were suggested for the development of the thyroid follicles; thyroid premordial cells, ultimobranchial cells and by budding from pre existing follicles (Krause and Cutts, 1983; El-Gharbawy, 1986; Elwan, 1994 and El-Shammaa, 1996). The third postulation by (Isler *et al.*, 1968) that the so-called budding was in fact tagential sectioning of the follicles was not accepted.

The development of the thyroid follicles was found to be interesting. In rabbits and rats (Mitskavitch, 1957), buffaloes (Fahmy and Moustafa, 1965) and camels (El-Gharbawy, 1986), the follicles began to develop in the periphery of the gland, and then extended centrally.

Primitive follicles with narrow empty lumina were demonstrated in 14 days-old rabbits fetuses. The cells were considered to be inactive since they contained few ill-developed rER, Golgi apparatus and mitochondria. The follicles became well developed in 20-22 days old fetuses and some of them contained a little amount of colloid at the 24<sup>th</sup> day. Similar sequences in rabbits were recorded by (Waterman and Gorbman, 1956; El-Shammaa, 1996; Taniguchi *et al.*, 1990), while follicles appeared in 15 days old fetuses of hamsters and started to secrete colloid 0-3 days after birth (Taniguchi *et al.*, 1990). In domestic animals, the development of

thyroid follicles and appearance of colloid was found to be variable (Mitskavitch, 1957 and Badawy *et al.*, 1983). It might extend to 3 weeks prior to birth in dogs (Nunez and Gershon, 1975).

The activity of the follicular cells to synthesis and to secrete thyroglobulins (colloid) and to reabsorb colloid to secrete active hormones was taken in consideration. In the literature, it had been found that the secretory activity of the gland cells began during the latter stages of fetal development in animals having a long gestation period as camels and buffaloes and postnatally in animals having short gestation period as rabbits and rats (Hall and Kaun, 1942 and Mitskavitch, 1957). This generalization could not be easily accepted since colloid started to be seen within follicles in 24-26 days old rabbit fetuses. The maximum rate of secretory activity, however, was detected in glands of adult rabbit. During this period (2-6 months), colloid accumulated in the follicles and vacuoles appeared in the colloid. Bank (1993) considered the presence of vacuoles in the colloid as a sign of secretory activity. Furthermore, so many cells of the rabbit's follicular cells had been found to contain numerous minute droplets of colloid in their apical cytoplasm.

Data gained from the EM graphs indicated that cells were actively secreting. They contained all cytoplasmic organoids that could synthesize glycoprotein (thyroglobulins). Numerous well-developed rER, mitochondria and Golgi apparatus were observed. Furthermore, short microvilli could be observed projecting from the follicular cells toward the colloid.

The development of parafollicular cells was found to be variable among different animals. Cells were demonstrated in opossum after birth (Krause and Cutts, 1983), they were described as light cells in the fetuses of rabbits (El-Shammaa, 1996), rats (Conde *et al.*, 1992), sheep (Jordon *et al.*, 1973), buffaloes (Elwan, 1994) and camels (El-Gharbawy, 1986). In rabbits, the parafollicular cells were recorded for the first time between the thyroid cord cells and between the epithelial cells of the ultimobranchial body cells at the 12<sup>th</sup> and 20<sup>th</sup> days old fetuses, respectively.

It had been suggested that the parafollicular cells were derived from the ultimobranchial body (Roy and Saigal, 1986 and Harash, 1987), neural crest and then carried by the

ultimobranchial body to the thyroid anlage (Pearse and Polka, 1971) or were derived from the follicular cells which have lost contact with colloid (Sarker and Isler, 1963 and Boyd, 1964). The last hypothesis could not be accepted since so many cells were seen coming in contact with colloid. The last finding had been observed in the glands of many animals including rabbits (Roy and Yadava, 1975; Badawy *et al.*, 1978; Ewis *et al.*, 1982 and Yanani *et al.*, 1982).

In 1990, Taniguchi and his co-workers stated that the UBB in hamster was derived from the 5<sup>th</sup> pouch and fused with the thyroid in the 12<sup>th</sup> day of gestation. In rabbits, the body was observed close to the thyroid anlage at the 20<sup>th</sup> day as a compact cell masses surrounded by a highly vascularized connective tissue. At the 22<sup>th</sup> day, the body became completely embedded in the thyroid tissue and at the 26<sup>th</sup> day, it was transformed into cystic structures similar to those which were derived from the thyroid anlage. Formation of thyroid follicles from the UBB was found to be a common finding in many other animals as rats (Conde *et al.*, 1992), sheep (Jordon *et al.*, 1973), buffaloes (Elwan, 1994) and camels (El-Gharbawy, 1986).

UBB as well as thyroid follicles in senile rabbits underwent cystic degeneration and numerous cell debris as well as many macrophage cells were recorded in their lumina. Similar degenerative changes were also demonstrated in the UBB of many other animals as rats (Conde *et al.*, 1992), dogs (Booth and Gloschal, 1978) and buffaloes (Elwan, 1994).

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