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EFFECT OF HYPERVITAMINOSIS D ON SOME ORGANS OF THE ALBINO RAT II- KIDNEY

(With 20 Figurs)

By

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تأثير زيادة فيتامين (د) على بعض أعضاء الفأر الأبيض

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في هذا البحث دُرس تأثير الجرعات المتزايدة من فيتامين (د) على كلية الفئران البيضاء وقد تمخضت هذه الدراسة عن النتائج التالية: في الفئران بعد الولادة أظهرت قطاعات الكلية مايلي : ضمور في بعض الأنيبيبات الملتفة القريبة والبعيدة والأنيبيبات الجامعة وانتفخت بعض الخلايا وضمرت أنويتها كما اختفت الحواف الحرة للأنيبيبات الملتفة القريبة. زادت كمية النسيج الضام. وجود رواسب الكالسيوم في الجُبَّع والأنيبيبات الملتفة والجامعة وانحناءات هنلي والأوعية الدموية . أظهرت الفئران البالغة: خاصة عمر ١١١ يوماً انفجار بعض الأوعية الدموية وخروج كرات دموية حمراء وظهور تجمعات من حبيبات بنية صدئية اللون على شكل ترسيبات صبغية. زادت كمية المواد الكربوهيدراتية في الفئران بعد الولادة عمرى ٢١ ، ٢٨ يوماً وكذلك في الفئران البالغة أما المحتوى البروتيني في كلى جميع فئران التجارب فقد أظهر زيادة طفيفة خاصة في الأعمار البالغة.

SUMMARY

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

A) Histological changes:

In the postnatal changes: Some convoluted tubules and collecting tubules show degeneration and the others are swollen with pyknotic nuclei. The brush borders of some proximal convoluted tubules are disappeared. The amount of collagenous fibers is markedly increased. Calcium deposits were observed in the glomeruler tufts, cells of convoluted and collecting tubules, loops of Henle and blood vessels.

In the adult stages: Hypervitaminosis D causes other histological changes in the kidney of rats. Some cells of the convoluted tubules and cellecting tubules showed necrosis. Extravasated red blood corpuscles are observed and cluster of rusty brown particles are occasionally encountered in the blood vessels.

B) Histochemical changes:

The carbohydrate substances are increased in the postnatal rats of 21-and 28-days old and adult stages. The proteinic content is slightly elevated specially in adult stages.

INTRODUCTION

The effects of hypervitaminosis D on the kidney of experimental adult animals have been extensively studied (HASS et al.,1958 and STERN, 1980). On the other hand, such studies were very few in postnatal life. POTVLIEGE (1962) found that the epithelium of proximal convoluted tubules in the kidney of rats exhibited basal cytoplasmic vacuolization and intracytoplasmic hyaline granules.

In this study, it is aimed to give an account on the histological and histochemical effects of hypervitaminosis D on the kidney of postnatal and adult albino rats.

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 & 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 daily injected intraperitoneally with the vitamin D which dissolved in sesame oil 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.

One day aftr the last injection, all the rats were dissected. Small pieces from the kidney were removed and immediately immersed in the fixatives which were Bouin's fluid, formal-alcohol and 10% formalin. They were dehydrated in ascending series of concentrated ethyl alcohol, then cleared in xylene, embedded in paraffin wax and sectioned at 5 um thicknes.

For the histological study, Harris' haematoxylin and eosin stain, Masson's trichrome stain and von Kossa method 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.

RESULTS

Histological Changes :

Normal kidney :

Stages of 14-and 21-days old:

The kidney is covered with a thin fibrous connective tissue capsule. Its renal tissue is divided into an outer cortex, and inner medulla. The uriniferous tubules are formed of nephrons and collecting tubules. Each nephron consists of: Malpighian or renal corpuscle, proximal convoluted tubule, Henle's loop and distal convoluted tubule (Figs. 1 & 2).

The renal corpuscle is rounded in shape and consists of a glomerulus and Bowman's capsule. The glomerulus is composed of tufts of capillaries which are formed of afferent arteriole enters and the efferent arteriole leaves the renal corpuscle. The bowman's capsule has a double walled epidthelial capsule that surrounds the glomerular blood capillaries (Fig. 1).

The proximal convoluted tubules are the most common components of the cortex. They are composed of simple cuboidal cells. The bases of the cells are broader than their apical parts. The free surfaces of these cells have brush borders (Fig. 1).

The distal convoluted tubule is made up of low cuboidal cells. The free borders of the cells having no brush borders and the lumina of the distal convoluted tubules are wider than of the proximal tubules (Fig. 1).

The collecting tubules and the Henle's loops are seen in the inner part of the cortex but the majority are seen in the medulla. The collecting tubule is composed of simple columnar cells. The lumen is wide with a thick wall (Fig. 2).

The thin segment (descending limb) of Henle's loop is similar to blood capillary as it is lined by simple squamous epithelium. The thick segment (ascending limb) of Henle's loop is lined with cuboidal epithelium (Fig. 2).

More collagenous fibers are found in the capsule. A sheath of delicate collagenous fibers are observed around the tubules. Both the proximal convoluted and collecting tubules have collagenous brush borders. More substantial fibers are found in association with the large vessels of the kidney (Fig. 3). Bundles of fibers were found to be delimit the Henle's loops and suspended in the interstitial connective tissue.

Stage of 28-days old:

The renal corpuscles, convoluted tubules (Fig. 4) and collecting tubules are enlarged. The capsule is slightly thicker than the previous stage. The amount of collagenous fibers is slightly increased.

Stages of 97-and 111-days old:

The kidney of these stages is more developed. It consists mainly of the previously described components which includes the capsule, the cortex and the medulla. The lumina of these tubules are narrow and show clear brush borders. The distal convoluted tubules having wide lumina and more cells without brush borders. The glomeruli are surrounded by more distinct double walled capsular epithelium (Bowman's capsule). The space between the two layers is called the capsular or Bowman's space (Fig. 5). The ascending and descending part of Henle's loop are more enlarged. The amount of the collagenous fibers in these stages is elevated than in the previous stages (Fig. 6). Von Kossa method showed no calcium precipitates could be observed in all normal stages.

Treated kidney :

Stage of 14-days old:

The convoluted tubules of the cortex are darkly stained. A slight changes including the nuclear enlargement of most cellular content in the cortex as well as some vacuolation are observed. The intertubular connective tissue contains thick walled blood vessels (Fig. 7).

The quantity of the collagenous fibers is elevated in the renal corpuscles and the lumina of convoluted tubules and collecting tubules if compared with the normal (Fig. 8). No calcium deposits could be observed.

Stage of 21-days old:

The brush borders of the proximal convoluted tubules are disappeared. Some cells are degenerated and some tubules showed marked destruction resulting the appearance of large vacuoles (Fig. 9).

The amount of collagenous fibers is similar to the previous stage.

Calcium deposits can be observed in the glomerular tufts (Fig. 10), the cells of convoluted tubules, and the collecting tubules as well as the wall of blood vessels in the cortex. Also, calcium deposits are observed in the cells of collecting tubules and in Henle's loops in the medulla. These deposits are shown in the form of patches of large size granules.

Stge of 28-days old:

Numerous vacuoles are clearly seen between the uriniferous tubules in the cortex. The tubules and their cells were found to be swollen. Some cells are degenerated, while some tubules lost their characteristic form (Fig. 11). The renal corpuscles and the lumina of the tubules showed a slight increased level of the collagenous fibers. In this stage, calcium deposits take the form of patches of large size granules as in the previous stage.

Stage of 97-days old:

Some of the uriniferous tubules lost their characteristic features where their boundaries are ruptured and showed necrosis. Numerous convoluted tubules appeared hypertrophic containing poorly stained cells. Some cells have pyknotic nuclei and others are swollen. The lumina of the uriniferous tubules are enlarged (Fig. 12). The glomeruli are stained deeply, while the capsular space is wide in certain areas. The level of the collagenous fibers is slighty increased than normal (Fig. 13).

The calcium deposits take the form of scattared granules in all the constituents of the renal units (Fig. 14).

Stage of 111-days old:

In addition to that observed in stage of 97-days old, this stage revealed the presence of extravasated red blood corpuscles and clusters of rusty brown particles. These particles are found in the blood vessels and intertubular connective tissue in the form of pigments. (Figs. 15 & 16). The uriniferous tubules showed a marked destruction and most cells are degenerated. Some cells are irregularly distributed without clear boundaries and containing darkly stained nuclei and cytoplasm. Necrotic areas are observed between the uriniferous tubules due to the degenerative changes (Fig. 15).

Histochemical Changes :

Carbohydrates :

The cortex of kidney in control rats of 14, 21 and 28-days old contains different stainable elements. In 14-days old rat, the capsule is moderately stained. The brush borders and the lumina of the convoluted and collecting tubules are deeply stained. Their boundaries are faintly stained. The glomeruli are moderately stained. Generally, the carbohydrate content is reduced from that age till 28-days old (Fig. 17). The uriniferous tubules of medulla of the previously decribed stages are faintly stained. In stage of 97-days old, the carbohydrate content is slightly elevated in the renal corpuscles, boundaries of the convoluted and collecting tubules, but it is slightly decreased from the brush borders and the lumina of the convoluted and collecting tubules. Also, themedulla showed a slight increase in its carbohydrate content, specially in the boundaries of the tubules. However, in 111-days old, the cortex showed a slight decrease in carbohydrate level, but in the medulla, the carbohydrate level is increased than that described in stage of 97-days old.

After treatment with vitamin D, the kidney of 14-days old rat revealed no change in the carbohydrate content. The kidney of 21-and 28-days old rat showed a great increase in carbohydrate substances which stained in a deep manner (Fig. 18), while of 97-and 111-days old revealed that the carbohydrate content is slightly increased in the renal corpuscles and boundaries of convoluted and collecting tubules.

It decreased from the brush borders of proximal convoluted tubules and lumina of the convoluted and collecting tubules.

General Proteins :

The 14-days old rats showed that, the capsule, glomeruli, convoluted tubules, collecting tubules and the constituents of the medulla revealed the presence of slight amount of protein. In 21 and 28-days old, the capsule and glomeruli showed moderate amount of protein, while the other constituents of cortex and medulla revealed the presence of slight amount of protein. In 97 and 111-days old, the capsule showed that the amount of protein is similar to that described in stages 21 and 28-days old, but the convoluted and collecting tubules and loops of Henle showed increase in the amount of protein which stained moderately by bromophenol blue (Fig. 19).

After treatment with vitamin D, the 14-days old rats showed that the proteinic content of th cortical constituents is increased, but the proteinic content of the medulla is unchanged other than that described in the control sections. In 21 and 28-days old the proteinic content of the cortex is not affected, but the proteinic content of the medulla is generally increased than that described in 14-days old. The kidney of 97 and 111-days old showed that the proteinic content is slightly elevated than the control group of the same age and concentrated in the capsule, and around the tubules and loops of Henle of the medulla, but the other constituents remained unchanged. (Fig. 20).

Sections of the kidney stained by bromophenol blue method revealed that the proteinic content is variable in postnatal stages but it is slightly elevated in adult stages.

DISCUSSION

The renal tissue showed calcium deposits in the form of patches of small granules and scattered granules. The degree of deposition is directly proportional in accordance with the increase of dose and the duration of treatment. HASS et al. (1958) found that the calcium was in the form of band-like granules in the kidney of rabbit.

The large doses of vitamin D caused swollen of the renal cells with cytoplasmic vacuolation. This finding is inagreement with POTVLIEGE (1962) who observed basal cytoplasmic vacuolation in the proximal convoluted tubules of rats and their of springs as result of hypervitaminosis D. In the present study, swelling, pyknosis of nuclei and necrosis in the renal tubules were observed. This result coincides with that obtained by EL-BANHAWY et al. (1986) on the kidney of rats as result of insecticide (nuvacron).

The lumina of the uriniferous tubules are enlarged. This result coincides with that of POTVLIEGE (1962) who found dilation of some nephrons and collecting tubules in the kidney of albino rats. The glomeruli, proximal and distal convoluted tubules, collecting tubules and intertubular connective tissue showed signs of degeneration, the exact of which depends on the intensity of hypervitaminosis D. This result was

recorded by STERN (1980) in human as result of hypervitaminosis D.

The presence of extravasated red blood corpuscles in the kidney of adult rats was due to lesions in the blood vessels, the rusty brown particles were mostly haemosiderin resulting from distruction of haemoglobin of the red blood corpuscles. Such result was reported by YOAKIM and EL-NAFFAR (1986) in the kidneys of the fish Tilapia nilotica infected with metacercaria of the parasitic trematode Euclinostomum ardeolae.

The renal tissue showed that the carbohydrate substances are greatly increased in postnatal stages, but in the adult it is slightly increased. This rsult is contradicted to what has been found by KAMEL et al. (1975) who reported that the carbohydrate substances are decreased in the cells of the treated kidney under the effect of hypervitaminosis A on the mother hens. In the present study, the proteinic content of the renal tissue is slightly elevated specially in adult stages. TAYLOR and WASSERMAN (1972) found that the calcium-binding protein content of the kidney was decreased in chicks fed on vitamin D-free diet from day of hatching up to 6 weeks and rapidly increased when vitamin D was administered to chicks.

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EXPLANATION OF FIGURES

Bowman's Capsule (BC), Blood Vessel (BV), Calcium Deposits (CD), Capsular Space (CS), Collecting Tubule (CT), Distal Convoluted Tubule (DCT), Glomerulus (G), Necrotic Area (NA), Proximal Convoluted Tubule (PCT), Pigment (PG), Parietal Layer of Bowman's Capsule (PW), swollen Cell (SC), Thick Segment of Henle's Loop (TCH), Thin Segment of Henle's Loop (TH) and Vacuole (V).

Figs 1 & 2: Transverse sections of the kidney of a control rat (14 and 21-days old) stained with haematoxylin and eosin. X 640.

1- Showing distal convoluted tubule, Bowman's capsule and proximal convoluted tubule.

2- Showing thin segment of Henle's loop, collecting tubule and thick segment of Henle's loop.

Fig. 3: The same section of the same rat stained with Masson's trichrome. X 500.

Fig. 4: Transverse section of the kidney of a control rat (28-days old), showing proximal and distal convoluted tubules. Haematoxylin and eosin. X 640.

Figs. 5 & 6: Transverse sections of the kidney of control rats (97 and 111-days old.

5- Showing distal and proximal convoluted tubules, capsular space and glomerulus. Haematoxylin and eosin. X 640.

6- Masson's trichrome. X 500.

Figs. 7 & 8: Transverse sections of the kidney of a treated rat (14-days old).

7- Showing distal and proximal convoluted tubules, blood vessel and vacuole. Haematoxylin and eosin. X 640.

8- Masson's trichrome. X 500.

Figs. 9 & 10: Transverse sections of the kidney of a treated rat (21-days old).

9- Showing degenerated tubules (arrows). Haematoxylin and eosin. X 640.

10- Showing calcium deposits. Von Kossa method. X 800.

Fig. 11: Transverse section of the kidney of a treated rat (28-days old), showing swollen cell. Haematoxylin and eosin. X 640.

Figs. 12-14: Transverse sections of the kidney of a treated rat (97-days old).

12- Showing proximal convoluted tubule. Haematoxylin and eosin. X 640.

13- Masson's trichrome. X 500.

14- Showing calcium deposits. Von Kossa method. X 800.

Figs. 15 & 16: Transverse sections of the kidney of a treated rat (111-days old) stained with haematoxylin and eosin. X 640.

15- Showing necrotic area and pigments.

16- Showing pigments.

Figs. 17 & 18: Transverse sections of the kidney of rats (21 and 28 days old). PAS technique. X 640.

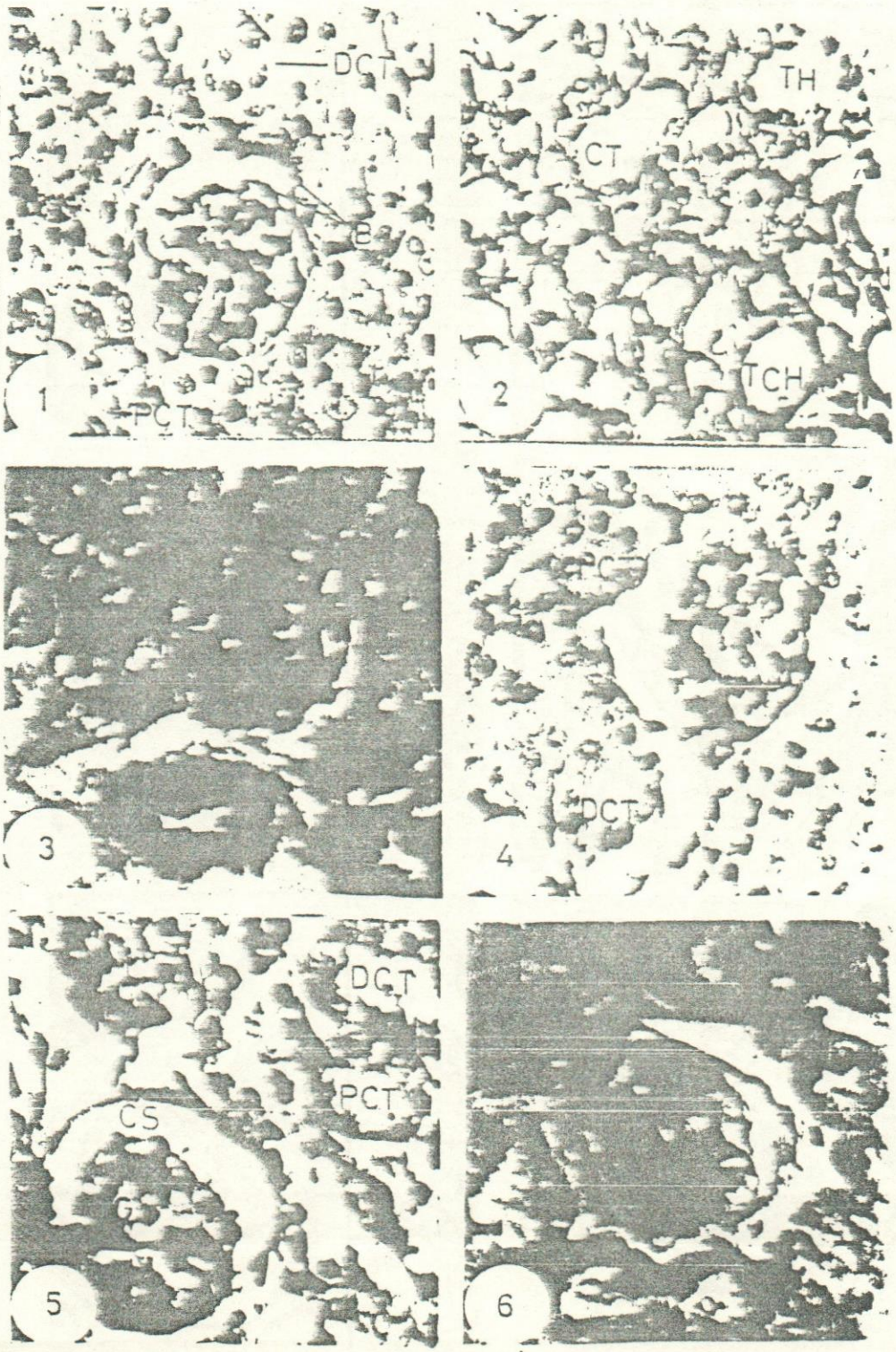
17- Control rat.

18- Treated rat.

Figs. 19 & 20: Transverse sections of the kidney of rats (97 and 111-days old). Bromophenol blue. X 640.

19- Control rat.

20- Treated rat.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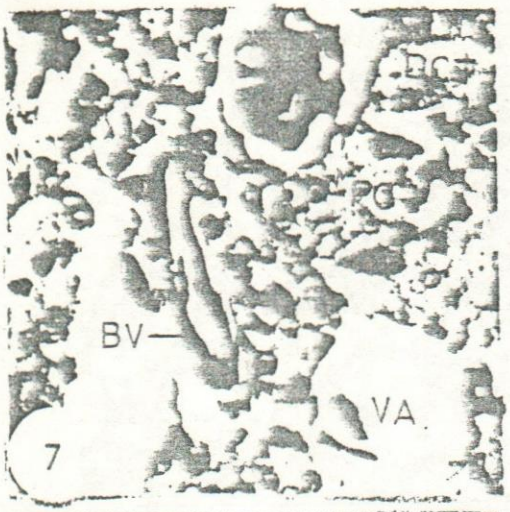

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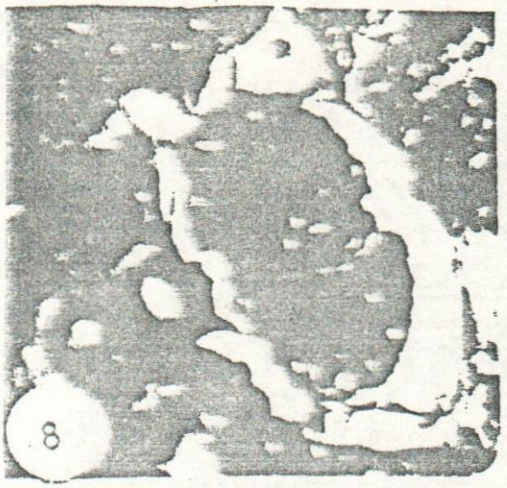

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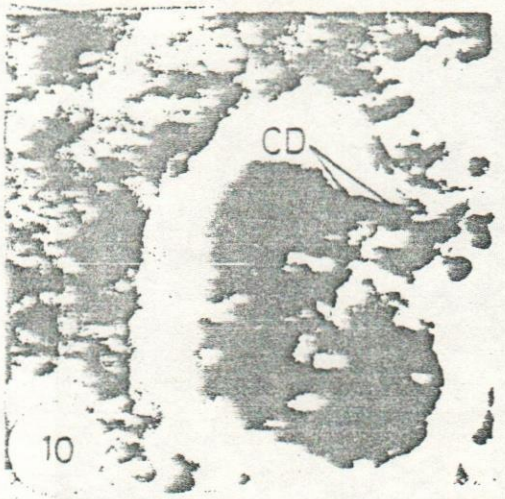

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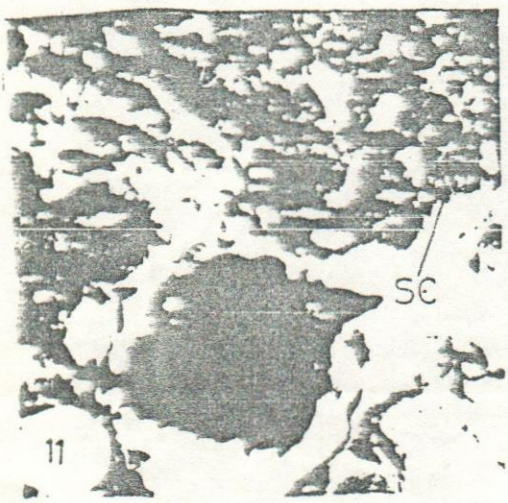

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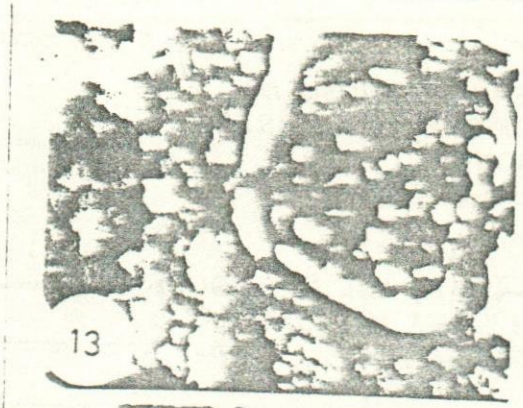
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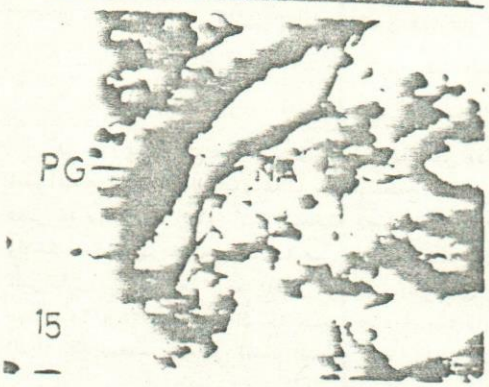
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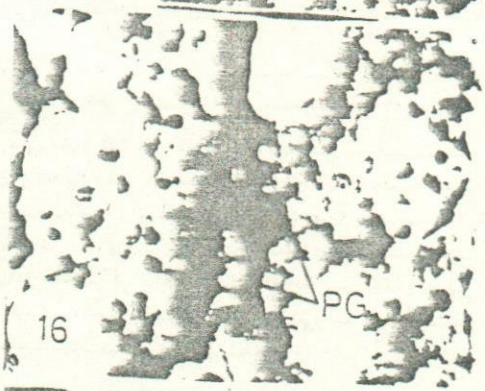
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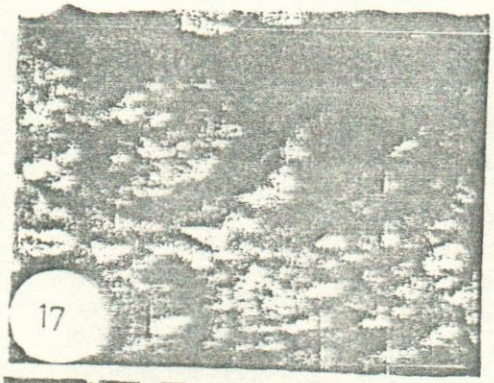
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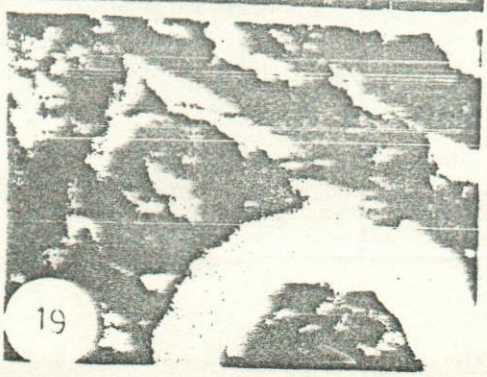
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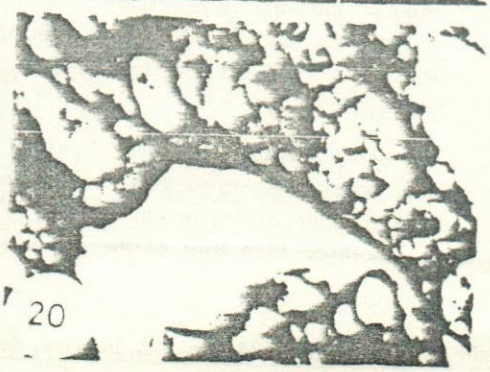
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