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Original Article

Effects of Hypothyroidism and Thyroxin Replacement on Postnatal Development of Lumbar Vertebrae in Albino Rats

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ABSTRACT

Background: Normal skeletal development and adult bone maintenance requires normal level of thyroid hormones. Growth arrest, delayed bone maturation, and epiphyseal dysgenesis are fundamental result of hypothyroidism in children.

Aim of the Work: To detect the effects of hypothyroidism and thyroxin treatment on the postnatal development of lumbar vertebrae in albino rats.

Materials and Methods: Sixty adult female rats were randomly subdivided into three equal groups; control group (received distilled water), carbimazole-treated group (received carbimazole in a dose of 6 microgram/gram body weight daily orally from 10th gestational day till the day 21 postnatally then carbimazole administration was continued to pups until the end of 8th week postnatally) and carbimazole+thyroxin treated group (received carbimazole on 10th gestational day in a dose of 6 microgram /gram body weight orally and thyroxin at birth in a dose of 10 microgram/kg/day orally and the administration of both drugs was continued until the day 21 postnatally then the administration was further continued to pups until the end of 8th week postnatally). The offsprings at ages (newborn, two weeks and eight weeks) anesthetized, sacrificed, their lumbar vertebrae were manibulated for histological examination. Morphometric and statistical studies were performed.

Results: The lumbar vertebrae markedly affected by hypothyroidism especially the proliferating zone and the collagen component of the matrix. Morphometric results revealed highly-significant decrease in the thickness of the epiphysis in hypothyroid rats. Most of these changes reverted by giving thyroxin.

Conclusion: Vertebral column growth is markedly affected by hypothyroidism and thyroxin replacement has a protective role.

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Key Words: Hypothyroidism, rat, thyroxin, vertebrae.

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INTRODUCTION

The basic structural and supportive tissue of the body is bone (or osseous tissue). Cells like (osteocytes, osteoblasts, osteoclasts) and extracellular matrix (ECM) are the main constituents of the bone tissue. Bone-forming cells are called osteoblasts which lay a matrix composed of collagen, but they also liberate calcium, magnesium, and phosphate ions, which harden and mix within the bone matrix into the mineral hydroxyl apatite. Bone is harder than cartilage without being brittle because it is formed of combination of hard mineral and flexible collagen. The organic component (collagen of type I and other proteins) and a nonorganic one (mainly hydroxyl apatite) are the main constituents of bone matrix[1-4].

Matrix of the Bone is formed of organic and non-organic constituents[5]. The combination of organic and non-organic components makes the bone harder and more resistant. Collagen fibers mainly type I collagen and glycosaminoglycans that are associated with proteins represent the organic component. The non-calcified organic matrix is formed of osteoid. Calcium, phosphorus, with smaller amounts of bicarbonate, citrate, magnesium, potassium, and sodium represent the non-organic components. These components form about 50% of the dry weight of bone matrix[6].

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The fetal skeleton develops by two different processes: intramembranous and cartilaginous (endochondral) ossification. The first one forms flat bones of the skull while endochondral ossification forms all long bones[7]. Many hormones, cytokines, and growth factors as thyroid hormone T3 (the active derivative of T4) tightly control endochondral ossification[3,8].

Growth plate (physis) in mammals is highly specialized mesoderm derived cartilaginous structure. It develops in the bone epiphysis, after appearance of the primary ossification centres and is essential for bone elongation. The plates are formed by numerous cells: reserve cells, proliferative cells, hypertrophic cells and apoptotic or degeneration cells. After puberty, cartilage is completely replaced by bone, and fusion between the epiphyseal plates with primary and secondary ossification centres is noticed as a result of reduction cell division in the epiphyseal cartilage[9].

Early in development, there is differentiation of each somite that contribute to the vertebral column and associated musculature[10,11].

Numerous genetic, nutritional, environmental, and hormonal factors affect growth[12]. Growth and differentiation of tissues in many mammals is markedly influenced by thyroid hormones[13].

Binding of triiodothyronine (T3), to its receptors (TRs) is the method by which thyroid hormone (TH) performs its actions[14]. Normal skeletal development and adult bone maintenance requires normal level of thyroid hormones[15]. TH action is essential requirement for bone development, maturation, growth and metabolism. Thyroid receptors such as TRα1, TRα2 and TRβ1 in osteoblasts[16], osteoclasts and chondrocytes, has been recognized and the marked affection of bone cells by TH when isolated in cell culture is evidence of a direct action of T3 on bone[17].

Carbimazole, belong to a thionamide group, which completely metabolized to the active form, known as Methimazole. Carbimazole suppress the synthesis of thyroid hormones by inhibition of the organification of iodide and the coupling of iodothyronine residues. Carbimazole is a good agent for inducing hypothyroidism[18]. Abnormal development or death results from exposure to inappropriate levels of TH[19].

Growth arrest, delayed bone maturation, and epiphyseal dysgenesis are fundamental results of hypothyroidism in children. Marked abnormalities in the epiphyseal cartilage plates (EGP), such as mal-arrangement of the proliferative zone, impaired differentiation of hypertrophic chondrocytes and abnormal formation of extra cellular matrix are founded as a result of inadequate levels of TH during post-natal development[20], leading to growth retardation and other abnormalities[21]. More ever, endochondral and intramembranous ossification showed a generalized delay[22]. In this study, we aimed to study the histological, ultrastructural, and morphometric alterations on the postnatal growth of axial skeleton, represented by the lumbar vertebrae, under the effect of hypothyroidism and thyroxin replacement.

MATERIALS AND METHODS

Chemicals and Drugs

Carbimazole: 5 mg round, pale pink tablets were used It was purchased from Amdipharm UK Limited, Capital House, 85 King William Street, London, EC4N 7BL, UK.

L- Thyroxin: 50 microgram white, circular and biconvex tablets were used. It was purchased from Glaxosmithkline pharmaceuticals Ltd. Actavis, Barnstaple, EX32 8NS,UK. All other chemicals used were of high commercially grade.

Animals

A total number of sixty adult female and 30 adult male albino rats (aged 3 months) weighting about (200) gram were obtained from animal house, Faculty of Medicine, Assiut University. The albino rats were kept in a room with a 12:12 hours light dark Cycle and were kept under controlled temperature. Food and water were available ad libitum. Matting was allowed between Female and male rats overnight in the ratio of 2 females for one male, gestational day one was recognized by presence of a vaginal plug. The experiment was carried out in accordance with the internationally accepted guidelines for the care and use of laboratory animals.

Experimental design

The pregnant female rats were randomly subdivided into three groups (twenty rats for each group):

Group I (Control group): In this group, rats received distilled water daily by means of oro-gastric tube from 10th gestational day until the end of 8th week postnatally.

Group II: (Carbimazole-treated group): In this group, rats (dams) were administered

carbimazole to make pups congenitally hypothyroid in a dose of 6 microgram /gram body weight daily by means of oro-gastric tube (the tablets were gridded and dissolved in water). The drug was administered from 10th gestational day till the day 21 postnatally then the administration was continued to pups until the end of 8th week postnatally[35].

Group III (Carbimazole + thyroxin treated group): In this group, rats (dams) received carbimazole before birth (on 10th gestational day) by means of oro-gastric tube in a dose of 6 microgram /gram body weight and thyroxin at birth (the tablets gridded and dissolved in water) by means of oro-gastric tube in a dose of 10 microgram/kg /day and the administration of both carbimazole and thyroxin was continued until the day 21 postnatally. The administration of both drugs was further continued to pups until the end of 8th week postnatally[35].

The male pups (offsprings) at (newborn, two-weeks and eight weeks old) were anaethesized by ether inhalation followed by excision of the chest wall and intracardiac perfusion with saline and 4% paraformaldehyde solution. The lumbar vertebrae were removed.

Histological techniques

Light microscopic study

For light microscopic examination, the lumbar vertebrae of all groups were rapidly excised and were fixed in 10% formalin for 48-72 hours. Then the tissues were decalcified, dehydrated in ascending grades of alcohol, put in xylene and embedded in paraffin. Serial sagittal sections about 5-7 µm- in thickness were cut and stained by Hematoxylin and eosin stain for demonstration of the general histological structure, Masson's trichrome stain for demonstration the collagen fibers and toluidine blue stain for demonstration glycosaminoglycan content of cartilage matrix[23].

Decalcification

It was done to make the bone easy to cut by the microtome. Two methods were used; (A) Decalcification was done by neutral ethylene diamine tetraacetic acid (EDTA). EDTA was prepared by dissolving 250 gram (gm) of EDTA (di-sodium salt) in 1750ml distilled water. The solution was neutralized by sodium hydroxide (PH. 7.0)[24]. The specimens were immersed in decalcifying fluid for about 4-40 days depending on the size and composition of the specimens. EDTA was renewed every 48 hours. (B) Another method of decalcification was used (formic acid-sodium citrate). This was done by adding 35ml formic acid in 65 ml sodium citrate (20 percent). A large volume of fluid was used and renewed every 48 hours. Decalcification took from 20 to 30 days depending on the size and composition of the specimens[24].

Electron microscopic technique

For electron microscopic examination, the lumbar vertebrae in each group were extracted and fixation in 4% cold gluteraldehyde was done after dissecting the animal for 24-48 hours then decalcification was done. Washing in phosphate buffer (PH7.2) 3-4times for 20 minutes each was done and post fixed in 1% OsO4 for 2h, after that washed in the same buffer 4 times. Dehydration by ascending grades of alcohol was done and embedding in epon araldite mixture. Semithin (0.5-1um) were obtained. Cutting off sections was done with KIB ultramicrotome. Staining was done with toluidine blue stain to identify tissue component such as nuclei, membranes, and intra cytoplasmic organelles of the cells[23]. Ultrathin sections (50-80 nm) from selected areas of reserve cells and proliferative cells of the epiphyseal cartilage plate of lumbar vertebrae were examined. The ultrathin sections were mounted on copper grids, examined by a transmission Jeol-JEM-100 CXII electron microscope and photographed at the electron microscope unit of Assiut University.

Morphometric and Statistical studies

Weight, tail length and crown rump length of 8 weeks old rats were measured. In addition, the thickness of epiphyses of lumbar vertebrae of 8 weeks old rats were measured at magnification of 100 using image analyzer (Leica Q 500 M.C) in histology department, Faculty of medicine, Assiut University. Comparison between control, treated and rehabilitated was done by ANOVA test. This technique was done by using Hematoxylin and eosin stained sections in about twelve sections chosen randomly belonging to six animals in each group. Mean values ±standard deviation (SD) were obtained and analyzed using ANOVA test.

RESULTS

I. Light microscopic results

A. New born rats

By light microscopic examination of the newborn control rats in the current study,

Haematoxylin and eosin and Masson's trichrome stained sections revealed that each lumbar vertebra is formed of a body, anteriorly, and a neural arch with a projecting spine, posteriorly. The body of each lumbar vertebra is composed of central part (centrum) and two peripheral areas of hyaline cartilage (epiphysis). The body of vertebra is seen surrounded by periosteum and exhibiting a primary ossification center (plate1; Figure1).The chondrocytes in the epiphysis are arranged forming zones traced from the periphery toward the centrum, zones are arranged as follow: (plate1; Figure1, plate2; Figure5).

1. Zone of reserve cartilage: in this zone the chondrocytes are arranged parallel to the articular surface. The cells are small and flat with rounded deep basophilic nuclei (plate1; Figure3).
2. Zone of proliferation: Chondrocytes in this area appeared slightly larger in size than those of the reserve zone. They are oval to round with rounded nuclei. They are enclosed inside lacunae with compressed matrix in between (plate1; Figure3).
3. Zone of hypertrophy: Chondrocytes are seen arranged in short longitudinal columns. The chondrocytes of this region are larger than the chondrocytes of the previous zone. They are enclosed inside large rounded lacunae. The extracellular matrix is seen compressed into linear bands between longitudinal rows of chondrocytes (plate2; Figure5).
4. Zone of degeneration: Hypertrophied chondrocytes became ruptured and the open ends of their vacated lacunae are being invaded by cells from the marrow filled spaces of the centrum (plate2; Figure5).

The centrum of each lumbar vertebra showed a well-developed primary ossification center consists of multiple spaces in the calcified cartilage representing empty confluent lacunae after chondrocyte degeneration. The centrum is enclosed by a thin collar of compact bone (the periosteal bone) (plate1; Figure1). The intervertebral disc appeared to be composed of an outer fibrous layer (annulus fibrosus) and a gelatinous core (nucleus pulpous) (plate2; Figure5).

Toluidine blue stained sections showed that the cartilage matrix appeared homogenous and composed of two regions; the darker territorial matrix which is the region surrounding the lacunae and the lighter interterritorial matrix (plate2; Figure7). In sections stained by Masson's trichrome, the cartilage matrix appeared homogenous and green in colour (plate2; Figure5).

In carbimazole treated group, H&E stained sections of the vertebrae of the newborn rats showed that, the chondrocytes of the proliferation zone in the epiphysis appeared smaller in size and had vacuolated cytoplasm with abnormally arranged and distorted cells (plate1; Figure4). Sections stained by toluidine blue and Masson's trichrome stains revealed that the matrix lost its differentiation into territorial and interterritorial matrix with diminished staining (plate2; Figures 6,8).

B. Two weeks old rats

By light microscopy, H&E stained sections of two weeks old control rats showed that the body of each lumbar vertebra is composed of central part (centrum) and two peripheral areas of hyaline cartilage (epiphysis) (plate3; Figure9). Examination of the proximal epiphysis of the vertebral body revealed that the hyaline cartilage consists of chondrocytes within their lacunae with an extensive extracellular matrix composed of fibers and ground substance (plate3; Figure12).

The chondrocytes in the epiphysis are arranged forming zones. Traced from the periphery toward the centrum, zones are arranged as follow (plate3; Figure12).

1. Zone of reserve cartilage (germinal zone).
2. Zone of proliferation (replication zone).
3. Zone of hypertrophy (transformation zone).
4. Zone of calcification (apoptotic zone).

Sections stained with H&E and Masson's trichrome stains showed that the intervertebral disc has two components: The annulus fibrosus and the nucleus pulposus. The annulus fibrosus has an outer layer of connective tissue, but it is primarily composed of overlapping layers of fibrocartilage in which collagen bundles are organized in adjacent layers .The nucleus pulposus is situated in the center of the annulus fibrosus and is formed of a few rounded cells embedded in a viscous matrix (plate3; Figure9, plate4; Figure15). Toluidine

blue stained sections showed that the territorial, or capsular, matrix, stained differently from the rest of the matrix (interrterritorial) (plate5; Figure18).

In carbimazole treated group, with H&E, examination of the epiphysis of the lumbar vertebrae showed that the cells of epiphysis appeared disorganized (plate3; Figure10). As regard the reserve cell zone, the cells appeared degenerated and distorted with areas of matrix devoid of cells. The chondrocytes in the proliferating zone, showed atrophy in some regions with loss of short longitudinal column arrangement (plate4; Figure13). The matrix in this group exhibited diminish staining of Masson's trichrome and toluidine blue stains (plate4; Figure16, plate5; Figure19). Nucleus pulposus of the intervertebral disc appeared shrunken as compared with control (plate3; Figure10).

In thyroxin treated group, examination of the lumbar vertebrae in sections stained with H&E showed maintainance of normal arrangement of condrocytes in the epiphysis. There are areas of matrix devoid of cells especially in the reserve cell zone where its cells are deformed and distorted (plate3; Figure11). As regard the proliferative cell zone, their chondrocytes appeared to be normal (plate4; Figure14). The matrix in this group exhibited a little diminished staining of Masson's trichrome and toluidine blue stains as compared to control group (plate5; Figures17,20). The intervertebral disc appeared normal (plate3; Figure11, plate5; Figure17).

C. Eight weeks old rats

By light microscopic examination, the epiphysis of lumbar vertebrae of eight weeks old control rats in H&E stained sections, revealed THE presence of epiphyseal cartilage (articular cartilage) which separates the epiphyseal growth plate cartilage from the intervertebral disc. At the periphery of hyaline cartilage, young chondrocytes have an elliptic shape, with the long axis parallel to the surface. In the epiphyseal growth plate cartilage the chondrocytes are arranged in zones as follow (plate6; Figures21,24).

1. Zone of reserve cartilage.
2. Zone of proliferation.
3. Zone of hypertrophy.
4. Zone of calcification.

The matrix in this group showed normal homogenous staining by Masson's trichrome and toluidine blue stains (plate7, Figure27, plate8, Figure30).

Carbimazole treated group showed that examination of the epiphysis of the lumbar vertebrae in H&E stained sections, revealed apparent decrease in the overall thickness of epiphyseal cartilage and the growth plate cartilage with loss of normal alignments of the chondrocytes (plate6; Figure22). There is wide areas of matrix devoid of chondrocytes and loss of the normal longitudinal arrangement of the proliferating zone with degenerated cells In the hypertrophied cell zone (plate7; Figure25).The matrix in this group exhibited diminished staining of masson's trichrome and toluidine blue stains (plate7; Figure28, plate8; Figure31).

In thyroxin treated group, examination of the epiphysis of the lumbar vertebrae by H&E stain showed presence of secondary ossification center. The epiphyseal growth plate showed increase in thickness and normal arrangement of chondrocytes (plate6; Figure23). In the proliferating cell zone, the chondrocytes appeared normal (plate7; Figure26).The matrix showed diminished Masson's trichrome and toluidine blue stains (plate8; Figures29,32).

II. Electron microscopic results of eight weeks old rats

The ultra structural features of the lumbar vertebrae in the control group showed that, the reserve cell zone (resting cartilage zone also known as germinal zone) is formed by small, uniform, spindle shape, compactly located chondrocytes rich in lipid and cytoplasmic vacuoles. The cell is encircled by a narrow clear pericellular zone called groove of Ranvier. Outside this zone a fine network of thin fibrils is seen in the extracellular matrix. The nuclei occupied large part of the cells. The endoplasmic reticulum is the best developed cytoplasmic organelle (plate9; Figures33,34). In the proliferating cell zone, the cells are flattened and lie in pairs. On the cell surface fine cytoplasmic projection appears. The nuclei are eccentrically located. The rough endoplasmic reticulum, Golgi vacuoles, and mitochondria can be observed. The individual cells in their lacunae are separated by transverse septa while the cell columns as a whole are separated by longitudinal septa which are parallel to long axis of bone (plate10; Figure38).

Carbimazole treated group showed that the chondrocytes in the reserve cell zone appeared shrunken with excessive vacuolization of the

cytoplasm. Abnormal shape and position of nuclei is observed with the rough endoplasmic reticulum less numerous and the collagen fibers in the extracellular matrix are less apparent (plate9; Figures35,36). In proliferating cell zone, the nuclei are eccentrically located and pyknotic, the outline of nuclear membrane not well demarcated. The rough endoplasmic reticulum appeared less numerous. Great part of the cytoplasm is occupied by vacuoles. The extracellular matrix showed poor staining and sparse collagen fibrils (plate10; Figure39).

Thyroxin treated group showed that cells In the reserve cell zone preserved their spindle shape; nucleus is large eccentric and occupying most of the cytoplasm. Intact nuclear membrane was detected. The cytoplasm showed vacuoles but less than that observed in carbimazole treated cells. The lipid vacuoles are less numerous than that is detected in control cells. The extracellular matrix maintained its abundant collagen fibrils (plate10; Figure37). In the proliferating cell zone, the cells maintain its flattened shape and the paired arrangement. The nuclei are intact and occupy eccentric position. The rough endoplasmic reticulum is well developed. The cytoplasm showed vacuolization but less than that observed in carbimazole treated cells (plate10; Figure40).

III. Morphometric and statistical results

In eight weeks old rats, there was a highly significant reduction in thickness of epiphysis of lumbar vertebrae, crown rump length and tail length in carbimazole-treated group as compared to control and thyroxin treated group (Table 1, Histograms 1,2,4) respectively. As regard the weight a moderate significant reduction was detected in carbimazole treated group as compared to control group and thyroxin treated group (Table 1, Histogram 3).

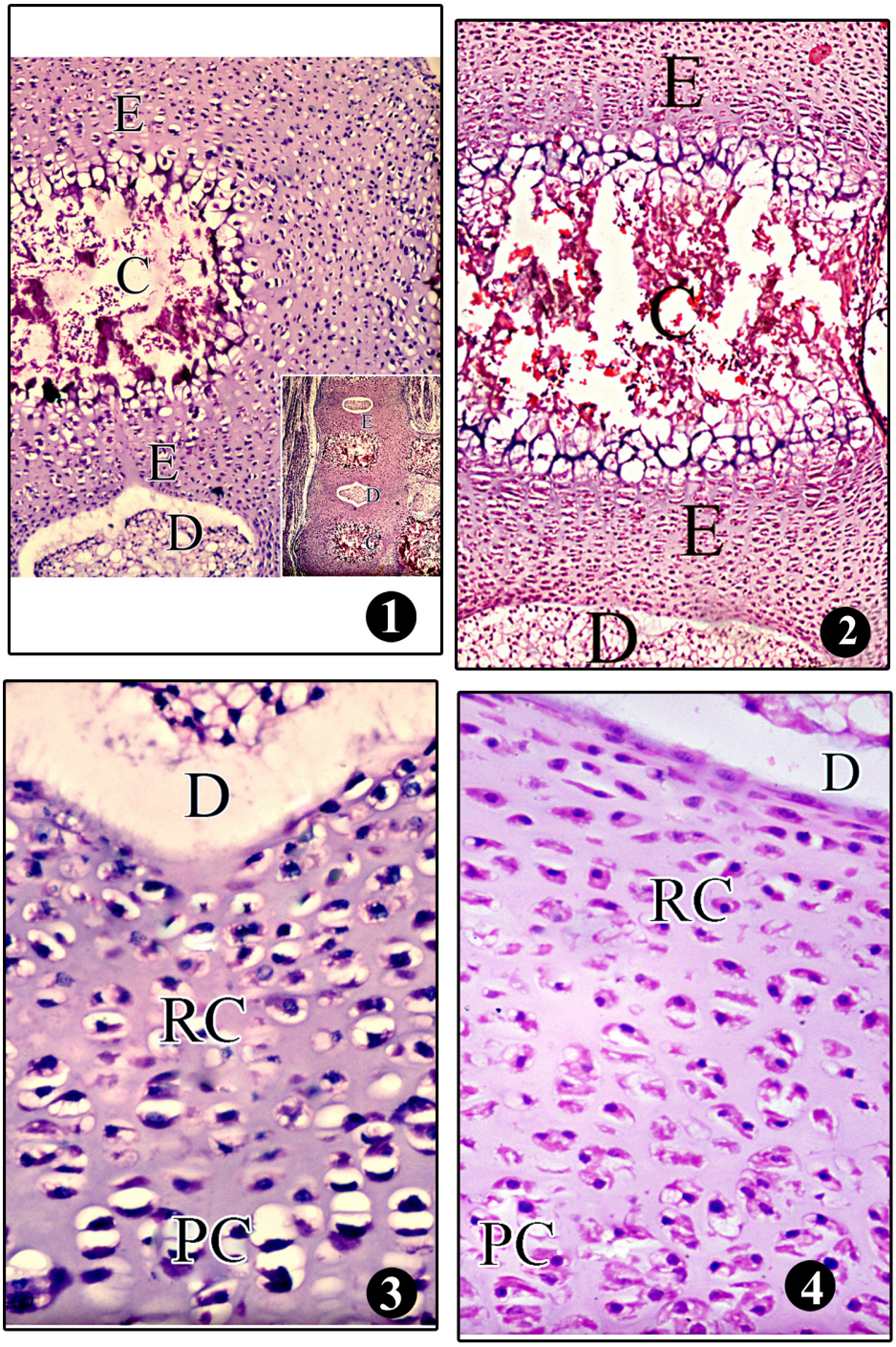


Plate 1

Fig. 1: A photomicrograph of a sagittal section of a lumbar vertebra of newborn albino rat (control group) showing, the epiphysis (E) with zones of growing chondrocytes and centrum (C) with well-developed primary ossification center. Part of the intervertebral disc (D) is apparent. Inset: showing lower magnification. (H &E; X 40, 100).

Fig. 2: A photomicrograph of a sagittal section of a lumbar vertebra of newborn albino rat (carbimazole-treated group) showing, the epiphysis (E) with zones of growing chondrocytes and the centrum (C) of the vertebral body with less developed primary ossification centre. The vertebra shows apparent shrinkage as compared to control group. (H &E; X 100).

Fig. 3: A photomicrograph of a sagittal section in the epiphysis of a lumbar vertebra of newborn albino rat (control group) showing, the growing chondrocytes : reserve cells (RC) and proliferating chondrocytes (PC). Part of the intervertebral disc (D) is apparent. (H &E; X 400).

Fig. 4: A photomicrograph of a sagittal section in the epiphysis of a lumbar vertebra of newborn albino rat (carbimazole-treated group) showing distortion and disorganization of cells including reserve cells (RC), proliferating chondrocytes (PC) and especially the proliferative cells (PC) which appear smaller with vacuolated cytoplasm. (H &E; X 400).

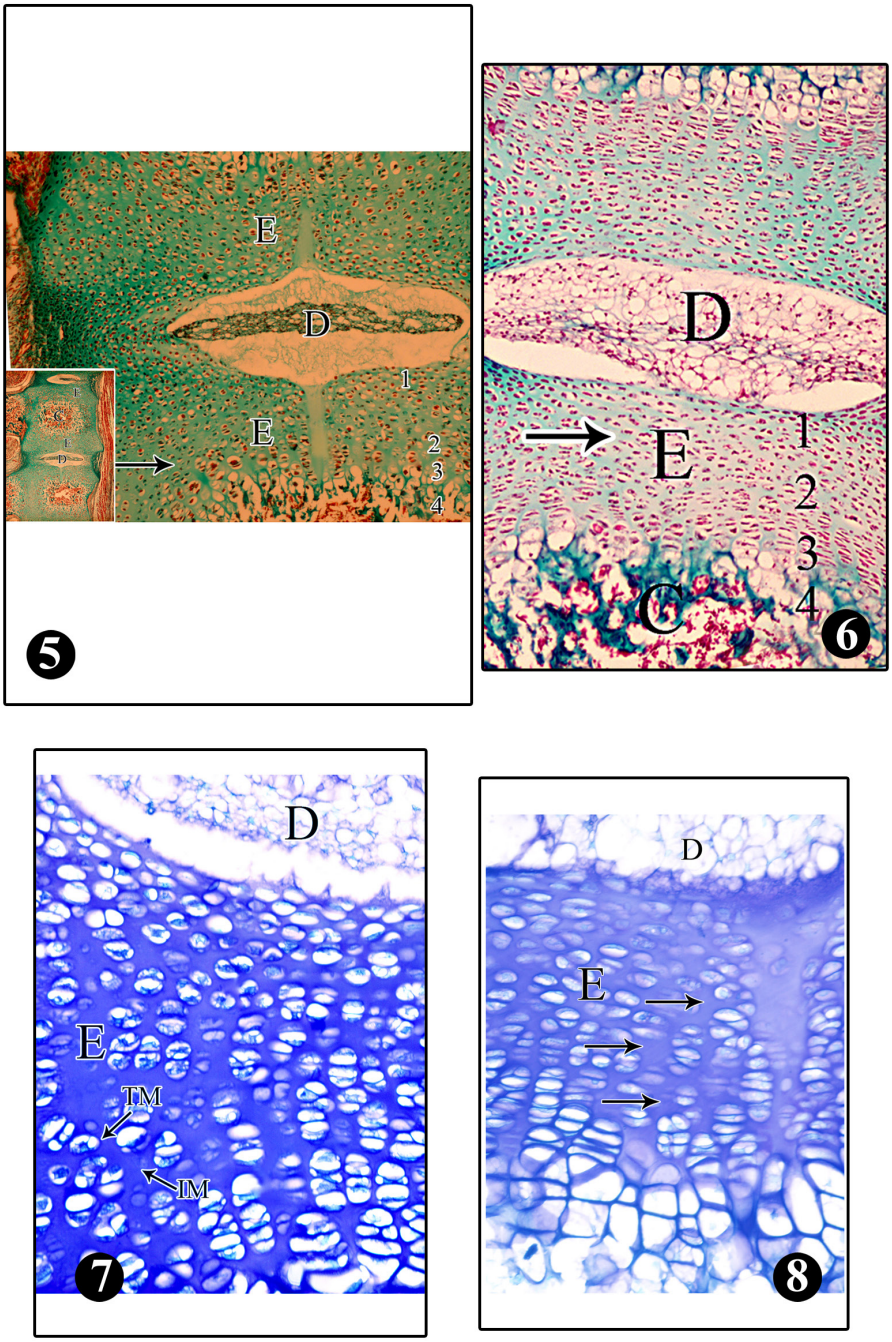


Plate 2

Fig. 5: A photomicrograph of a sagittal section of lumbar vertebrae of newborn albino rat (control group) showing the epiphysis (E) with zones of growing chondrocytes; reserve cells (1), proliferating cells (2), hypertrophied cells (3) and degeneration cells (4) with normal homogenous distribution of the stain showing green colour of the matrix (arrow). The intervertebral disc (D) is evident. Inset: showing lower magnification. The centrum (C) of the vertebral body is evident. (Masson's trichrome; X 40, 100).

Fig. 6: Aphotomicrograph of a sagittal section of lumbar vertebrae of newborn albino rat (carbimazole-treated group) showing the epiphysis (E) with diminished staining of matrix (arrow) in between the growing chondrocytes; reserve cells (1), proliferating cells (2), hypertrophied cells (3) and degeneration cells (4). The intervertebral disc (D) is evident. (Masson's trichrome; X 100).

Fig. 7: A photomicrograph of a sagittal section of a lumbar vertebra of newborn albino rat (control group) showing the epiphysis (E) with zones of growing chondrocytes; with normal distribution of the stain. The matrix is differentiated into territorial matrix (TM) and inter territorial (IM). part of the intervertebral disc (D) is evident. (Toluidine blue; X 400).

Fig. 8: Aphotomicrograph of a sagittal section of a lumbar vertebra of newborn albino rat (carbimazole-treated group) showing, the epiphysis (E) with zones of growing chondrocytes. There are areas of diminished staining (Arrow) in between chondrocytes as compared to control group. (Toluidine blue; X 400).

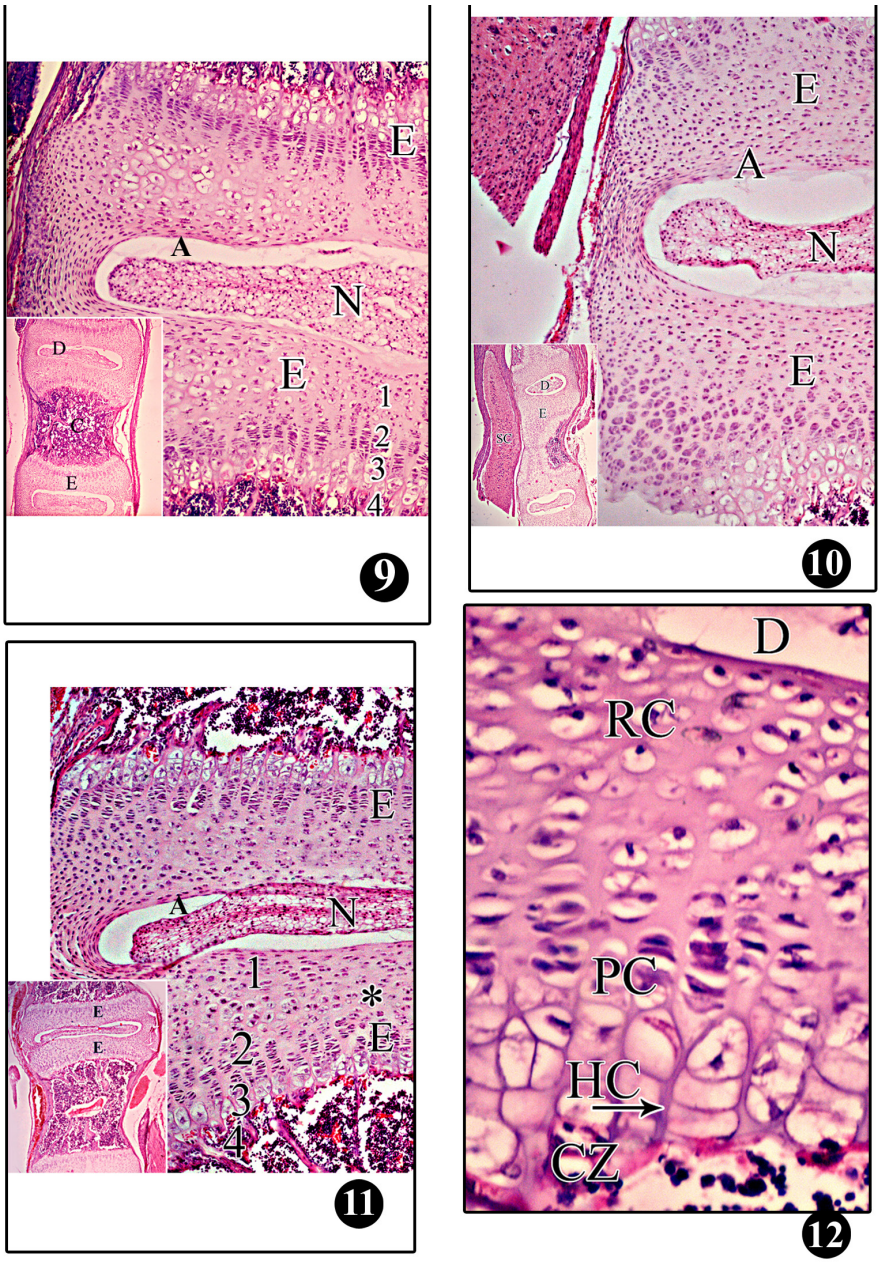


Plate 3

Fig. 9: A photomicrograph of a sagittal section of lumbar vertebrae of two weeks old albino rat (control group) showing, the epiphyses (E) with the growing chondrocytes : reserve cells (1), proliferative cells (2), hypertrophied cells (3) and calcification zone(4). Annulus fibrosus (A) and nucleus pulposus (N) of the intervertebral disc are observed. Inset: showing lower magnification. The centrum (C) and the intervertebral disc (D) are seen. (H&E; X 40, X 100).

Fig. 10: A photomicrograph of a sagittal section of umbar vertebrae of two weeks old albino rat (carbimazole-treated group) showing, the epiphyses (E) with disorganization the growing chondrocytes. Note shrunken nucleus pulposus (N) of the intervertebral disc as compared to control group. Inset: showing lower magnification. The spinal cord (SC) and the intervertebral disc (D) are evident. (H&E; X 40, X 100).

Fig. 11: A photomicrograph of a sagittal section of lumbar vertebrae of two weeks old albino rat (carbimazole + thyroxin treated group) showing that the epiphyses (E) are maintaining its normal arrangement of cells as control group including reserve cells (1), proliferating cells (2), hypertrophied cells (3) and degeneration cells (4). However, there are areas of matrix devoid of cells especially in the reserve cell zone (star). Well developed annulus fibrosus (A) and nucleus pulposus (N) of the intervertebral disc are noticed. Inset: showing lower magnification. (H&E; X 40, X 100).

Fig. 12: A photomicrograph of a sagittal section in the epiphysis of a lumbar vertebra of two weeks old albino rat (control group) showing, the reserve cells (RC) are close to the inter vertebral disc (D), proliferating cells (PC) are larger than reserve cells and arranged in longitudinal column, hypertrophied cells (HC) are larger than the proliferative cells with compressed matrix in between (arrow) and calcification zone (CZ). (H&E; X 400).

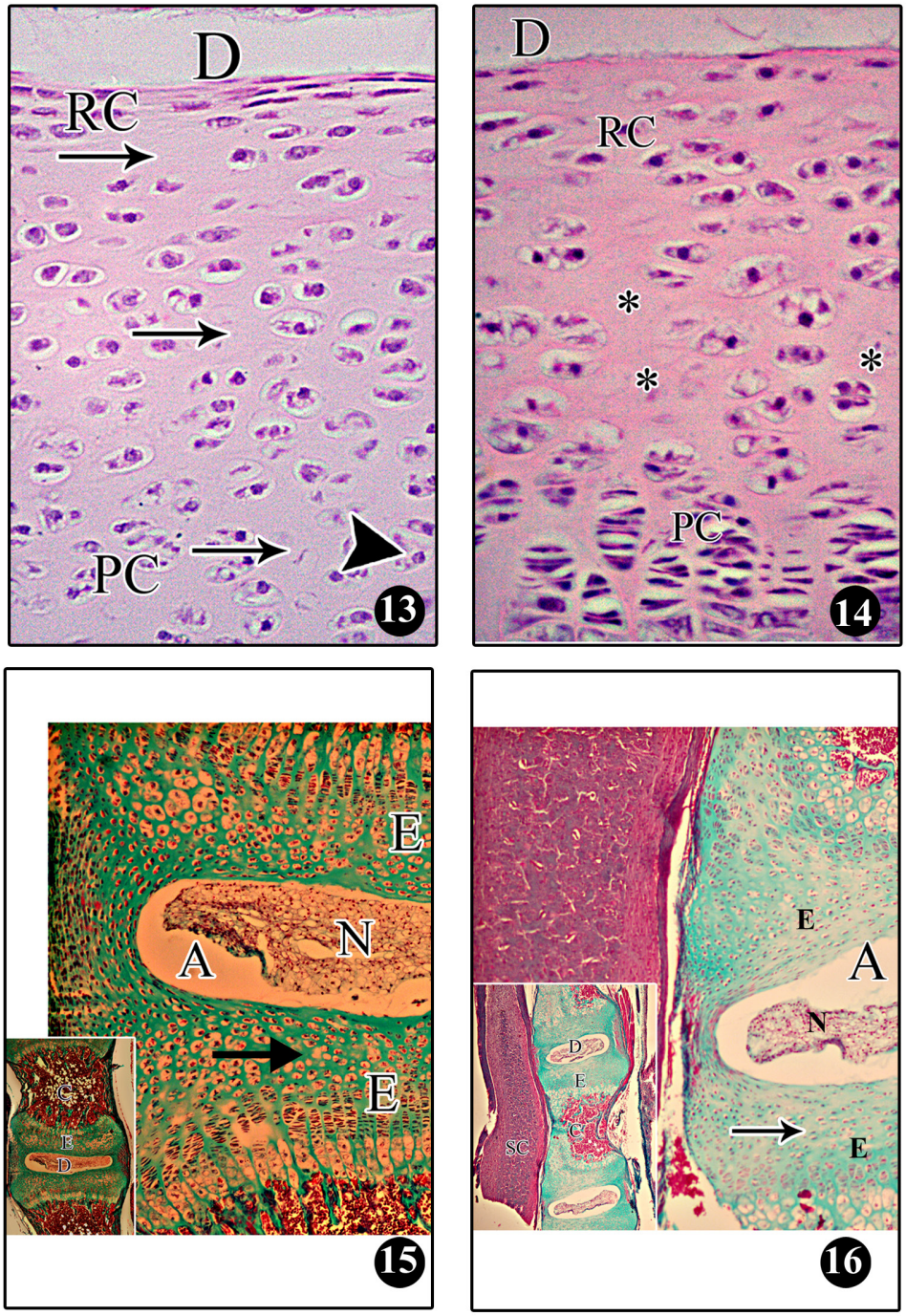


Plate 4

Fig. 13: A photomicrograph of a sagittal section in epiphysis of a lumbar vertebra of two weeks old albino rat (carbimazole-treated group) showing that, the reserve cells (RC) are close to inter vertebral disc (D), marked distortion and loss of arrangement of cell column in the proliferating cells (PC) with areas of matrix devoid of cells (arrow) , some cells are atrophied (arrow head). (H&E; X 400).

Fig. 14: A photomicrograph of a sagittal section in the epiphysis of a lumbar vertebra of two weeks old albino rat (carbimazole + thyroxin treated group) showing, marked deformity and distortion in the reserve cells (RC) with areas of matrix devoid of cells (stars). Note more or less normal arrangement of the proliferating cells (PC). The intervertebral disc (D) is noticed. (H&E; X 400).

Fig. 15: A photomicrograph of a sagittal section of lumbar vertebrae of two weeks old albino rat (control group) showing, the epiphysis (E) with normal homogenous distribution of the stain and greenish staining of the collagen fibers (arrow). Inset: showing lower magnification. Note the centrum (C) and the intervertebral disc (D). (Masson's trichrome; X 40, X 100).

Fig. 16: A photomicrograph of a sagittal section of lumbar vertebrae of two weeks old albino rat (carbimazole-treated group) showing, the epiphyses (E) with diminished heterogenous staining (arrow) as compared to control group. Inset: showing lower magnification. The centrum (C), the intervertebral disc (D) and the spinal cord (SC) are noticed. (Masson's trichrome; X 40, X 100).

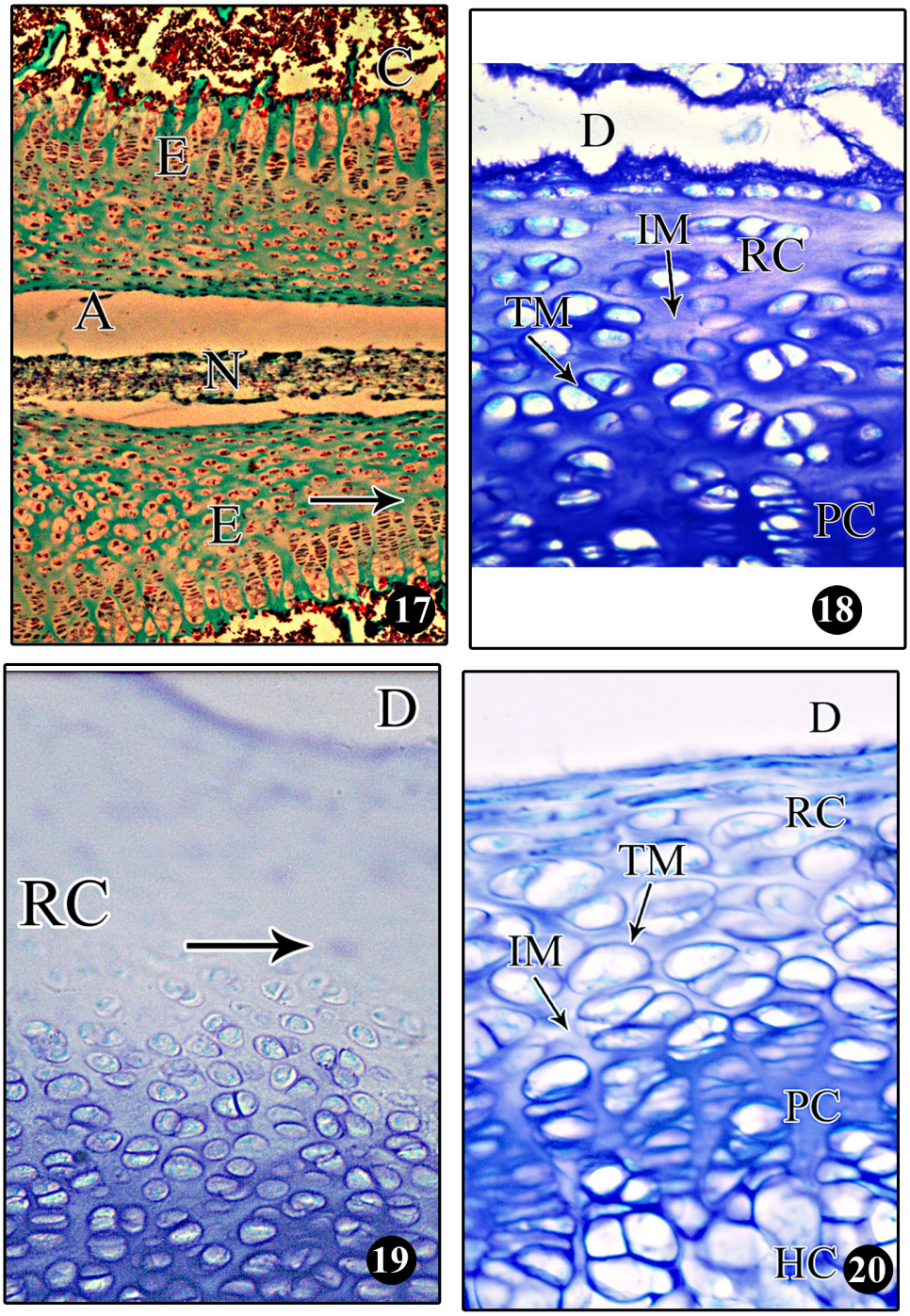


Plate 5

Fig. 17: A photomicrograph of a sagittal section of lumbar vertebrae of two weeks old albino rat (carbimazole + thyroxin treated group) showing the epiphysis (E) with diminished staining (arrow) as compared to control group. Note the centrum of the vertebral body. Annulus fibrosus (A) and nucleus pulposus (N) of the intervertebral disc are observed. (Masson's trichrome X 100).

Fig. 18: A photomicrograph of a sagittal section of a lumbar vertebra of two weeks old albino rat (control group) showing normal distribution of the stain in cartilage matrix. The matrix is differentiated into darker territorial (TM) and lighter inter territorial (IM) matrix. Note the reserve cells (RC), the proliferative cells (PC) and The intervertebral disc (D). Toluidine blue; X 400).

Fig. 19: A photomicrograph of a sagittal section of a lumbar vertebra of two weeks old albino rat (carbimazole-treated group) showing, areas of diminished staining (Arrow) in between chondrocytes in the reserve cell zone(RC). (Toluidine blue; X 400).

Fig. 20: A photomicrograph of a sagittal section of a lumbar vertebra of two weeks old albino rat (carbimazole + thyroxin treated group) showing, diminished staining of the cartilage matrix as compared to control group. The matrix is differentiated into darker territorial (TM) and lighter interterritorial (IM) matrix. Note the reserve cells (RC), proliferative cells (PC) and hypertrophic cells (HC).The intervertebral disc (D) is evident. (Toluidine blue; X 400).

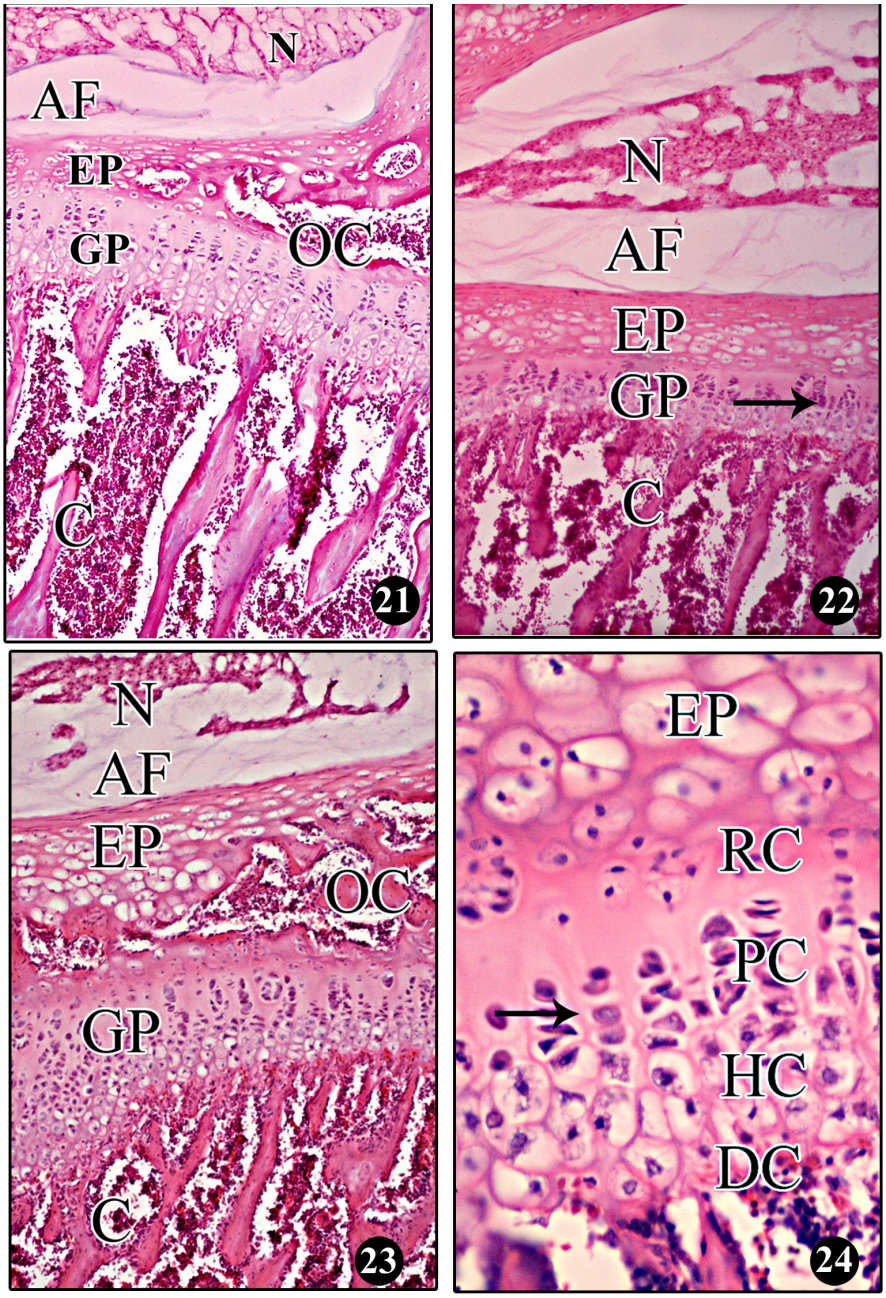


Plate 6

Fig. 21: A photomicrograph of a sagittal section in a lumbar vertebra of eight weeks old albino rat (control group) showing the epiphyseal cartilage (EP) with secondary ossification center (OC), growth plate cartilage (GP) and completely ossified centrum of the vertebral body (C). Notice the nucleus pulposus (N) and annulus fibrosus (AF) of the intervertebral disc. (H&E.; X 100).

Fig. 22: A photomicrograph of a sagittal section in a lumbar vertebra of eight weeks old albino rat (carbimazole-treated group) showing apparent reduction in thickness of epiphyseal cartilage (EP) and growth plate cartilage (GP) as compared to control group. In addition, the cells in growth plate cartilage lose its normal alignment (arrow). Note the centrum of the vertebral body (C). The nucleus pulposus (N) and annulus fibrosus (AF) of the intervertebral disc are observed. (H&E; X 100).

Fig. 23: A photomicrograph of a sagittal section in a lumbar vertebra of eight weeks old albino rat (carbimazole + thyroxin treated group) showing the epiphyseal cartilage (EP) with well developed secondary centre of ossification (OC), the growth plate cartilage (GP) shows increase in thickness and normal alignment of chondrocytes and completely ossified centrum of the vertebral body (C). Notice the nucleus pulposus (N) and annulus fibrosus (AF) of the intervertebral disc. (H&E; X 100).

Fig. 24: A photomicrograph of a sagittal section in growth plate cartilage of a lumbar vertebra of eight weeks old albino rat (control group) showing, the reserve cell zone (RC) appears decreased in thickness as compared with the previous ages, proliferating cell zone (PC) arranged in longitudinal column within elliptical lacunae and compressed matrix (arrow) in between the columns, hypertrophied cells (HC) lies next to the proliferating cells and larger and degenerating cells (DC) of calcification zone are the nearest to the centrum. Part of the epiphyseal cartilage is apparent (EP). (H&E; X 400).

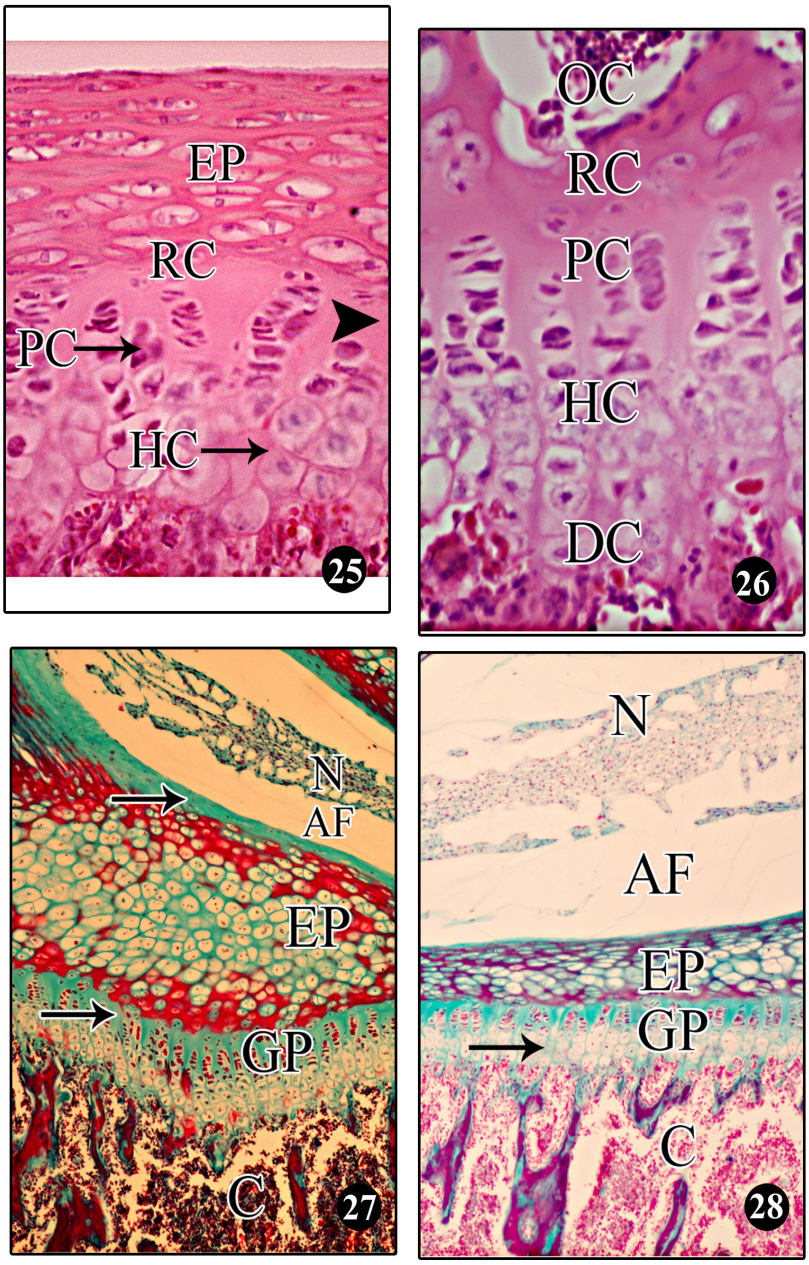


Plate 7

Fig. 25: A photomicrograph of a sagittal section in the growth plate cartilage of a lumbar vertebra of eight weeks old albino rat (carbimazole-treated group) showing the reserve cell zone (RC) with marked decrease in thickness as compared to control group, the proliferating cell zone (PC) exhibits wide areas of matrix devoid of cells (arrow head) and loss of the normal alignment of proliferating cells (arrow), hypertrophied cells (HC) appear degenerated with dilated lacunae. The overall thickness of these layers appears less than that observed in control group. (H&E; X 400).

Fig. 26: A photomicrograph of a sagittal section in the growth plate cartilage of a lumbar vertebra of eight weeks old albino rat (carbimazole + thyroxin treated group) showing the proliferating cell zone appears more or less normal (PC) and maintains its normal alignment as compared to control group. Note the reserve cell zone (RC), hypertrophied cells (HC) and degenerating cells (DC) of calcification zone. Secondary centre of ossification (OC) is seen. (H&E; X 400).

Fig. 27: A photomicrograph of a sagittal section in a lumbar vertebra of eight weeks old albino rat (control group) showing normal homogenous distribution of the stain with green colour of collagen (arrows). Notice epiphyseal cartilage (EP), growth plate cartilage (GP), centrum of the vertebral body (C). The nucleus pulposus (N) and annulus fibrosus (AF) of the intervertebral disc are observed. (Masson's trichrome; X 100).

Fig. 28: A photomicrograph of a sagittal section in a lumbar vertebra of eight weeks old albino rat (carbimazole-treated group) showing diminished staining of the growth plate (GP) as compared to the control group (arrow). The epiphyseal cartilage (EP), centrum of the vertebral body (C), the nucleus pulposus (N) and annulus fibrosus (AF) of the intervertebral disc are noticed. (Masson's trichrome; X 100).

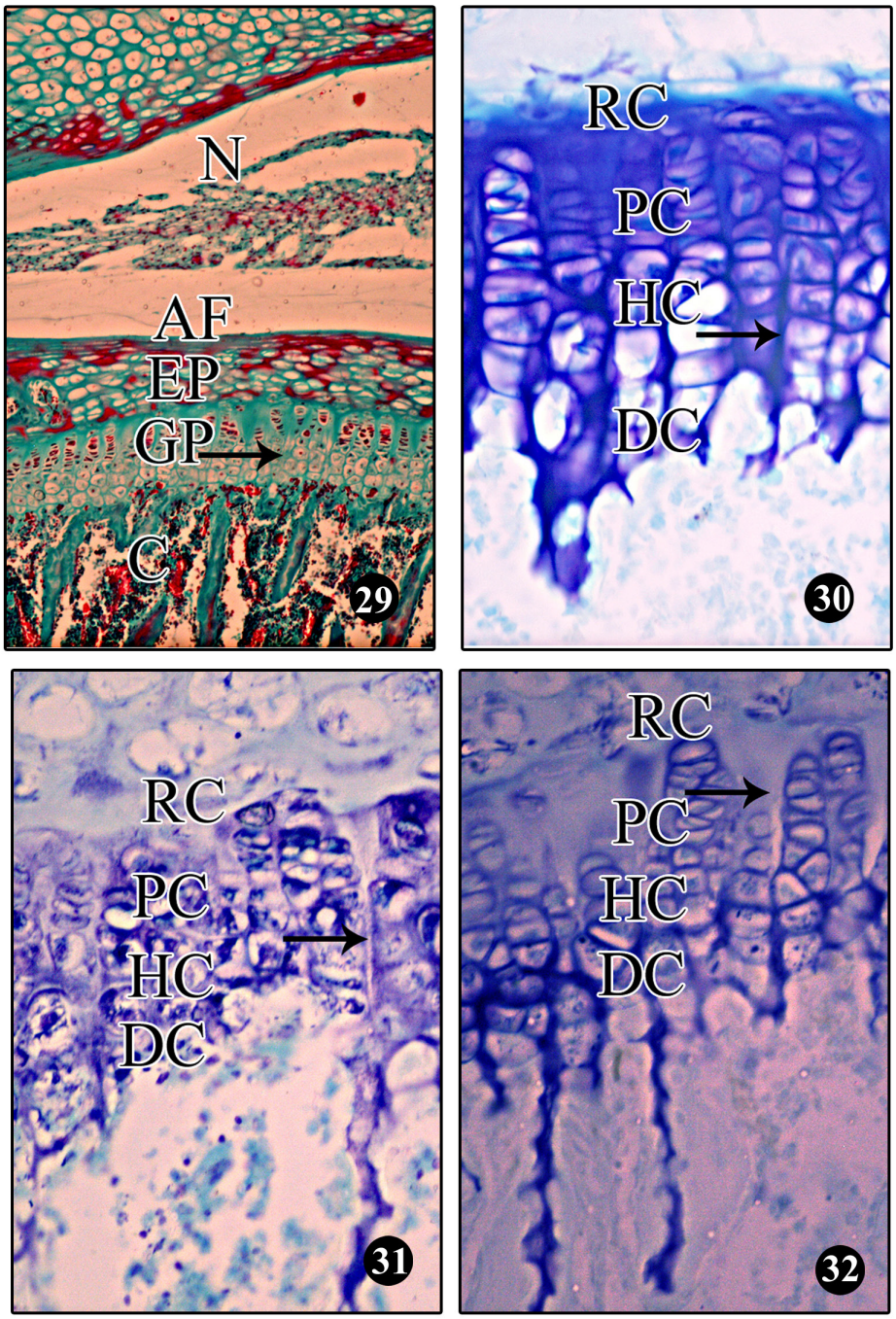


Plate 8

Fig. 29: A photomicrograph of a sagittal section in a lumbar vertebra of eight weeks old albino rat carbimazole + thyroxin treated group) showing more or less normal homogenous distribution of the stain with green colour of collagen (arrow). Notice epiphyseal cartilage (EP), growth plate cartilage (GP), centrum of the vertebral body (C), nucleus pulposus (N) and annulus fibrosus (AF) of the intervertebral disc. (Masson's trichrome; X 100).

Fig. 30: A photomicrograph of a sagittal section in the growth plate cartilage of a lumbar vertebra of eight weeks old albino rat (control group) showing normal distribution of the stain in cartilage matrix (arrow). Notice the reserve cells (RC), proliferative cells (PC), hypertrophic cells (HC) and degenerated cells (DC). (Toluidine blue; X 400).

Fig. 31: A photomicrograph of a sagittal section in growth plate cartilage of a lumbar vertebra of eight weeks old albino rat (carbimazole-treated group) showing marked diminished staining of the cartilage matrix (arrow) as compared to control group. (Toluidine blue; X 400).

Fig. 32: A photomicrograph of a sagittal section in the growth plate cartilage of a lumbar vertebra of eight weeks old albino rat (carbimazole + thyroxin treated group) showing a little diminished staining of the cartilage matrix (arrow) as compared to control group. (Toluidine blue; X 400).

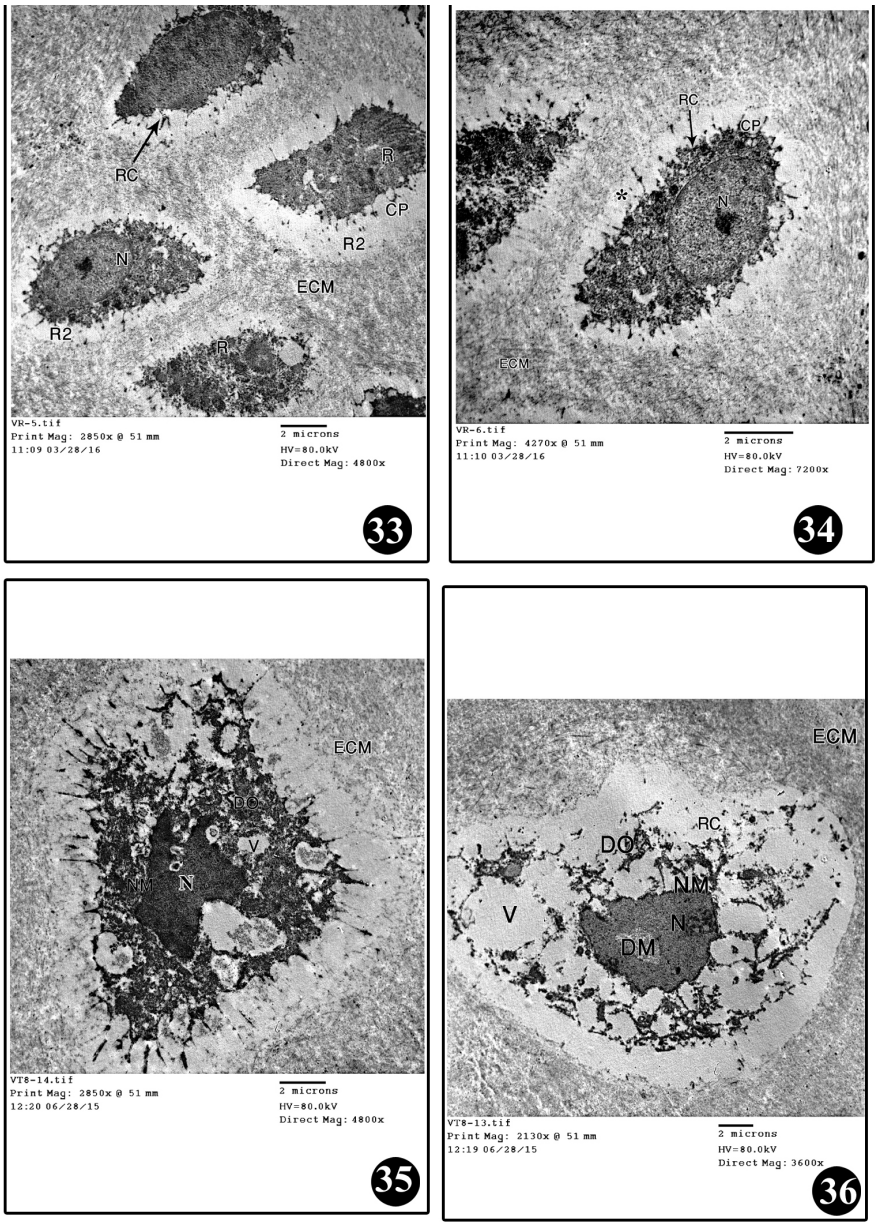


Plate 9

Fig. 33: An electron micrograph of reserve cell zone of growth plate cartilage in a lumbar vertebra of eight weeks old albino rat (control group) showing spindle shaped reserve cells (RC), each cell is surrounded by a clear pericellular zone (R2), outside this clear zone the extracellular matrix (ECM) is formed of a large number of collagen fibrils. The nucleus (N) is eccentrically located and occupying a large part of the cell. Well-developed rough endoplasmic reticulum (R) is observed in peripheral part of the cytoplasm. On the cell surface small fine cytoplasmic processes (CP) project into the pericellular zone. (TEM; X4800).

Fig. 34: An electron micrograph of reserve cell zone of growth plate cartilage in a lumbar vertebra of eight weeks old albino rat (control group) showing reserve cell (RC) spindle in shape and surrounded by clear pericellular zone (star). The nucleus (N) is large, eccentric and occupying most of the cytoplasm. On the cell surface the cytoplasmic processes (CP) are apparent. The cell is surrounded by extracellular matrix (ECM) rich in collagen fibrils. (TEM; X7200).

Fig. 35: An electron micrograph of reserve cell zone of growth plate cartilage in a lumbar vertebra of eight weeks old albino rat (carbimazole-treated group) showing extensive vacuolization (V) of the cytoplasm, Abnormal shaped electron dense nucleus (N), irregular nuclear membrane (NM) and degenerated cytoplasmic organelles (DO). The extracellular matrix (ECM) exhibits few collagen fibrils and poor staining. (TEM; X4800).

Fig. 36: An electron micrograph of reserve cell zone of growth plate cartilage in a lumbar vertebra of eight weeks old albino rat (carbimazole-treated group) showing reserve cell (RC) with extensive vacuolization (V) of the cytoplasm and marked degeneration of most of cytoplasmic organelles (DO). Abnormal shaped electron dense nucleus (N), irregularities in nuclear membrane (NM) and degenerated material (DM) is accumulated inside the nucleus. The extracellular matrix (ECM) exhibits few collagen fibrils and poor staining. (TEM; X3600).

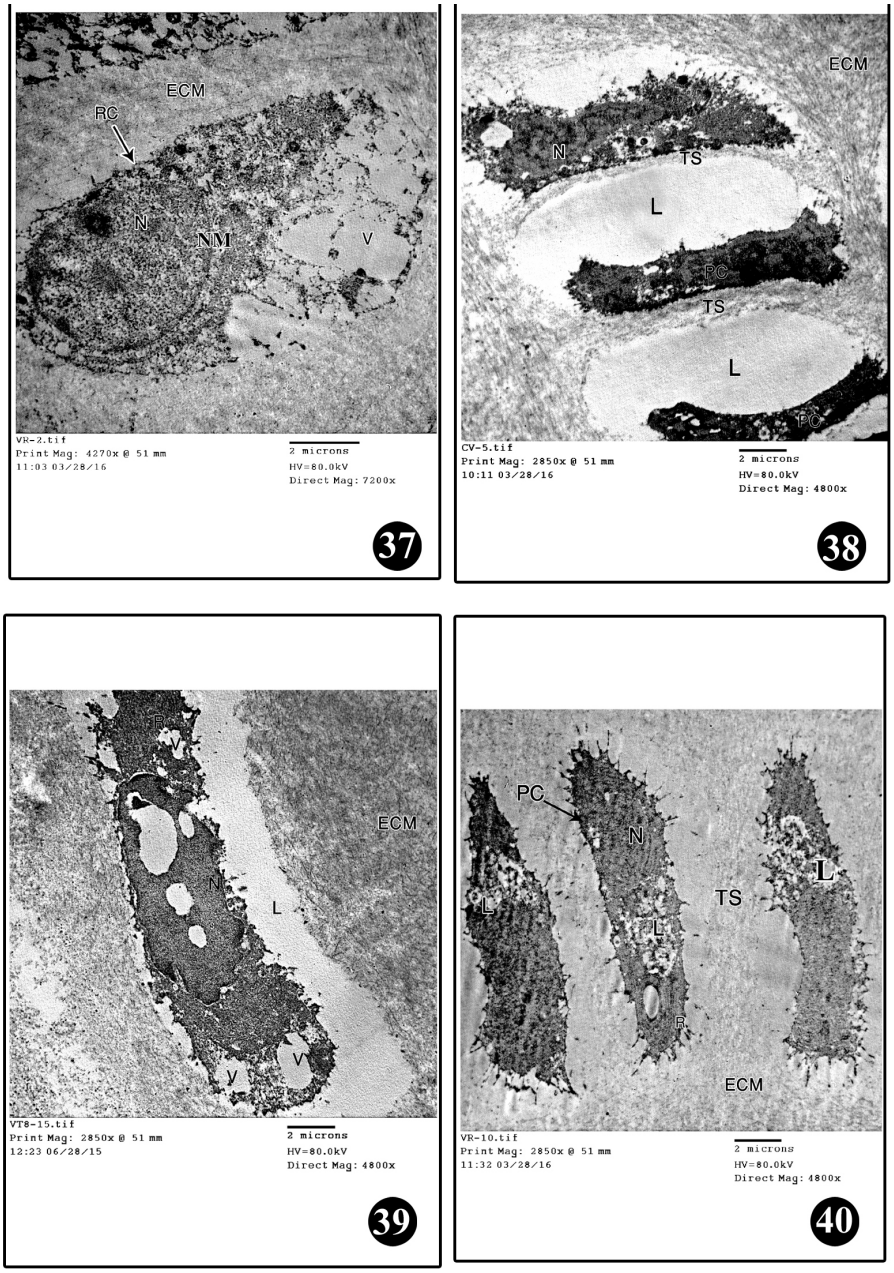


Plate 10

Fig. 37: An electron micrograph of reserve cell zone of growth plate cartilage in a lumbar vertebra of eight weeks old albino rat (carbimazole+thyroxin-treated group) showing the reserve cell (RC) preserves its spindle shape but lacking the clear pericellular zone. The cell is surrounded by extracellular matrix (ECM) rich in collagen fibrils. The cytoplasmic processes on the cell surface are absent. The nucleus (N) is large eccentric and well formed with regular intact nuclear membrane (NM). The vacuoles (V) in the peripheral part of the cytoplasm are disturbing the cell membrane. (TEM; X7200).

Fig. 38: An electron micrograph of proliferative cell zone of growth plate cartilage in a lumbar vertebra of eight weeks old albino rat (control group) showing flattened proliferating cells (PC) enclosed in lacunae (L) these lacunae are separated from each other by transverse septa (TS). Note the eccentric nucleus (N) and collagen fibrils of the extracellular matrix (ECM) is abundant. (TEM; X4800).

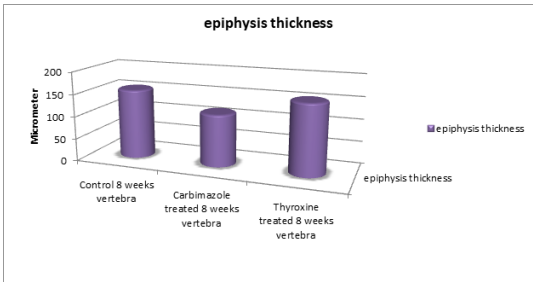
Fig. 39: An electron micrograph of proliferative cell zone of growth plate cartilage in a lumbar vertebra of eight weeks old albino rat (carbimazole-treated group) showing that the proliferating cell is flattened and enclosed in lacunae (L). Extensive vacuoles (V) in the cytoplasm and inside the nucleus (N) which is abnormally placed are noticed. The cell is surrounded by extracellular matrix (ECM). (TEM; X4800).

Fig. 40: An electron micrograph of proliferative cell zone of growth plate cartilage in a lumbar vertebra of eight weeks old albino rat (thyroxin treated group)showing, a number of the proliferating cells (PC) which appear flattened, separated from each other by transverse septa (TS). The cells contain large eccentric nucleus (N) and extensive amount of lipid granules (L). Outside the cells, the extracellular matrix (ECM) shows scanty collagen fibrils. (TEM; X4800).

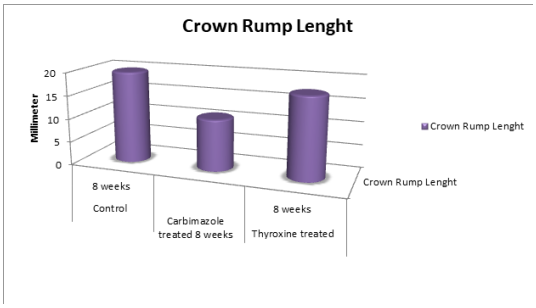
Table 1: Showing comparison of thickness of epiphysis in lumbar vertebrae, crown rump length, tail length and weight in 8 weeks old control, carbimazole - treated and carbimazole + thyroxin treated groups

group	Control 8 weeks lumbar vertebrae	Carbimazole-treated 8 weeks lumbar vertebrae	Carbimazole+Thyroxin treated 8 weeks lumbar vertebrae	ANOVA	N
	mean± SD	mean± SD	mean± SD	*P value*	
epiphysis thickness	155.81±13.287	116.09±10.369	157.19±15.238	0.000***	6
Weight	187.24±44.792	128.89±29.990	157.6±12.149	0.001**	6
Crown Rump Length	19.78±0.667	11.09±2.427	17.5±0.707	0.000***	6
Tail length	16.22±1.302	8.55±2.583	17±1.054	0.000***	6

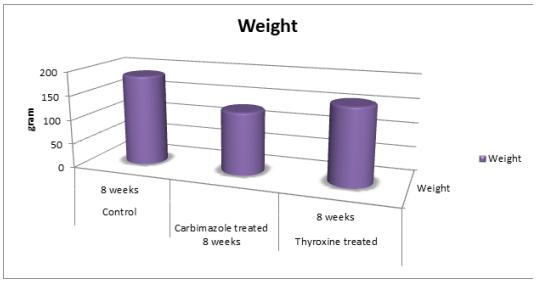
(SD) standard deviation
***$P \leq 0.0001$ highly significant
**$P \leq 0.001$ moderately significant



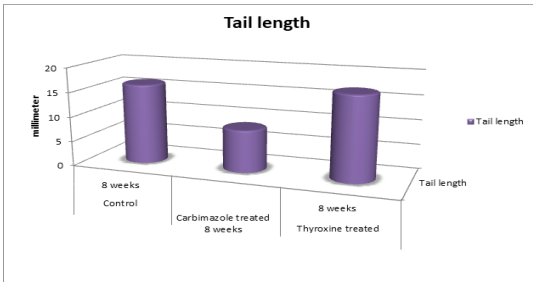
Histogram 1: Epiphyseal thickness of of lumbar vertebrae in 8 weeks old albino rats of ontrol, carbimazole-treated and carbimazole + thyroxin treated groups



Histogram 2: Crown rump length in 8 weeks old albino rats of control, carbimazole-treated and carbimazole + thyroxin treated groups



Histogram 3: Weight in 8 weeks old albino rats of control, carbimazole-treated and carbimazole + thyroxin treated groups



Histogram 4: Tail length in 8 weeks old albino rats of control, carbimazole-treated and carbimazole + thyroxin treated groups

DISCUSSION

Postnatal skeletal development, linear growth, and bone maintenance are tightly regulated by thyroid hormones[25], so the current work is carried out to show the effect of hypothyroidism and thyroxin treatment on the postnatal development of the lumbar vertebrae in albino rat.

Thyroid disorders may occur at any age, however, your risk of having this disease increases after the age of 60. Undiagnosed thyroid problems may cause heart disease, osteoporosis and infertility[26].

The most common congenital endocrine disorder is known to be congenital hypothyroidism (CH). Better prognosis and management of hypothyroidism is greatly achieved by early treatment of CH patients. To ensure long-term health, optimal treatment and management throughout the patient's life, beginning in the neonatal period, are essential[27].

Fragility fractures, dropped quality of life and increased mortality are a fundamental results of

loss of bone mineral density due to osteoporosis. The balance between bone formation and bone resorption is essential and its disturbance can lead to loss of bone mass and disruption of its structure. Optimal development, bone mass and normal functioning of human skeleton relies mainly on different factors. Thyroid hormones play essential role in bone metabolism. Both excess and deficiency of fT4 and fT3 can be potentially deleterious for bone tissue[28,29].

Growth retardation or even growth arrest, abnormality in endochondral ossification with delayed bone age and persistent short stature are the end results of untreated hypothyroidism in childhood[30,19,31]. Hypometabolism generally arise from hypothyroidism. Bone formation processes are slowed in 50% while bone resorption processes are slowed in 40%[32].

Almost all of antithyroid drugs cross the placental barrier and secreted into the milk. Thus, they affect the thyroid status of the growing fetus and the newborn[33]. Carbimazole, an antithyroid drug used during pregnancy and lactation is known to suppress the thyroid hormone synthesis of both mother and fetus even the therapeutic dose taken during pregnancy can induce hypothyroidism[34].

In the present work, carbimazole was administered to the experimental animals in a single daily oral dose of 6 µg/gm body weight[35]. The treatment was continued from 10th day of pregnancy to 21st day of lactation then the carbimazole was administered to offsprings up to 8 weeks of age.

In this work, ages that were studied had chosen as follow, newborn, two weeks, and eight weeks chosen to detect the effect of carbimazole and thyroxin replacement on the growing bone. because in rats, the rate of longitudinal bone growth increases between one to five weeks and then declines between 11.5 to 13 weeks[36].

To address this issue, albino rats were chosen. This choice was depends on the shortness of their gestational period, about 21 days and the rate of their bone growth which is substantially higher in comparison to human[37]. The present work was performed on the lumbar part of the vertebral column as an example of an irregular bone in the axial skeleton.

The present work revealed that, the body of each lumbar vertebra appears to be composed of central part (centrum) and two peripheral areas of hyaline cartilage (epiphysis). The body of vertebra is seen surrounded by periosteum and exhibiting a primary ossification center.

The chondrocytes in the epiphysis are arranged forming zones traced from the periphery toward the centrum, the zones are arranged as follow: Zone of reserve cartilage, Zone of proliferation with its cells appeared slightly larger in size than those of the reserve zone, hypertrophic zone. The cells of this area appeared larger than the cells of the previous zone and degeneration zone. The centrum of each lumbar vertebra showed a well-developed primary ossification center.

These results are in accordance with previous studies[38-43] who described the same zones of chondrocyte maturation in the epiphysis of the vertebrae.

In the present work, examination of the epiphysis in carbimazole treated newborn vertebrae showed, zone of proliferation with smaller chondrocytes and vacuolated cytoplasm. Masson's trichrome and toluidine blue stained sections showed that the matrix lost its differentiation into territorial and interterritorial matrix with diminished staining.

These results coincideD with the results reported by Capelo *et al*.[44] who observed that at embryonic day18.5, clearer differences in the morphology of the spine were observed between euthyroid and hypothyroid fetuses. At this fetal age, both groups presented developed primary ossification centers in the vertebra However, whereas residual cartilage was detected in the spine of hypothyroid fetuses, it was not detected in fetuses with normal thyroid hormone level. Moreover, hypothyroid fetuses presented malalighnment in the proliferative zones of the vertebra in comparison to euthyroid fetuses.

The current results are in accordance with Senecal *et al*.[45] Who stated that absence of secondary ossification is consider one of the hallmarks exhibited at birth in human newborns with congenital hypothyroidism.

Some authors explained that decrease in the mRNA expression of collagen1 and ossification center which are two markers of osteoblast phenotype, and Collagen 10, a marker of chondrocyte differentiation, at embryonic day (E18.5) is attributed to decreased levels of TH. This is in accordance with the morphological changes of the skeleton at the end of the fetal development. This strongly support that thyroid hormone signaling performs an important role in

the skeletal development only at the end of the fetal life and during the postnatal development[44,46].

Two weeks old control vertebrae in the present work revealed that, the matrix showed normal staining by masson's trichrome and toluidine blue stains. The epiphyseal cartilage plate at this age consists of the following zones: Zone of reserve cells, Zone of proliferating cells, zone of maturing and hypertrophied cells and Zone of calcification.

These results are in agreement with other authors[38,47,48] who reported that the growth plate was divided into several zones clearly demarcated on the basis of their cell morphology and arrangement of cells. From epiphysis to diaphysis these successively merging zones were distinguished as the resting, proliferative and hypertrophic zone.

Carbimazole-treated two weeks vertebrae in this work showed that, in the proliferating cell zone; the normal longitudinal arrangement is lost. The matrix shows diminished heterogenous staining by Masson's trichrome and toluidine blue stains. After thyroxin replacement the the normal longitudinal arrangement in the proliferating cell zone was restorated in most cells.

The present results coincided with some authors[49] who reported that hypothyroidism delays ossification in mice as showed in X-ray imaging and CT analysis. This was emphasized by alcian blue/alizarin red stains, showing a greater portion of blue-stained cartilage remains unossified in the (hypothyroid) skeleton (humerus, tibia, vertebrae, and ribs) at postnatal ages P14, P21 and P28. Vertebrae of hypothyroid mice appeared shorter and undermineralized. Also the epiphyses were not apparent in the tibias and femurs of hypothyroid mice both at P14 and P35.f inally, the results of the author revealed that hypothyroid mice showed growth retardation with a delayed endochondral ossification.

In human the same finding were detected by Williams *et al*.[50] who added that, short stature, delay in bone age, skeletal dysgenesis, and delayed development of teeth, with abnormally thin epiphyseal growth plates and delayed chondrocyte hypertrophy are the end result of hypothyroidism in children.

Stevens *et al*.[31] reported that, hypothyroid rats displayed a disorganized growth plate with a decreased hypertrophic zone and this is in accordance with the present work.

Diminshed staining of matrix can be clarified on the basis of that T3 seems to act in cartilage at several levels. It stimulates the clonal expansion of resting chondrocytes. It also stimulates hypertrophic chondrocyte differentiation, as shown by induction of collagen X expression, upon T3 stimulation[20,51]. Hypothyroidism results in epiphyseal dysgenesis due to reduction in T3 which is necessary to proliferation and differentiation of reserve cell zone and for blood vessels invasion of the growth plate[52,53].

The present study revealed well developed secondary ossification center in eight weeks old control lumbar vertebrae. The epiphyseal growth plate showed that the zones have the same differentiation as in the previous age but the thickness is decreased due to marked extension of the secondary ossification center.

Many investigators[54-57] reported that each growth plate is a sandwich-like, structure showing four zones: resting, replicative, transformation and apoptotic and this is in accordance with the present work.

The this work, carbimazole treated vertebrae revealed abnormality (malalighnment) in the arrangement of growing chondrocytes especially in the proliferating zone. There was a reduction in thickness of epiphyseal cartilage plate as compared to the control. The matrix showed diminished staining by Masson's trichrome and toluidine blue stains.

After thyroxin replacement in the present study, the epiphysis of the lumbar vertebrae showed the appearance of secondary ossification center. The epiphyseal growth plate showed increase in thickness and normal arrangement of chondrocytes. The matrix showed a little diminished staining.

Morphometric results in the current work revealed a decrease in weight, epiphyseal thickness, crown rumb length and tail length in hypothyroid group. The present results are in accordance with Marino *et al*.[58] Who reported that, the architecture of the epiphyseal growth plate affected by hypothyroidism. The resting zone of hypothyroid animals was wide due to delayed appearance of secondary center of ossification in epiphsis.

At eight weeks of age in this study, hypothyroid animals revealed a greater number of resting zone chondrocytes and a larger resting zone height with a smaller total growth plate height with less

numerous proliferative chondrocytes number per column as compared to control animals. Similar to these morphometric results,[58] reported that, hypothyroidism reduces growth of body mass; tail length; tibial length; and organ weight (heart, liver, and kidney). The decrease in body weight can be clarified as follow: rat pups of hypothyroid mother feed less than normal with delayed weaning, and thus inhibition of growth is attributed to both the direct influence of thyroid hormone deficiency and the indirect effects of under nutrition[59].

Diminished staining of cartilage matrix in the present study was explained by some researchers[60] who stated that in hypothyroid rats, the proliferating chondrocytes showed an abnormal increase in sulfation of extracellular matrix. This matrix is abnormally deposited in a patchy irregular manner supposing that thyroid hormones affect extracellular matrix architecture. Treatment of hypothyroid rats with thyroid hormones reversed these changes this is due to the direct actions of T3 on bone, and it is not mediated by growth hormone.

In the current study, electron microscopic examination of reserve and proliferating cell zones in the lumbar vertebrae of 8 weeks old control rats showed that, resting cartilage zone are formed of spindle shape, chondrocytes rich in lipid and cytoplasmic vacuoles. The endoplasmic reticulum is the best developed cytoplasmic organelle. The proliferating cell zone, are flattened cells and lie in pairs.

The present electron microscopic results are in harmony with Howllet, Burdan *et al*. and Mackie *et al*.[61,55,62] but Howllet performed his study on tibia in avian.

The present electron microscopic results in carbimazole treated 8 weeks old vertebrae showed that the reserve cells appeared shrunken with excessive vacuolization of the cytoplasm with less numerous rough endoplasmic reticulum. The collagen fibers in the extracellular matrix are less apparent. In proliferating cell zone, the nuclei are pyknotic with less demarkated nuclear membrane outline. After thyroxine replacement in this work, the reserve cells preserved their spindle shape. The cytoplasm showed vacuoles but less than that observed in carbimazole treated cells. These electron microscopic results are in harmony with other investigators[63].

Soliman *et al*.[64] hypothesized that thyroid hormones act by affection of growth plate chondrocytes proliferation, differentiation, and invasion by blood vessels. This action was done through thyroid receptors. Several mechanisms by which thyroid hormone perform its action including direct action on the chondrocytes, osteoblasts and mast cells, as well through interaction with other hormones and growth factors

It was concluded that hypothyroidism has marked influence on the bone growth, especially epiphyseal growth plate in the epiphyses of lumbar vertebrae. So once hypothyroidism is diagnosed thyroxin replacement must be started immediately.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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تأثيرات نقص نشاط الغدة الدرقية واستبدال هرمون الغدة الدرقية على تطور الفقرات القطنية في مرحله ما بعد الولادة في الجرذان البيض

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ملخص البحث

الخلفية: يعتبر المستوى الطبيعي لهرمون الغدة الدرقيه ضروريا لنمو الهيكل العظمي الطبيعي وصيانة عظام البالغين. يؤدي نقص افراز هرمون الغدة الدرقية في الاطفال الي توقف في النمو وتاخر في نضج العظام وخلل في التنسج المشاشي.

الهدف من البحث: اجريت هذة الدراسة لتوضيح تاثير نقص افراز هرمون الغدة الدرقية والعلاج بهرمون الثيروكثين علي نمو ما بعد الولادة في الفقرات القطنيه في الجرذان البيض.

المواد وطرق البحث:: تم اجراء البحث علي 60 فارا من الاناث البيضاء البالغة وتم تقسيمها عشوائيا الي ثلاث مجموعات متساوية (1) مجموعه ضابطه امهات:(20) تلقت الماء المقطر (2) مجموعه معالجة بالكاربيمازول (20) تم اعطاء هذة المجموعه عقار الكاربيمازول بجرعه 6 ميكروجرام \جرام يوميا من اليوم العاشر من الحمل وحتى اليوم 21 بعد الولادة واستمر اعطاء الدواء للمواليد حتي نهايه الاسبوع الثامن بعد الولادة (3) مجموعه معالجة بالكاربيمازول+ الثيروكثين (20) الفئران في هذة المجموعه تم اعطاؤها عقار الكاربيمازول بجرعه 6 ميكروجرام \جرام يوميا من اليوم العاشر من الحمل يوميا حتى الولادة وعند الولادة اعطي عقار الثيروكثين بجرعه 10 ميكروجرام \ كجم واستمر اعطاء كل من عقار الثيروكثين والكاربيمازول حتى اليوم 21 بعد الولادة واستمر اعطاء هذين العقارين للمواليد حتى نهايه الاسبوع الثامن بعد الولادة. لكل مجموعه من المجموعات السابقه تم دراسه المواليد في الاعمار الاتيه عمر حديث الولادة,اسبوعين , 8اسابيع.

تم اخذ الفقرات القطنيه في كل عمر وبعد استخراجها وضعت في محلول فورمالين 10% لمدة 48 ساعه تم ازاله الكالسيوم وتجفيف العينات وغمسها في البارافيين وتقطيعها بسمك 5 ميكروميتر ثم صباغتها بصبغات الهيماتوكسلين والايوسين ,ماسون تراي كروم وتلودين بلو وتم فحص العينات بالميكروسكوب الضوئي , واخذت بعض العينات من االفقرات القطنيه وجهزت للفحص الهستولوجى والدراسة المورفومترية ولإحصائيةة في عمر ثمانيه اسابيع.

النتائج: واوضحت النتائج ان نقص افراز هرمون الغدة الدرقية له تاثير واضح علي نمو في الفقرات القطنيه وخاصه في منطقه الانتشار والياف الكولاجين في المصفوفه.واوضحت النتائج المورفومتريه في عمر 8 اسابيع نقص ملحوظ في سمك المشاش في الفقرات القطنيه مقارنه بالمجموعه الضابطه والمجموعه المعالجه بهرمون الثيروكثين لذلك اعطاء هرمون الثيروكثين مباشرة بعد الولادة يودي الي تعديل معظم هذة التغيرات.

الاستنتاج: لوحظ من هذة الدراسه ان نقص افراز هرمون الغدة الدرقية له تاثير واضح علي النمو في الفقرات القطنيه وأن التعويض بهرمون الثيروكثين له تأ\أثير وقائى.