Dept. of Zoology,
Fac. of Science, Assiut University.
Head of Dept. Prof. Dr. E.G. Yoakim.

EFFECT OF HYPERVITAMINOSIS D ON SOME ORGANS OF THE ALBINO RAT
III- BONE
(With 2 Table and 21 Figures)

By

M.K. EL-NAFFAR; I.M. ANWAR; A.B. MAHMOUD; M.S. EL-HAWARY and AMNA M. MOSTAFA

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تأثير زيادة فيتامين (د) على بعض أعضاء الفأر الأبيض

٣ـ العظـــام

محمـد النفـار ، ابراهيم أنور ، عبدالله بكر ، مصباح الهوارى ، آمنـة مصطفى

في هذا لبحث تم دراسة تأثير زيادة فيتامين (د) على عظام الفئران البيضاء وقد أسفرت هـــــذه الدراسة عن النتائج التالية: غلاف العظم أصبح سميكا وزاد عدد الخلايا المكونة للعظم والخلايا العظمية فـــى عمـر ١٤ يوماً بعد الولادة كما انتفخت بعض الخلايا العظميه وضمرت أنويتها في عمر ٢١ يوماً بعد الـــولادة واتخذ العظم غير الناضج خصوصا عمر ٢٨ يوماً شكل العظم الكثيف وزادت فيه نسبة المادة . العظميـــة عـــن الفراغات النخاعية هذا وقد زادت الألياف الغروانيه زيادة ملحوظة في غلاف العظم والمادة بين الخلويـة فـي عمـرى ٢١ ، ٢٨ يوماً بعد الولادة ونقص عدد أجهزة هافرس في الأعمار البالغة بالاضافة الى أنه لم تتأثـــر المواد الكربوهيدراتيه في العظم المعالج بينما زادت المواد البروتينية به في كل الفئـران .

SUMMARY

The effect of hypervitaminosis D on the bone of the albino rats has been studied. The following results have demonstrated:

A) Histological Changes :

In stage of 14-days old, the periosteum becomes more thicker, vascularized and the number of osteoblasts and osteocytes are increased. Some osteocytes become swollen and showed pyknotic nuclei in stage of 21-days old. At the age of 28-days old, the bone having the appearance of a compact bone more than being cancellous unlike the control group, and the proportion between the bone substances and marrow cavities is elevated. The collagenous fibers are markedly increased in the periosteum and in the bone matrix at the ages of 21 and 28-days old.

In adult stages, excessive doses of vitamin D cause other changes in the bone. The osteocytes become more elongated with narrow lucunae. The number of Haversian systems is decreased. The collagenous meterial is increased than that of the normal.

B) Histochemical Changes :

The carbohydrate content in the bones of postnatal and adult stages is not affected, while the proteinic content is increasd in all stages.

C) Physiological Changes :

The amount of calcium in bone-ash and serum is increased than normal.

INTRODUCTION

The previous studies on the effect of hypervitaminosis D on the bones of mammals in postnatal life were few. JEANS (1950) found that the excessive doses of vitamin D cause abnormal deposits of calcium in the diaphysis of long bones. SEBRELL and HARRIS (1954) observed some histological changes in the bone.

The present study is mainly concerned with histological, histochemical and physiological changes in the bones ofalbino rats due to hypervitaminosis D.

MATERIAL and METHODS

Fifty-four postnatal rats were divided into six groups, each with 9 rats. Three groups of these animals which constitute the treated animals were daily injected intraperitoneally with vitamin D (Devarol-S) dissolved in sesame oil at a dosage of 0.2 ml (12.000 i.u.). This dose was administered for seven successive days to 7, 14 and 21-days old. The other three groups served as controls. They were received the same dose of sesame oil only by a similar manner.

Thirty-six mature adult rats of 90-days old were divided into four groups each of 9 rats. The first two groups were given vitamin D in the same manner at a dosage of 1 ml (60,000 i.u.). The doses were used for 7 successive days to one group and 21 successive days to the second one. The other two groups were served as the control groups.

On day after the last injection all the rats were dissected. Samples of blood were collected for obtaining the serum. The bone of the hind limb was dissected free from the soft tissue. Small parts of diaphysis were fixed in buffered formalin and decalcified in ethylendiamine-tetra-acetic acid (EDTA) (HILLEMAN and LEE, 1953). They were dehydrated, cleared, embedded in paraffin wax and sectioned at 5 um. thickness.

For the histological study, Harris' haematoxylin and eosin stain and Masson's trichrome stain were used. All the methods were applied according to DRURY and WALLINGTON (1980).

For the histochemical study, PAS reaction (HOTCHKISS, 1948) was used for the demonstration of carbohydrates in general and bromophenol blue method (PEARSE, 1980) was used for the demonstration of general proteins.

For the physiological studies, calcium in serum of control and treated rats was measured according to the reaction of cresophalein complexone with calcium to form coloured complex compound which is measured colorimetrically according to the technique of GITELMAN (1967). Also calcium was determined in bone-ash of normal and treated rats according to SCHWARZENBACH and BIEDERMAN (1948). This method involves the titration of amount of calcium in solution of bone-ash against versene solution forming a purple colour. The amount of calcium is given by the amount of reagent.

RESULTS

A) Histological Changes :

Normal Bone :

Stage of 14-days old:

The bone in this stage is cancellous. It consists of bone trabeculae and enclose irregular marrow cavities of various sizes. Cancellous bone is characterized by possessing more cavities than bone substance (Fig. 1 & 4).

Cancellous bone is surrounded by the periosteum and consists of two layers: The outer fibrous layer and the inner osteogenic layer which is formed of osteogenic cells that stained deeply. The endosteum is a less marked layer on the internal surfaces of the bone lining the large central cavity of this bone (Figs. 1 & 2).

The osteogenic cells are flat and less differentiated. The osteoblasts are present immediately below the osteogenic layer of periosteum and surrounding the bone marrow cavities. The osteoblasts are oval in shape having faintly stained cytoplasm and darkly stained nuclei. The nuclei are oval and eccentric (Fig. 2). The osteocytes are situated beneath the osteoblasts, embedded in bone matrix and present inside lacunae. Thelarge osteocytes are ovoid in shape and situated near the endosteum. Their nuclei are densely stained. The cytoplasm is faintly stained and their lacunae are notwell-defined (Figs. 2 & 3).

The periosteum and bone matrix are rich in blood vessels and contain dense bundles of collagenous fibers (Fig. 4).

Stage of 21-days old:

In this stage, the bone cells are more defined and characterized by increased number of osteoblasts that become relatively large. The cavities become correspondingly smaller in size, therefore the matrix having bone substances more than cavities (Figs. 4 & 5). New layers of bone are added and characterized by the appearance of fine lines known as cementing lines. The osteocytes are increased in number and enlarged in size. The majority of them are ovoid in shape (Figs. 5 and 6). The bone matrix contains great amount of collagen substances. The endosteum is stained deeply with Masson's trichrome.

Stage of 28-days old:

The periosteum is thicker than that of the previous stages. All the cavities in the cancellous bone of this stage are lined with osteogenic cells which multiply and differentiate into osteoblasts. The endosteum is similar to the previous stage (Fig. 7). Both the periosteum and the matrix having thick bundles of collagenous fibers as described before.

Stage of 97-days old:

The bone in this stage represents the more adult type, known as compact bone, where Haversian systems are common. The matrix stains lightly. The mature bone is characterized by new layers of bone cells. The periosteum is not prominent, however the osteogenic layer is formed of thin, pale, spindle shaped cells lying on the outer surface of the bone. These are resting osteogenic cells. The osteocytes are well established and found embedded in their lacunae. They are ovoid in shape (Figs. 8 & 9). More collagenous fibers are shown mainly in the periosteum and the bone matrix (Fig. 10).

Stage of 111-days old:

In this stage, the periosteum is referred to as resting periosteum where neither apposition nor resorption is occuring.

Haversian systems are well-developed and so this stage represents the fully mature adult stage. Each system has a central Haversian canal which is surrounded by very crowded osteocytes (Fig. 11). The collagenous fibers of the periosteum showed dark stainability, while the matrix showed moderate amount of collagenous fibers which is moderately stained.

Treated Bone:

Stage of 14-days old:

Comparing the treated rats with the control ones, the periosteum is more thicker, having large number of osteogenic cells. Below the periosteum, numerous osteoblasts could be observed. The periosteum becomes vascularized. Both the number of osteoblasts and osteocytes are increased. The matrix seemed loosely and faintly stained. Some osteocytes are slightly enlarged (Fig. 12).

The quantity of collagenous fibers is similar to that of the normal.

Stage of 21-days old:

The osteocytes are relatively swollen ad show pyknotic nuclei (Fig.s 13). More collagenous fibers are markedly observed in the periosteum and the matrix if compared to the normal (Fig. 14).

Stage of 28-days old:

It revealed that the bone having the appearance of a compact structure more than being cancellous unlike the normal stage of the same age. The marrow cavities are decreased, and the proportion between bone substances and marrow cavities is elevated. The osteocytes are increased in number more than the control groups. The Haversian system begins to appear in this stage (Figs. 15 & 16).

The quantity of collagenous fibers in the periosteum and the matrix is similar to that of the previous stage.

Stages of 97 and 111-days. old:

A slight histological changes could be observed in these stages if compared to normal. The osteocytes become more elongated with darkly stained nuclei and narrow lacunae. The number of Haversian systems is decreased (Fig. 17). The matrix revealed the presence of more collagenous fibers (Fig. 18).

B) Histochemical Changes :

Carbohydrates :

After treatment with large doses of vitamin D, the bone constitutents in post-natal stages and adult stages are stained in a similar manner as the control (Fig. 19).

General Proteins :

In general, the bone of the normal rats contains different stainable elements. The periosteum contains high amount of general proteins. However, the matrix exhibits a moderate amount. In 14-days old rat, the periosteum and bone matrix show moderate amount of protein (Fig. 20). In 21 and 28 days old, the bone matrix is less stained while that of the bone marrow trabeculae is similar to the stage of 14-days old. In stages of 97 and 111-days old, the proteinic content is greatly in the periosteum and bone matrix.

After treatment with vitamin D, the periosteum and the matrix of postnatal stages showed great increase in proteinic content (Fig. 21). In adult stages it showed a slight increase in their proteinic content than the control groups.

C) Physiological Changes :

The calcium level is measured in the serum and bone-ash of normal and treated animals.

1) Determination of total serum calcium:

The calcium is determined in serum according to the method of GITELMAN

(1967). The concentrations of calcium in serum samples of normal and treated rats were calculated in table (1).

2) Determination of bone-ash calcium:

The calcium is determined in bone-ash according to SCHWARZENBACH and BIEDERMAN (1948). The levels of calcium in bone-ash of normal and treated rats can be summerized in table (2).

Table (1): Levels of calcium serum of normal and treated rats.

Serum specimens	Conc.of calcium/ 100 ml. serum of normal rats	Conc.of calcium/ 100 ml. serum of treated rats
14-days old.	5.83 mg.	9.50 mg.
21-days old.	12.33 mg.	14.67 mg.
28-days old.	14.67 mg.	15.33 mg.
97-days old.	10.26 mg.	16.41 mg.
111-days old.	12.82 mg.	14.87 mg.

Table (2): Concentrations of calcium in bone-ash of normal and treated rats.

Specimen of bone-ash	Conc.of calcium/ gm.dry wt. of normal rats	Conc.of calcium/gm. dry wt. of treated rats
14 - days old.	58.8 mg.	62.4 mg.
21 - days old.	62.8 mg.	68.8 mg.
28 - days old.	72.8 mg.	78.8 mg.
97 - days old.	58.0 mg.	68.8 mg.
111- days old.	53.8 mg.	78.6 mg.

EXPLANATION OF FIGURES

Cementing Line (CL), Endosteum (E), Fibrous Layer (FL), Haversian Canal (HC), Matrix (M), Marrow Cavity (MC), Osteoblast (O), Osteocyte (OC), Osteogenic Layer (OL), Periosteum (P) andTrabecula (T).

Figs. 1-4: L.S. of the diaphysis of the hind limb of a control rat (14-days old).

1- Showing periosteum, marrow cavity and endosteum. Haematoxylin and eosin. X 256.

2- Showing the fibrous layer, osteogenic layer and osteoblast. Haematoxyiin and eosin. X 640.

3- Showing osteoocyte. Haematoxylin and eosin. X 640.

4- Showing the matrix, marrow cavity and trabecula. Masson's trichrome. X 200.

Figs. 5 & 6: L.S. of the diaphysis of the hind limb of a control rat (21-days old) stained with haematoxylin and eosin.

5- Showing osteoblast and marrow cavity. X 256.

6- Showing cementing line and osteocyte. X 640.

Fig. 7: L.S. of the diaphysis of the hind limb of a control rat (28-days old) stained with haematoxylin and eosin. Showing periosteum. X 256.

Figs. 8 & 9: L.S. of the diaphysis of the hind limb of a control rat (97-days old) stained with haematoxylin and eosin.

8- Showing periosteum and Haversian canal. X 256.

9- Showing osteocyte. X 640.

Fig. 10: The same section of the same rat stained with Masson's trichrome. X 200.

Fig. 11: L.S. of the diaphysis of the hind limb of a control rat (111-days old), showing periosteum and osteocyte. Haematoxylin and eosin. X 200.

Fig. 12: L.S. of the diaphysis of the hind limb of a treated rat (14-days old), showing periosteum, osteoblasts and osteocyte. Haematoxylin and eosin. X 256.

Fig. 13: L.S. of the diaphysis of the hind limb of a treated rat (21-days old), showing osteocyte. Haematoxylin and eosin. X 640.

Fig. 14: The same section of the same rat stained with Masson's trichrome. X 200.

Figs. 15 & 16: L.S. of the diaphysis of the hind limb of a treated rat (28-days old), showing Haversian canal and osteocyte. Haematoxylin and eosin. X 256 and 640 respectively.

Fig. 17: L.S. of the diaphysis of the hind limb of treated rats (97 and 111-days old), showing osteocyte. Haematoxylin and eosin. X 640.

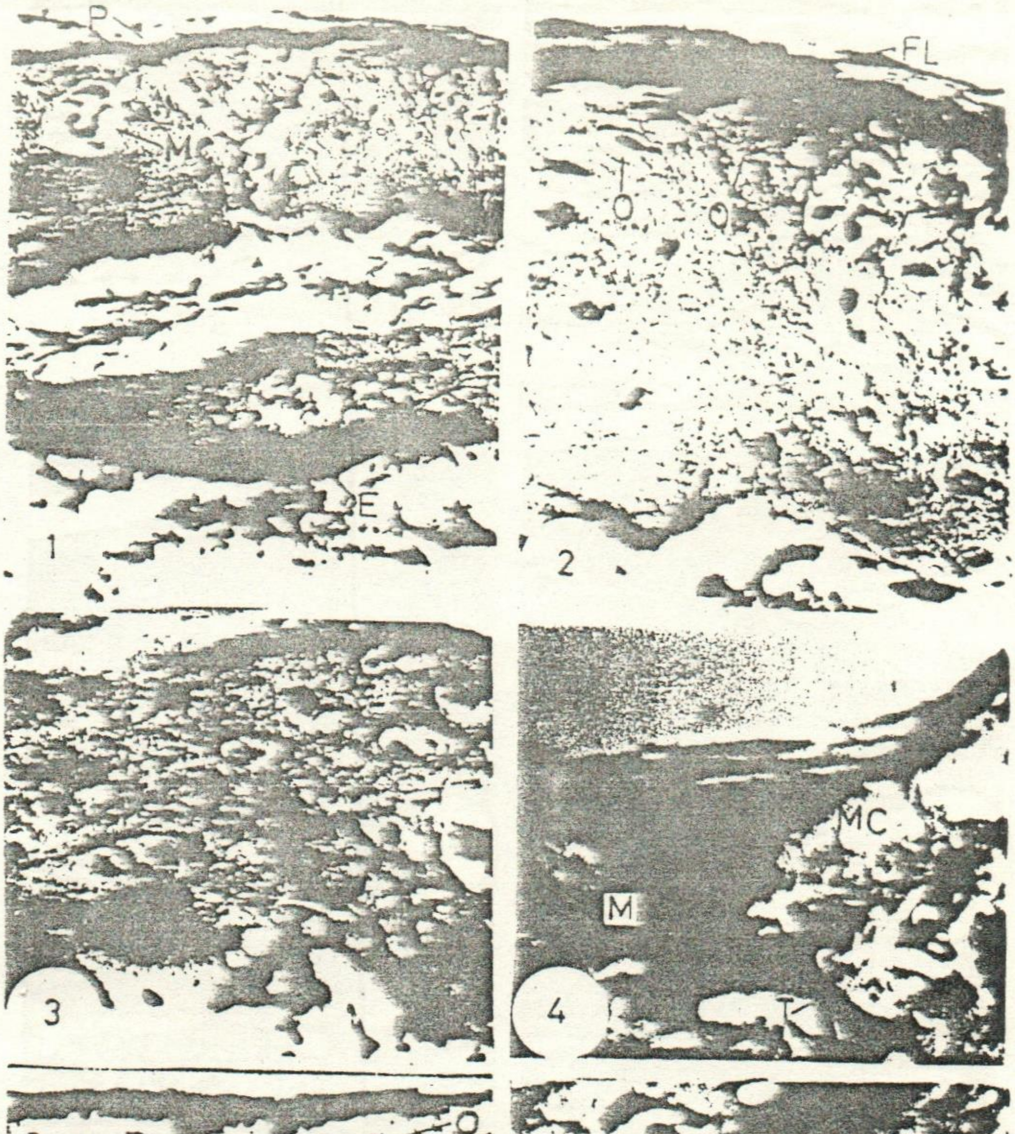
Fig. 18: The same section of the same rat stained with Masson's trichrome. X 200.

Fig. 19: L.S. of the diaphysis of the hind limb of a treated rat (14-days old). PAS technique. X 640.

Figs. 20 & 21: L.S. of the diaphysis of the hind limb of rat (14-days old). Bromophenol blue.

20- Control rat. X 640.

21- Treated rat. X 680.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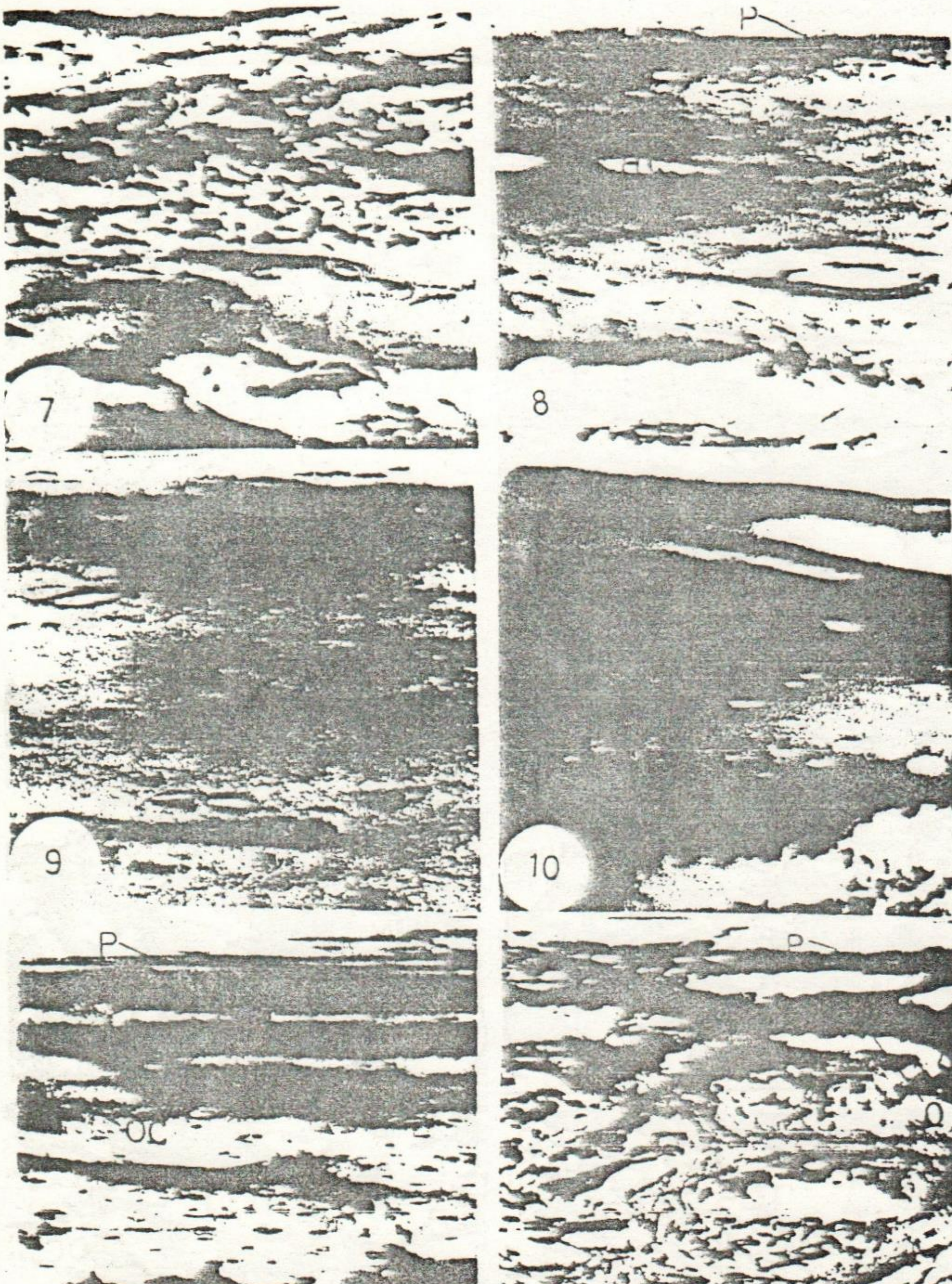
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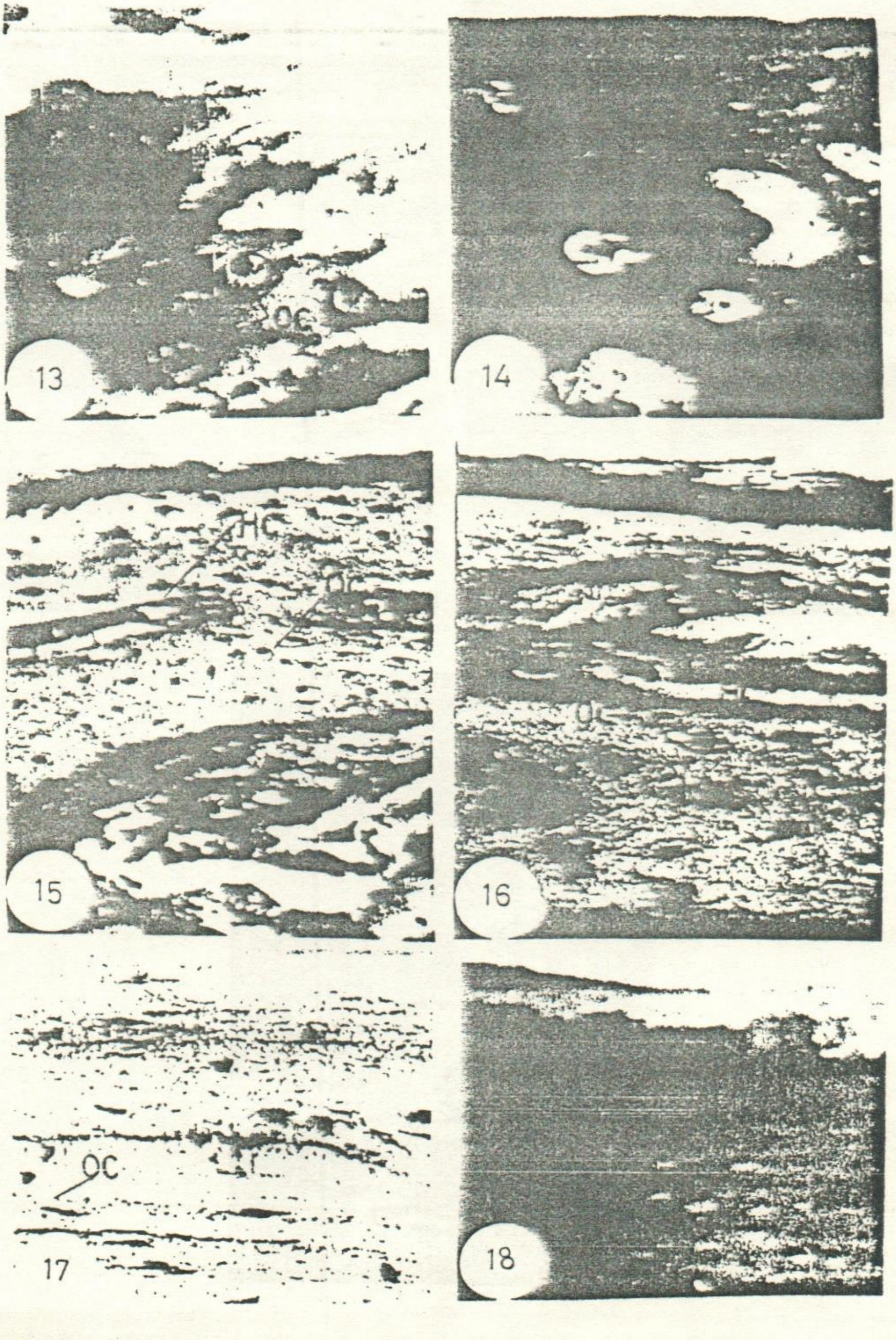
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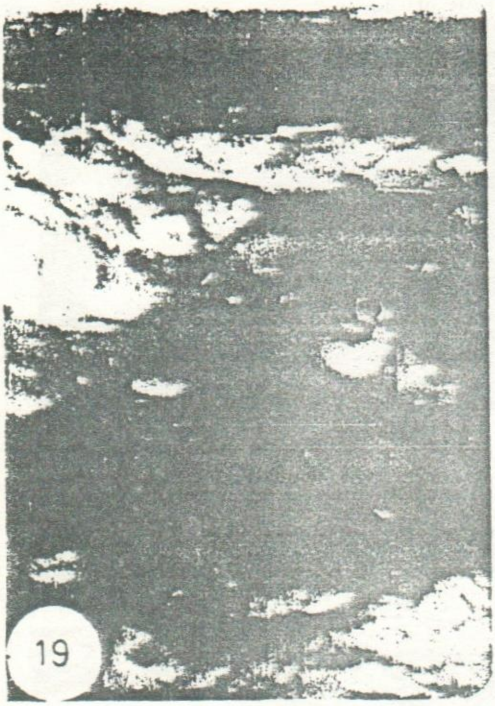
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DISCUSSION

The present study showed that the periosteum is thicker. Such result was observed in the early stages of children by SEBRELL and HARRIS (1954) who reported that the bones show accelerated calcification with thickening of the periosteum under the effect of hypervitaminosis D. In the present study, the bone matrix of 14-days old seemed to be lossely due to bone resorption. STERN (1980) noted that the excess of vitamin D results in resorption of bone with loss of both mineral and matrix in which bone resorption involves the removal of both the mineral and organic phases of the bone matrix.

Rresults of the present study revealed that some osteocytes are swollen with pyknotic nuclei and the number of osteoblast and osteocytes are increased. Similar results were observed by WEISBRODE et al. (1977 & 1979) in rats.

In 28-days old rat, the bone having the appearance of a compact structure more than being cancellous. The proportion between bone substnces and marrow cavities is elevated due to acceleration of calcification as a result of hypervitaminosis D.

The bone collagenous material is increased in the present study. ISAEVA et al. (1978) found that vitamin D exerts a favorable effect on the state of the collagen matrix in the bone tissue of rat. CANAS et al. (1969) and RAISZ et al. (1978) found that the vitamin D-deficient chick showed enhanced collagen systhesis in cartilage after treatment with vitamin D.

The bone of the present study revealed that the carbohydrate content is not affected under the effect of hypervitaminosis D. However, the proteinic content is increased. WIENTROUB et al. (1987) found that vitamin D deficiency has no effects on bone cell-derived noncollagenous proteins in rats.

In th present work, there is an increased in the serum calcium level. Such observation was recorded by SWARUP et al. (1984) who administered vitamin D to the male catfish, Clarias batrachus for 17 days and BALSAN et al. (1970) who gave a single oral dose of vitamin D to children. The calcium in bone-ash is increased in the present study which coincides with the result recorded by WEISBRODE et al. (1977 & 1979).

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